Tomocube

TomoStudio™

Software User Manual – ver. 3.0

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Table of Contents

Introduction

TomoStudio works in conjunction with Tomocube's holographic microscope to acquire, visualize, and analyze 2-D and 3-D images of microscopic objects such as living cells and tissues — though it is not limited to viewing these. The microscope supports three types of modalities – holotomography, fluorescence imaging, and grayscale brightfield imaging (2-D only).

1.1 System Requirements

TomoStudio uses GPU acceleration for fast processing and visualization. Here are the hardware requirements for running TomoStudio:

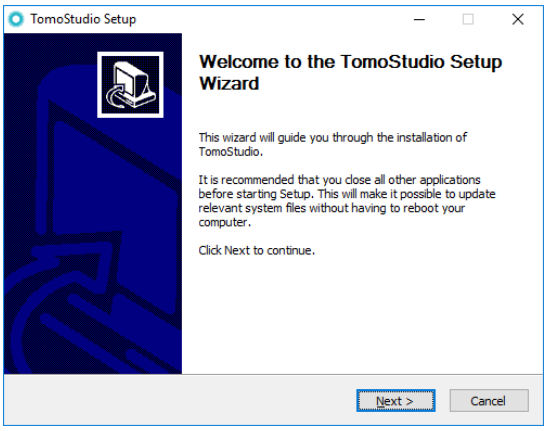
	Recommended	Minimum
CPU	Intel Core i7	Multi-core CPU
Memory[1)]	64 GB	16 GB
GPU/Graphics Card	GeForce RTX 2070	GeForce GTX 980
Hard Disk	8 TB (4TB x 2)	500 GB
Operating System	64-bit version of Microsoft (MS) Windows 10	64-bit version of MS Windows 7

1) Normally it requires a large amount of memory, at least twice the file size.

1.2 Installing TomoStudio

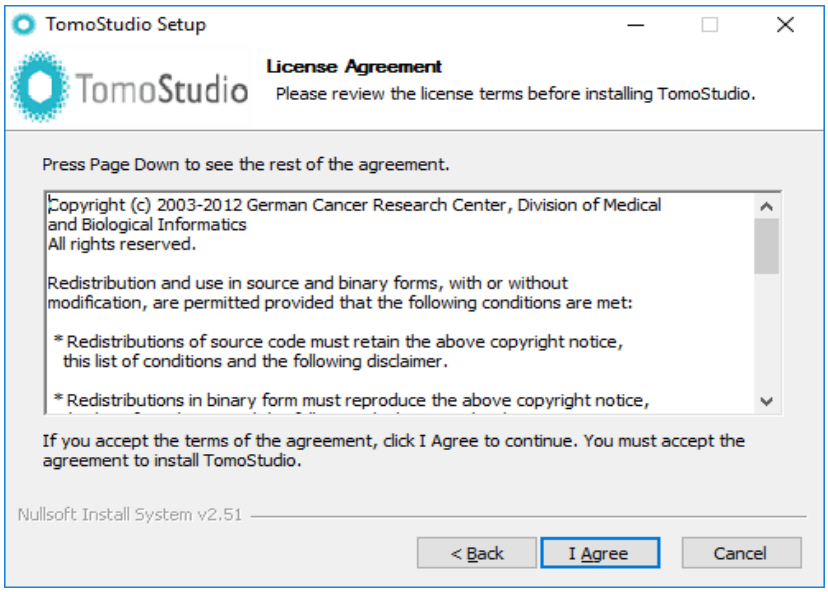
To install TomoStudio, please follow the directions below:

1. Double-click the installation file, and the **Setup Wizard** will pop up.

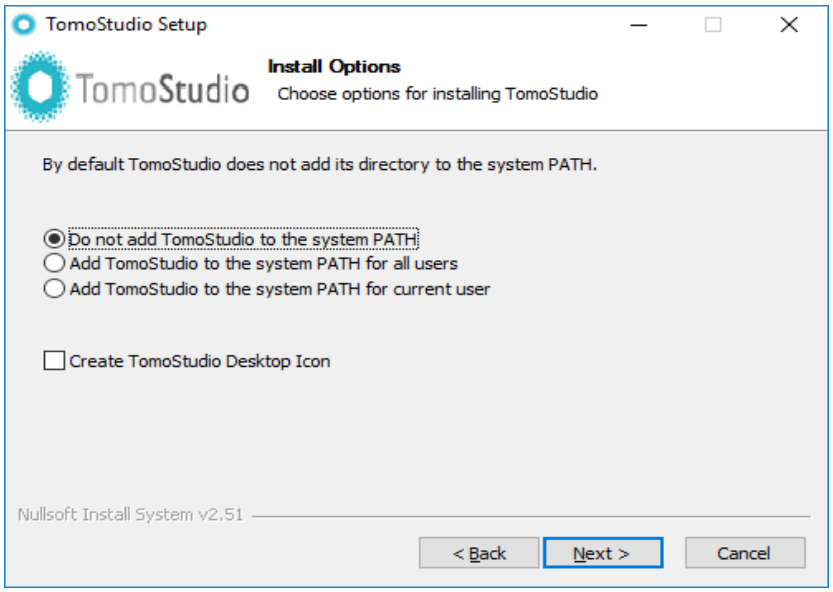


2. Click **Next** to continue.

3. To install TomoStudio on the user's system, it is required to accept the terms and conditions of TomoStudio License Agreement. Please read the agreement carefully, and then click **I Agree** to continue. If you do not want to accept the agreement, please click **Cancel** to exit the installation process.

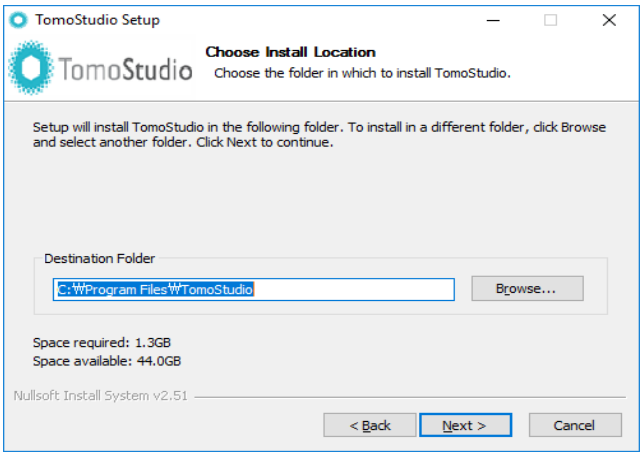


4. Choose options for installing TomoStudio, and then click **Next** to continue.

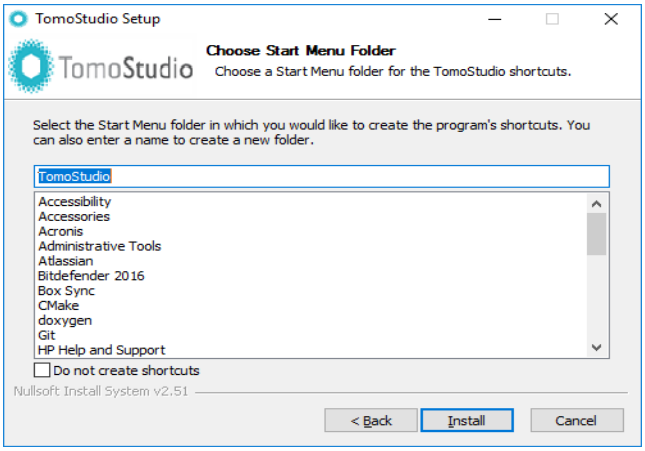


5. TomoStudio's default destination folder will be "C:\Program Files\TomoStudio\".

6. To change the install path, please click **Browse** and select a different folder.

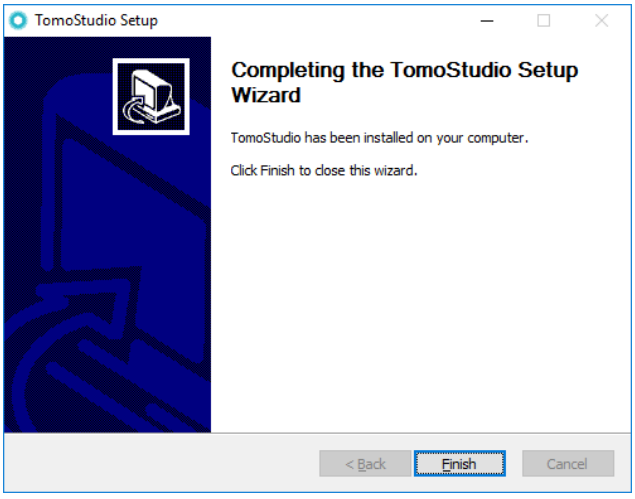


7. The default folder name in the start menu is ‘TomoStudio’ and you can rename it.



8. Click **Install** to start installing TomoStudio.

9. Finally, a message will appear to inform that the installation is complete. Click on **Finish** to exit.



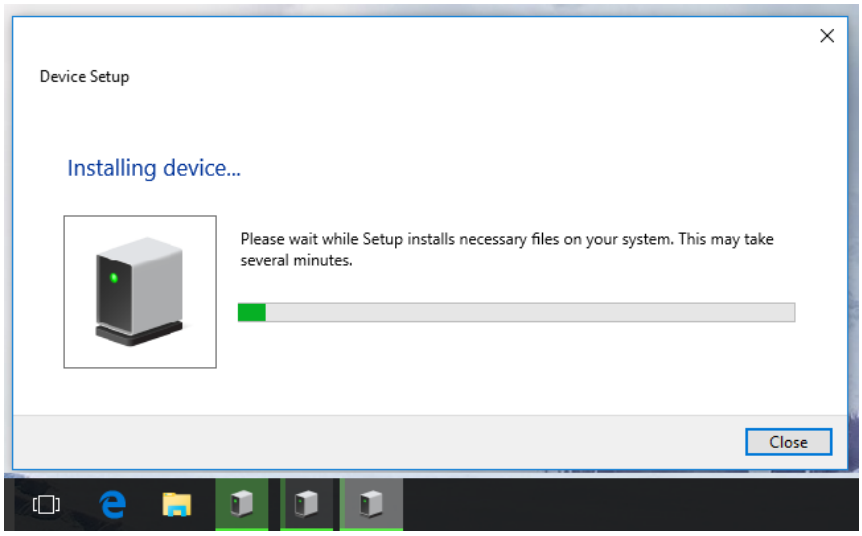
1.3 Connecting to Microscope

1. Connect the USB cable of the microscope to the computer.

> ***To safely install the device driver, the microscope power MUST be off before it is connected to the computer.***

2. Turn on the microscope

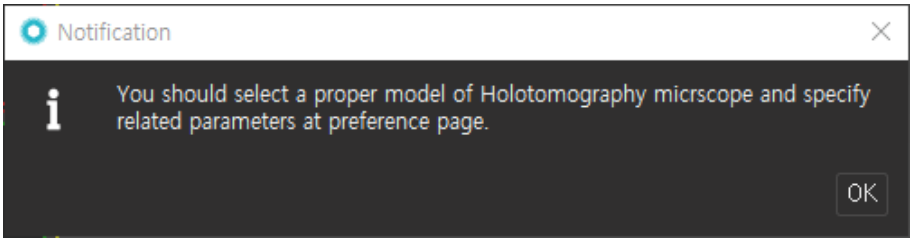
3. Wait for several minutes until it finishes installing device drivers on the system.



1.4 Microscope Configuration

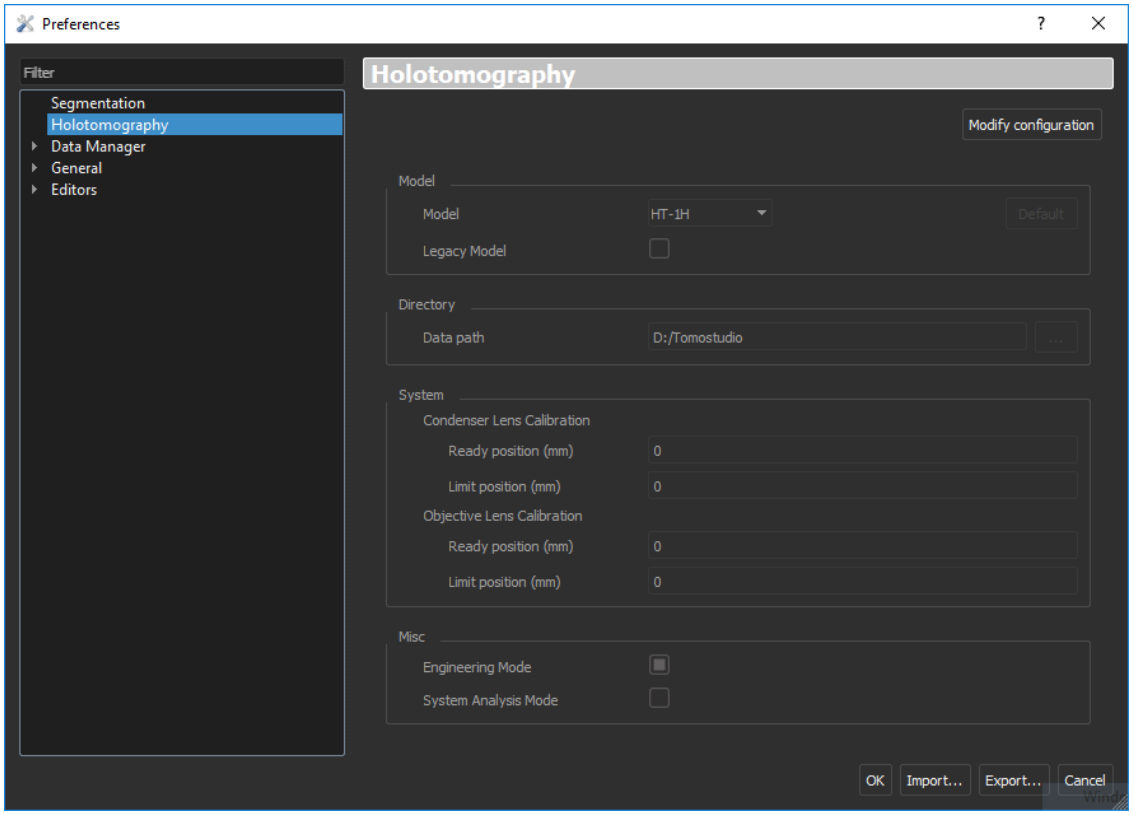
1. Start TomoStudio by clicking on its icon in the program menu

2. If this is the first time TomoStudio is being operated on this system, a notification window will appear.

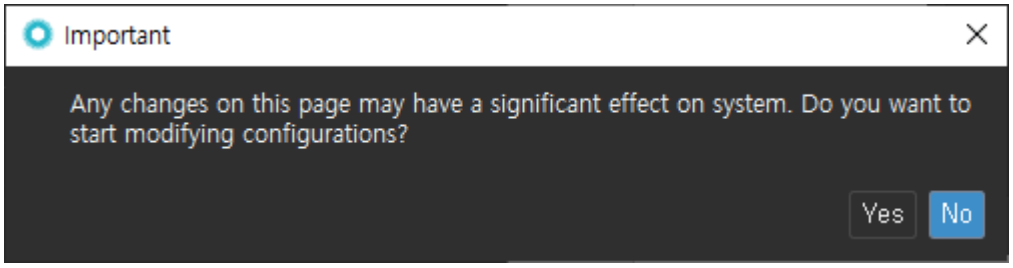


3. Once you click the **OK** button, Holotomography configuration window will be shown. You can re-open this window whenever you want by clicking **Window > Preferences** on the menu.

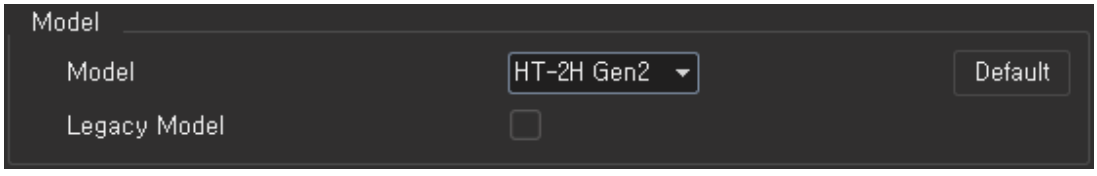
4. Select **Holotomography** on the left panel then the configuration page will appear.



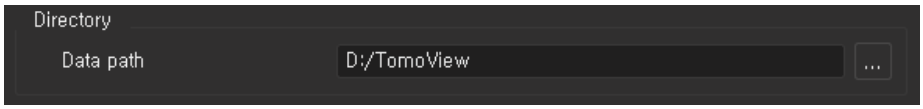
5. Press **Modify configuration** button and an information message will appear as shown below. Click **Yes** to continue.



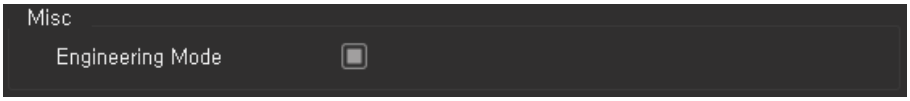
6. Select the proper model and click **Default** button; then the software will be filled up with the recommended values for the selected model.



7. Select a path to store data and configurations. We recommend choosing the drive which has the largest storage space in the system.



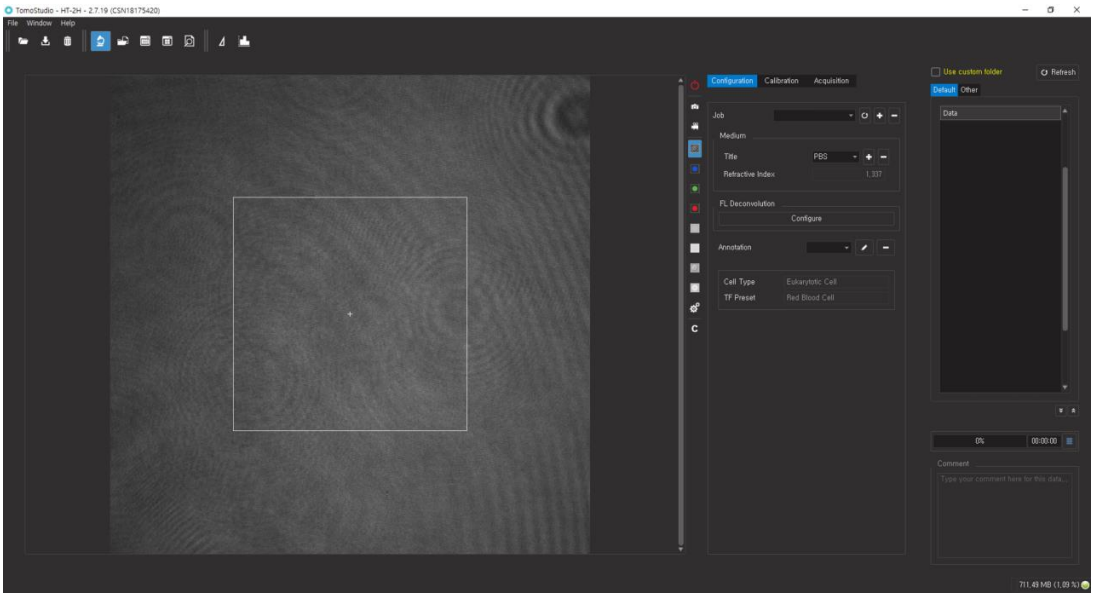
8. The engineering mode is only for maintenance purposes therefore, this step can be skipped for ordinary users.



9. Click **OK** to continue.

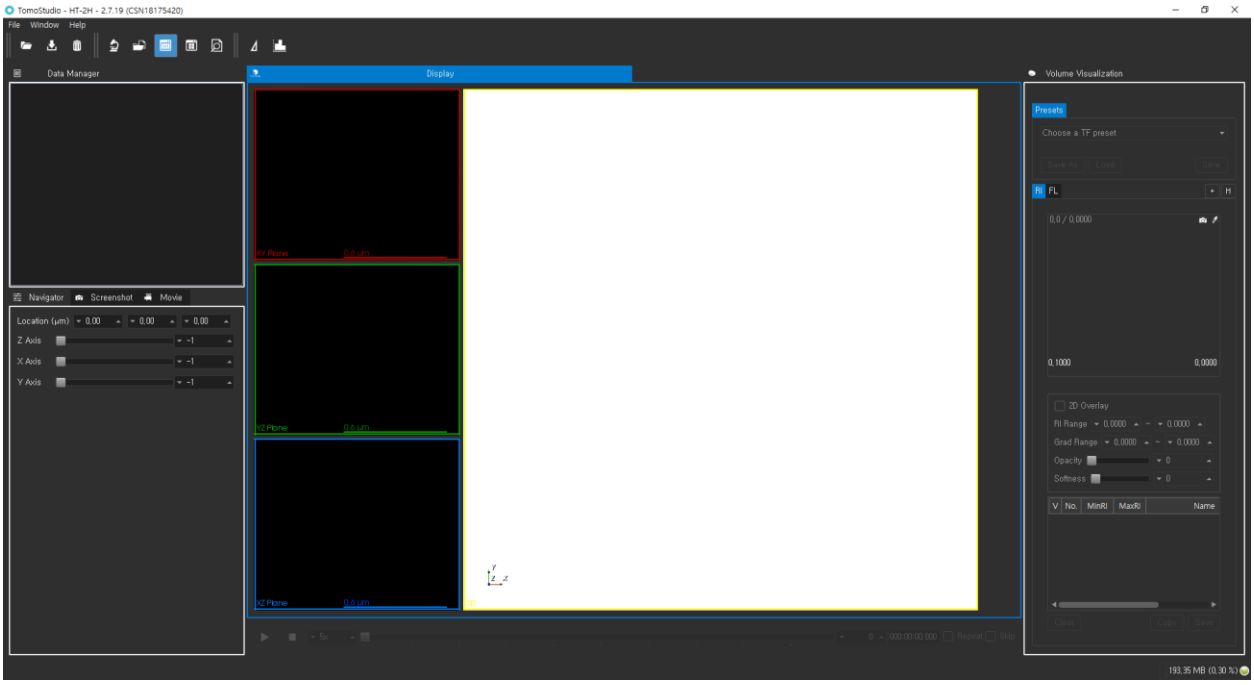
10. Finish and close TomoStudio to apply changes, and then restart TomoStudio.

11. Click **Microscope** to check the microscope connection status and its configuration. If the user can see the image below without any error messages, it means the program has been installed properly.



1.5 User Interface Overview

The following figure gives an overview of the user interface.



TomoStudio provides five different points of view to create and provide separate environment for each function as shown below.

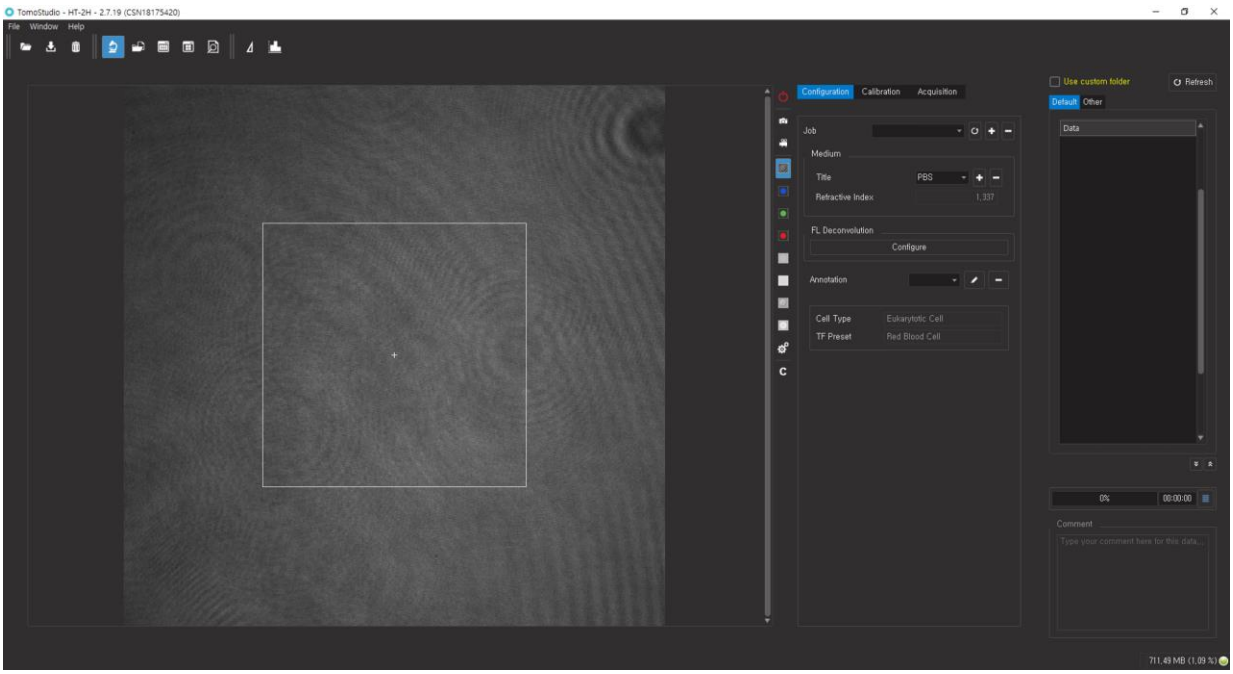
From the left to the right in the figure above,

Icon	Name	Key features
	Microscope perspective	Instrument calibration Image acquisition
	Data navigation perspective	Data information Image preview
	Research perspective	2D/3D/4D image visualization.
	Viewer perspective	Display panels that show the image in full screen.
	Analysis perspective	3D segmentation and quantification

Microscope

2.1 Overview

When Microscope perspective is selected, initialization process automatically begins. The software makes a connection to the microscope controller and initializes all its components. This process may take time depends on the PC specification and the circumstances. If no error occurs during initialization, the user will see the perspective like below.



Microscope perspective consists of multiple panels for capturing and processing images. There are three panels: display, control, and data management.

1. Display panel – Real-time raw hologram and processed images (e.g. phase shift and brightfield) are shown on this panel.

2. Control panel – User can control the microscope and capture images by using control buttons on this panel.

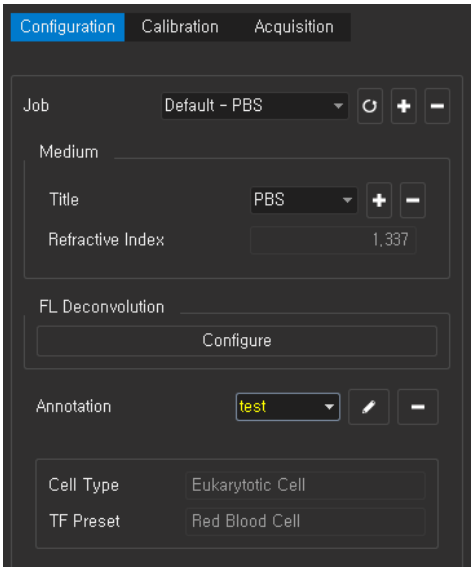
3. Data management panel – All captured images are listed on this panel.

Live holographic images or processed images such as a phase map are shown on the display panel in real time. Images can be easily acquired by following the flows in the control panel. Acquired images are listed on the data management panel.

⚠ *Sample needs to be prepared to follow the next steps.*

2.2 Configuration Step

In the configuration step, parameters for the image acquisition can be adjusted. The parameter set can be saved into a job file for later use, so the user can use that stored job file for another image acquisition tasks with the same conditions.



2.2.1 Job Selection

If the user would like to use a previous job, just select the required job from the drop-down list. After loading the job file, all the modified parameters will be updated to the task automatically.

2.2.2 Add/Delete Job

By clicking on the **plus** button beside the job drop-down list or **Save As** button, a new job can be added to the list. The existing job can be deleted by clicking on **minus** button beside the **plus** button.

2.2.3 Parameter Adjustment

Medium

The RI value of the medium (e.g. PBS – phosphate-buffered saline) in which a sample is immersed is required. The user can select an existing medium or add new medium here.

⚠ *The RI value of a medium is critical for building tomogram correctly. If the user has no information about the medium for measurements, please contact its manufacturer or Tomocube.*

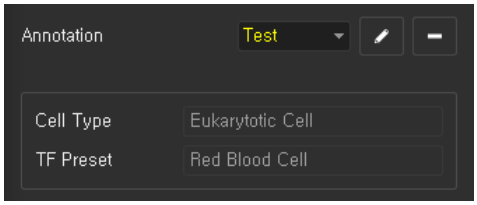
FL Deconvolution

Deconvolution removes the noises coming from incorrect focus planes. Iteration number refers to the number of times the deconvolution cycle goes on. Large number makes data sharper and small number makes data blurrier. The range is between 1 to 100 with the default number of 40.

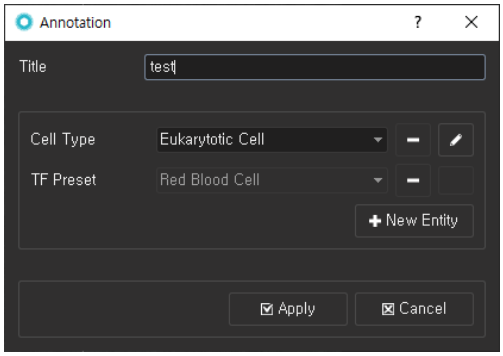
2.2.4 Annotation

Before capturing images, the user may want to put annotations for the data. All annotation entities will be saved in a TCF file with images.

Annotation

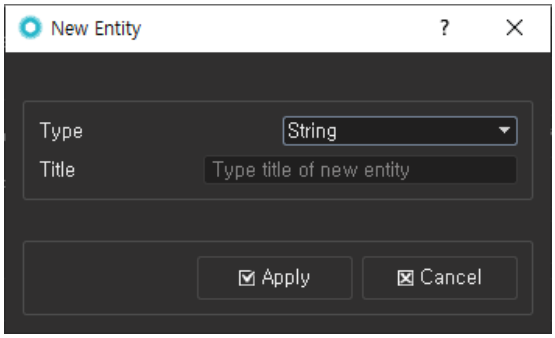


Annotation entities can be modified by clicking on the **pencil** button above. Once the user clicks the button, the annotation window pops up.



In this window, the user can add/delete annotation entities and enter new values on it. If the user specifies **TF Preset** entity, which is a preset transfer function, it will be included in the image file so it can be applied to the volume visualization automatically.

By clicking on **New Entity** button, the New Entity window pops up. Here the user can define a new annotation entity. Type and title of the new entity need to be specified.



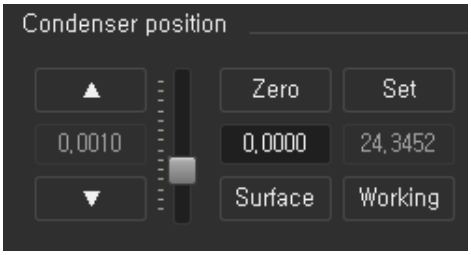
✔ *Later TomoStudio will provide data management capability. Once the feature is available, the annotation data the user entered will be more useful.*

2.3 Calibration Step

The position of the condenser lens needs to be calibrated and for this process, background images need to be acquired. Here is a recommended workflow:

1. Before beginning calibration, the sample should be prepared and placed on the sample stage of the microscope properly.

2. Move down the condenser lens to the sample by clicking on the **Surface** button. Then the user will be able to see the blurred shape of the cells on the screen if the samples are in the field of view.

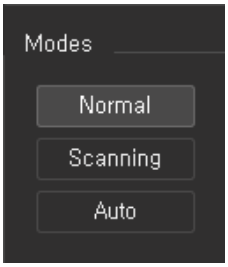


   By clicking on **Up** and **Down** buttons, the location of the condenser lens can be precisely adjusted.

3. Use **arrow** buttons on the screen or 3D controller to place the sample into the field of view. Using the X and Y knobs on the microscope also provides additional way to move the sample.

4. Use **arrow** buttons or 3D controller to find the best focus position. Using the Z knob is also possible.

5. Move the condenser lens to its best vertical position by clicking on the **Auto** button.



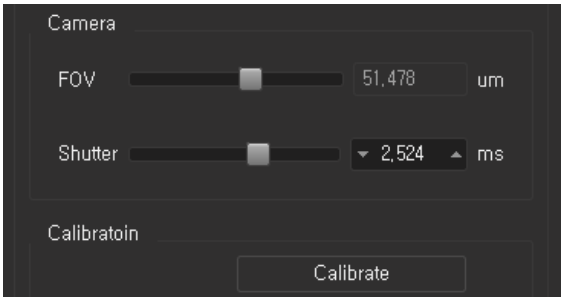
   When the user clicks on the **Scanning** button, the square-shaped illumination beam should appear on the screen. The illumination beam should remain at a fixed position on the sample plane if the focus is correctly set up. If the beam laterally translates in a circular trajectory, or if it flickers, it means that the vertical location of the condenser is not fully

optimized. In this case, the user can manually find the best position by moving the condenser lens using **Up** and **Down** buttons.

6. Using arrows on the screen or 3D controller or X&Y knobs, move to a blank spot with no cells in the field of view.

> ✔ *TomoMotion Server provides more ways to control the lenses and stage as well as the camera shutter. Refer to TomoMotion Server in Appendix.*

7. Adjust FOV and Shutter. Please refer to Field of View and Camera Shutter sections below.



8. Once a blank spot with nothing in FOV is found, click on the **Calibrate** button to capture background images.

Field of View

Field of view represents the size of an area which will be captured. When the user moves the slider of **FOV** bar, the size of the white color box on the screen is changed. The inside area of the box will be captured and saved into an image file later.

> ⓘ *To know the proper size of an area for a specific sample, finding a sample adjusting a focus roughly before changing the field of view can be necessary.*

Camera Shutter

The brightness of the image is determined by laser power, sample status, and camera shutter speed. In this microscope, the user can adjust the brightness by changing the camera shutter speed.

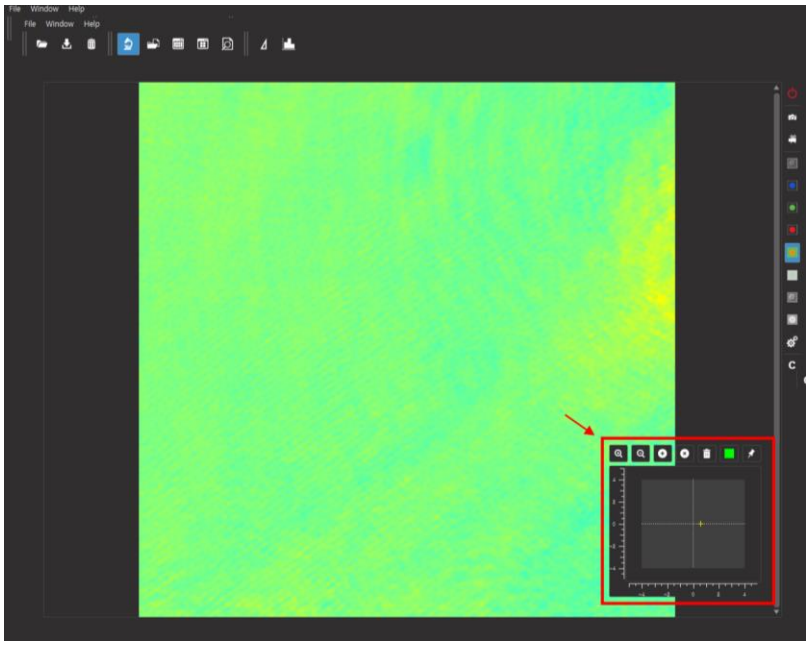
> ⓘ *Right after the microscope is turned on, the laser power might be unstable for several minutes, so waiting for about 30 minutes before capturing the images is highly recommended.*

2.4 Acquisition Step

In the acquisition step, the user can monitor samples using various imaging modes and then acquire 3D images. In order to find a desired sample among others, the user may need to utilize various view modes. The user can also enter the annotation information for sample images in this step.

2.4.1 Mini-map

The **mini-map** allows quick navigation of the sample and it records multiple objective positions for re-visiting. Mini-map can be displayed by clicking the Acquisition tab and hovering mouse curser over the bottom-right corner of the display panel. The sample area can be navigated by double-clicking a spot on the mini-map to move the objective to that position.



Detailed description of the mini-map functions is listed below:

Icon	Name	Description
	Zoom in	Zooms in mini-map
	Zoom out	Zooms out mini-map
	Add point	Records current position of the objective
	Delete point	Deletes selected position (red point)
	Delete all points	Removes all recorded positions on mini-map
	Pin mini-map	Fixes the mini-map on the display panel.

2.4.2 View mode

TomoStudio can show three different real-time images: hologram, phase and brightfield for HT-1 — and it additionally shows 3-channel fluorescence images for HT-2. The view mode options are displayed as icons to the right of the display panel. Clicking one of the options will change the display to the selected camera mode.

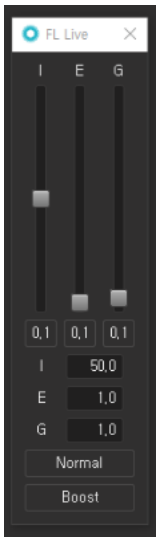
Icon	Title	Description
	Laser On/Off	Turns the laser on/off
	Capture	Saves a screenshot of current display panel into a .png file
	Record	Saves multiple screenshots of current display panel into .png files
	Bright-field	Shows real-time Bright-field images
	Phase	Shows real-time Phase images
	Hologram	Shows real-time Hologram images
	Fluorescence	Shows real-time fluorescence images; blue, green, and red can be selected
	Control	Displays the view mode control dialog
C	Calibration	Perform re-calibration at the current position

View mode control dialog

In the view mode control dialog, the image quality can be adjusted for Phase and Bright-field images using the **Tuning** slider. The options for Holography (FOV and Shutter) and Fluorescence (Intensity, Shutter, Gain, and Fluorophore) can also be adjusted.

Fluorescence live control

When you click a fluorescence channel icon, a new window called **Fluorescence live control** is appeared. All parameters related to fluorescence imaging can be adjusted in this panel: Intensity (I), Exposure time (E), Gain (G).



All changes on **Normal** mode in this panel are stored into the job configuration. If you select **Boost** mode, the camera gain is increased to the maximum, intensity to 1.0 and shutter to 1.0 millisecond. It is to minimize photodamage of samples while user is searching proper imaging area.

2.4.3 Title

The name of data folder containing all the raw files and the processed image file (.TCF, see page 29) is made with the following format: “Date.Time.Title-index (yyyyMMdd.HHmmss.SSS.Title-index)”. When a user does not change the default title before data acquisition, the file name will automatically be saved in the aforementioned format ex.) “20201029.171010.225.Default-001”.

⚠ *Note that special characters, which cannot be used in Microsoft windows folder name, are also not allowed in Title.*

2.4.4 Acquisition

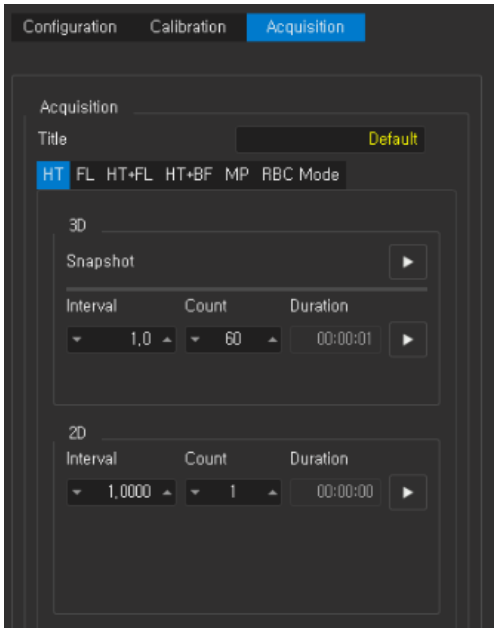
TomoStudio supports various acquisition modes. For HT-1, it supports three modes: HT and RBC. For HT-2, it supports five modes in total with two additional modes: FL and HT+FL. There are different acquisition configurations in each acquisition mode tab. Once each image set is captured, all files will be stored automatically and listed upon **Data Manager**.

Tab	Description
HT	Acquires Holotomographic images
FL	Acquires Fluorescence images
HT+FL	Acquires Holotomographic and Fluorescence images
HT+BF	Acquires Holotomographic images with Bright-field images
MP	Acquires images at multi points: including tile-scanning and matrix-scanning
RBC Mode	Acquires 2D Phase Time-lapse and a 3D Holotomographic image

HT

In the **HT** tab, there are three arrow-shaped capture buttons. The two buttons in the 3D section are **3D Single capture** and **3D Time-lapse capture** buttons. The first one is used to acquire one 3D image and the second is used to acquire multiple 3D images with the designated time interval and time duration.

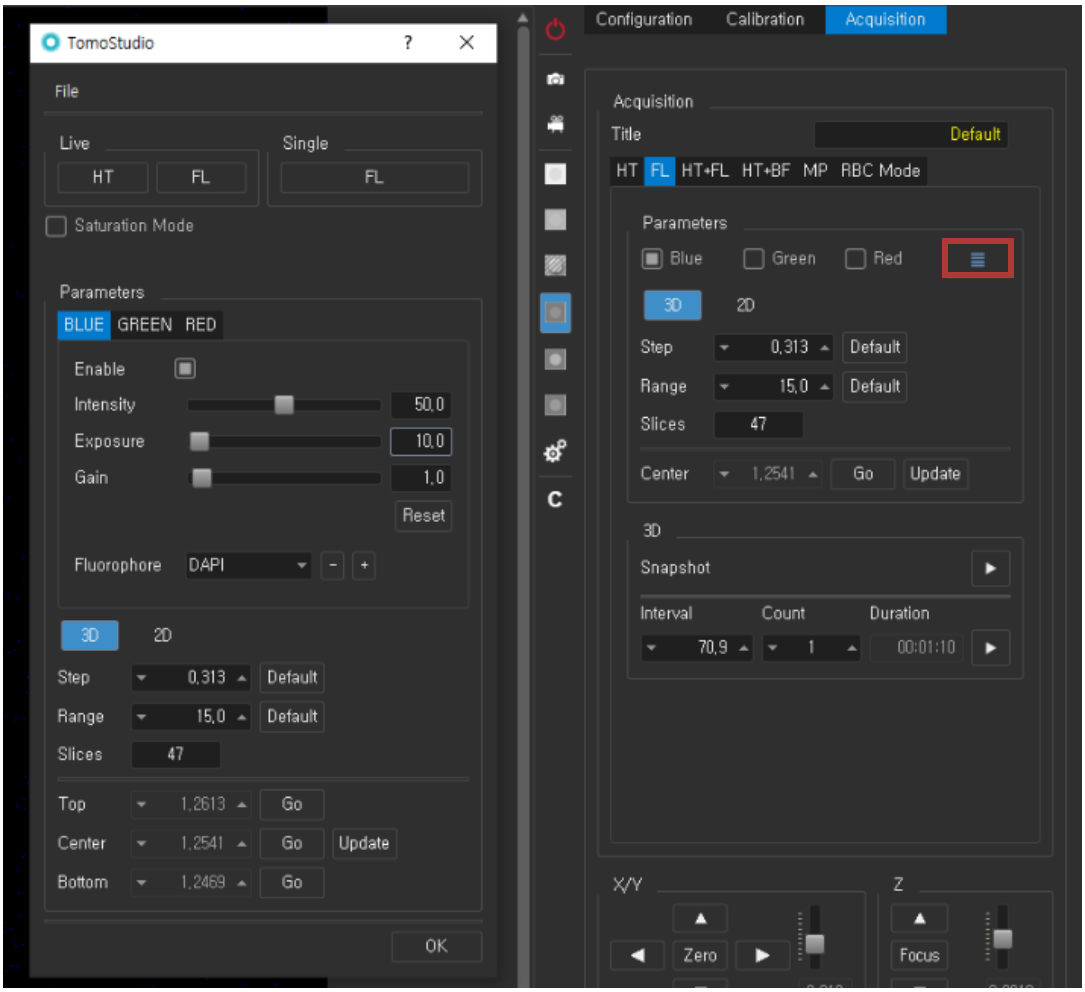
The button in the 2D section is for 2D time-lapse capture, which is used to acquire multiple 2D images with the designated time interval and time duration.



FL

In the **FL** tab, capturing one or multiple 3D fluorescence images is supported. In the case of fluorescence imaging, additional parameter adjustments are required: illumination, camera condition, z-axis scan range and step size.

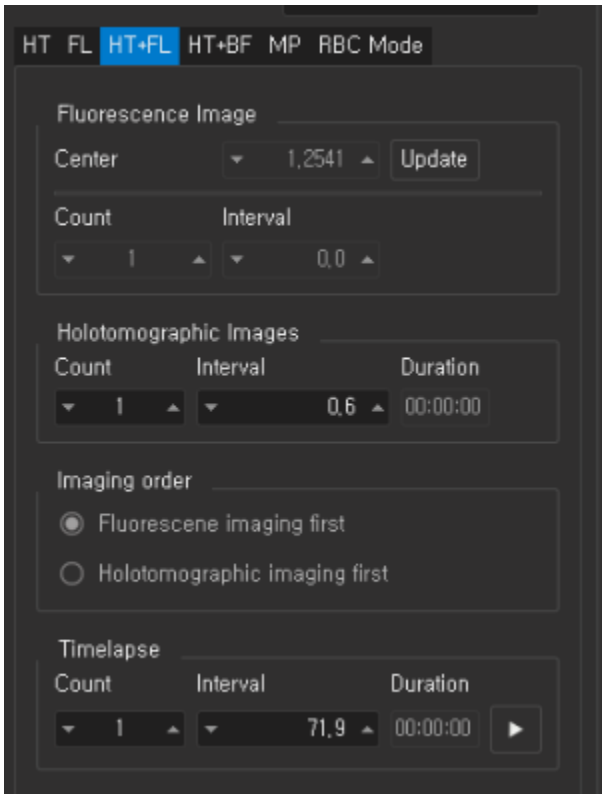
Channels and the z scan range and the step size can be configured on the FL tab directly. More parameter details can be adjusted on the advanced fluorescence configuration dialog, which is displayed by pressing the blue button in the upper right corner (“hamburger-like” icon).



On each channel tab, the imaging conditions such as illumination intensity, camera shutter speed, and camera gain value can be configured. Live image with current parameters can be monitored by clicking the **Live** button on the above. Scanning step and range are same for all channels. By clicking the **Go** button beside **Top**, **Center** and **Bottom** positions, the changed parameters will be applied.

HT+FL

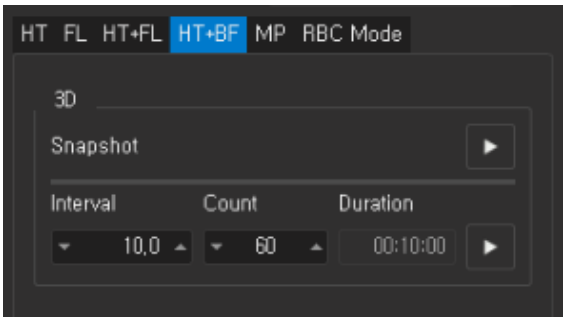
In **HT+FL** tab, a sequential acquisition of holotomogram and fluorescence images is supported. A fluorescence image and one or multiple HT images form one set of acquired images. It is possible to acquire multiple sets in the **Time-lapse** section.



Because same types of parameters as the FL tab are used, adjusting the parameters on the FL tab before acquiring images on this tab is required.

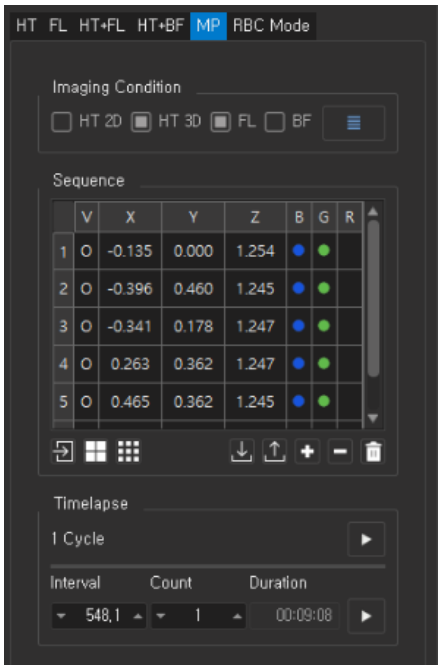
HT+BF

HT+BF tap is for the sequential acquisition of RI tomogram and the bright-field image. The minimum interval is 1 seconds.



MP

In the **MP** tab, multi-point imaging acquisition is supported. One or multiple imaging options can be selected in the **Imaging Condition** tab. In the **Sequence** tab, the information of set positions for MP imaging is listed.

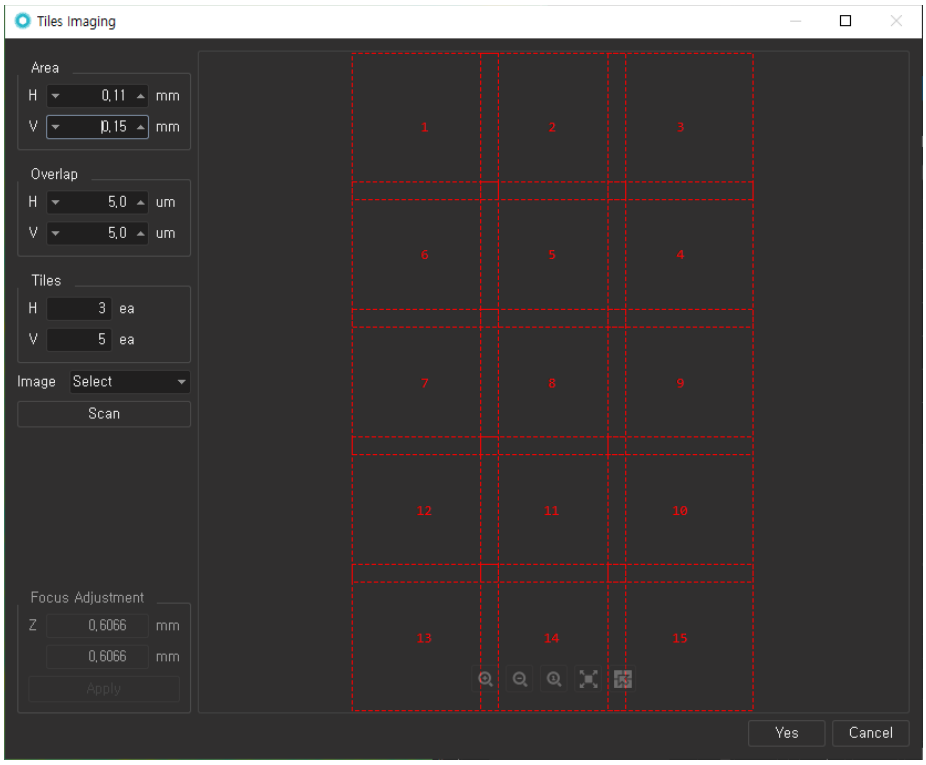


Icon	Title	Description
	Import positions	List of positions are imported from the mini-map
	Tile-scanning	Captures multiple images to produce an image with larger area
	Matrix scanning	Samples images in a set area
	Save positions	Saves the list of positions into a .csv file
	Load positions	Loads autosaved positions, or the list of positions from a .csv file
	Add position	Adds the information of current position of the objective
	Delete position	Removes a selected position
	Delete all positions	Removes the whole list

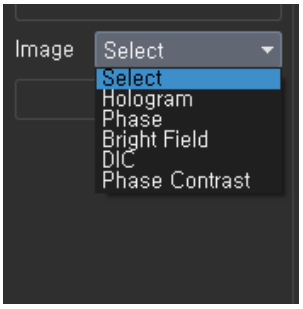
After setting the positions, capture the images by snapshot or by **time-lapse**. To acquire one snapshot of multiple points, click the capture button next to '**1 Cycle**'. To capture time-lapse images, set the interval as the number of seconds between each cycle and set the count as the number of cycles to be captured. '**Duration**' shows the estimated time required for completing the time-lapse MP imaging. Clicking the capture icon will begin the time-lapse imaging.

MP > Tile-scanning

Tile-scanning offers acquisition of images larger than the maximum field of view.



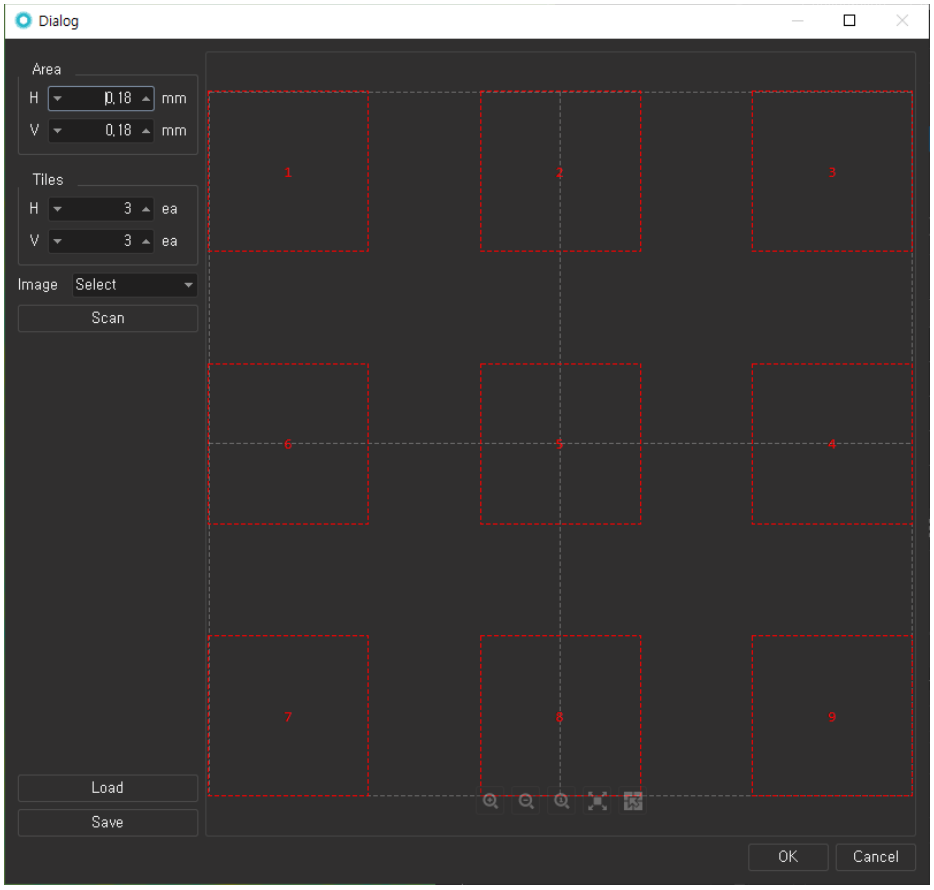
Clicking the tile-scanning icon in the MP tab opens the tile-scanning window. Sizes of total image area and overlap region can be adjusted on the left panel. The overlap determines the area overlapped between each tile image, and it cannot exceed 50% of the width of FOV. The number of tiles cannot be set manually and will come out automatically along the size of the tile-scanning area.



The imaging scheme will appear on the display panel automatically after adjusting the image area. Note that the tile in the center is the current FOV. The type of image (Hologram, Phase, Brightfield, DIC, Phase contrast) for scanning can be chosen by clicking '**Select**' next to **Image**. Then, clicking '**Scan**' will produce quick scan of the area and display it on the window. Clicking '**Yes**' will import the position list into the MP mode. Begin imaging by clicking 'Snapshot' or 'Timelapse'.

MP > Matrix-scanning

Matrix-scanning can be used to capture sample images of a certain area.



In matrix-scanning, the **Area** section determines the size of the area to be sampled. Both the area size and the number of tiles can be set vertically and horizontally, and the imaging matrices will be positioned automatically within the set area. Clicking **Scan** will produce quick scan of the area and display it on the window. Clicking ‘Yes’ will import the position list into the MP mode. Begin imaging by clicking ‘Snapshot’ or ‘Timelapse’.

RBC Mode

In the **RBC Mode** tab, there is only one **capture** button. It is used to acquire one 3D image and one set of phase maps. The phase map is captured for 2 seconds with a speed of 150 frames per second. It is used to measure and analyze dynamic membrane fluctuation.

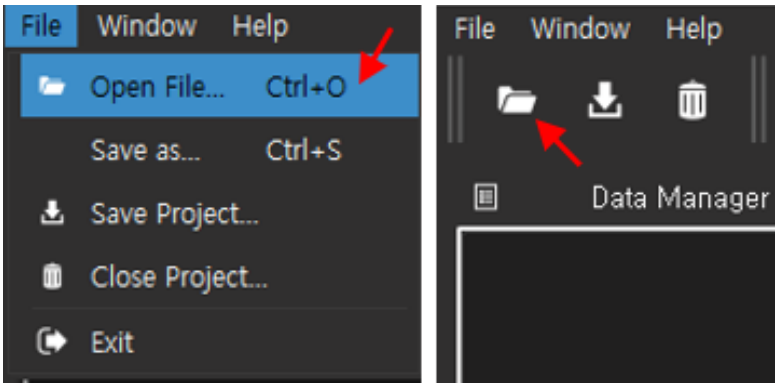
Visualization

3.1 Overview

TomoStudio utilizes a customized file format, TCF (Tomocube Common File) which is based on Hierarchical Data Format (HDF5). The file stores not only images but also annotation datasets which include multiple types of images such as RI tomogram, MIP (Most Intensity Projection) image, phase map, and fluorescence (FL) images.

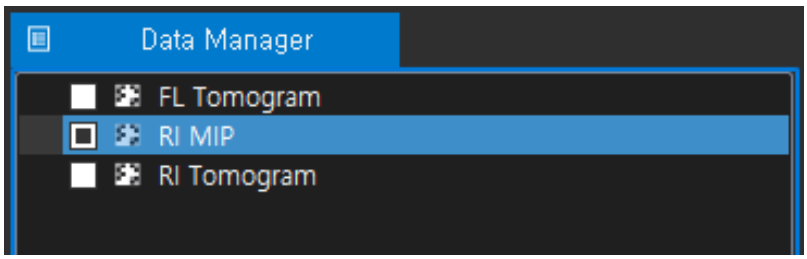
The **RI tomogram** represents the 3D refractive index (RI) distribution of a sample and the MIP image shows the highest RI value for each lateral position. The 2D/3D/4D FL images for each light channel are also available in the **FL tomogram**. For now, up to 3 channels (red, green, and blue) can be utilized for fluorescence imaging.

The user can open a TCF file by clicking **File > Open file** on the menu or by clicking the data files icon on the toolbar.

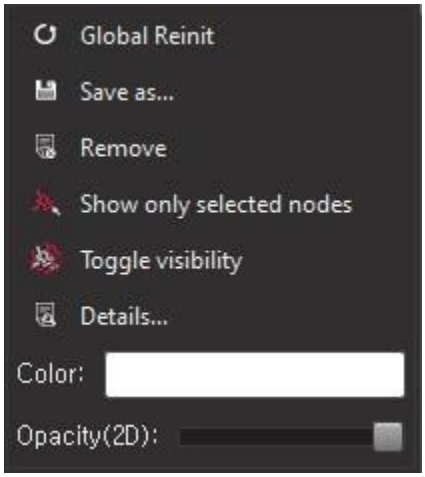


3.2 Data Manager

Once a TCF file is opened, image nodes of the file are displayed on the data manager panel. The image shown on the display panel is updated when the user checks a different image node on the data manager panel.



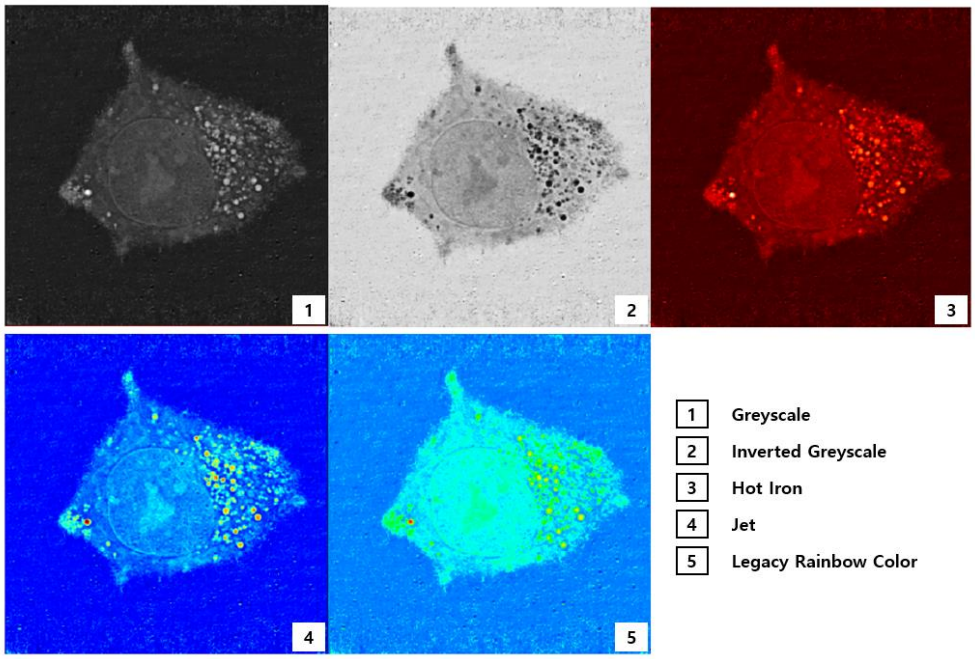
Clicking the right mouse button on an image node opens a pop-up menu (context menu) as shown below.



Menu	Function
Global Reinit	Re-initialize display status to default.
Save as…	Export image into another format. Several common file formats such as NRRD (Nearly Raw Raster Data), NIfTI (Neuroimaging Informatics Technology Initiative), VTK Image, VTK Legacy Image, Mha (Meta Image) file, and multipage TIFF image are supported.
Remove	Remove the selected image from the data manager panel.
Show only selected nodes	Show only the selected nodes.
Toggle visibility	Toggle on/off the visibility of the selected node
Details	Show detailed information for the selected node
Color	Change color of the selected node
Opacity	Change opacity of the selected node

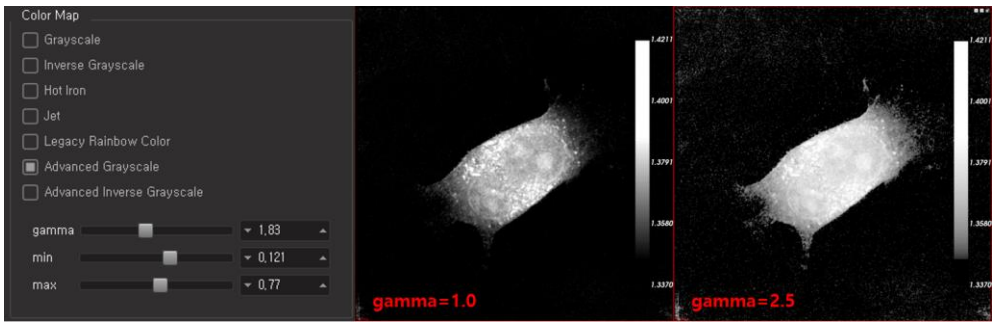
3.3 Color Map

Colorize 2D image by using pre-defined colormaps such as Grayscale (default), Inverse grayscale, Hot iron, Jet, and Legacy rainbow color. Below images are examples of each color map.



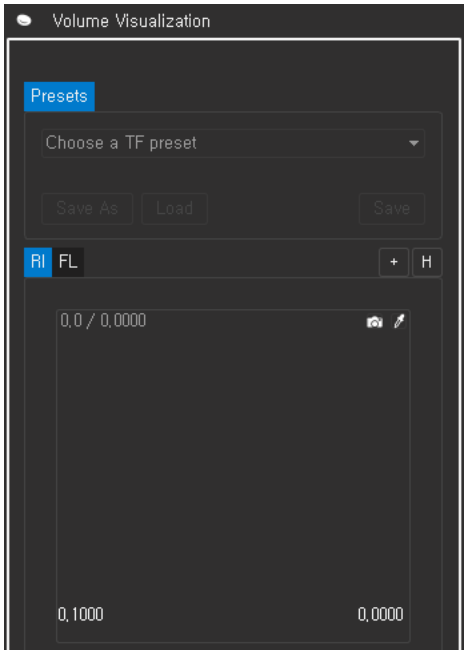
In 2D planes (XY, YZ, XZ), the scalar bar item is added that makes user can come up with relationship between an intensity of original HT image and a visualized color of the 2D render window.

Non-linear grayscale colormap for HT image is added according to the gamma correction theory in image/video processing research. Properties to modify the non-linearity of grayscale map can be managed by using the parameter controller in both **Advanced Grayscale** and **Advanced Inverse Grayscale** options.



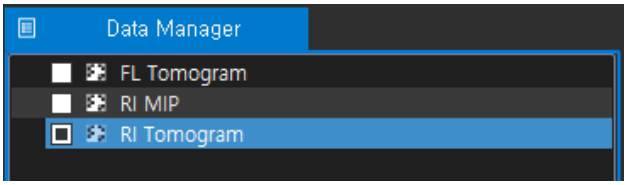
3.4 Refractive Index Volume Visualization

Volume visualization is a basic tool for visualizing three-dimensional images. There are two different types of three-dimensional images, RI tomogram and FL tomogram, so TomoStudio provides two different volume visualization functions for these two types of images. In this section, the volume visualization of RI tomogram will be described.



3.4.1 Enable Volume Rendering

1. Select a tomogram (3D/4D image) on Data Manager panel.



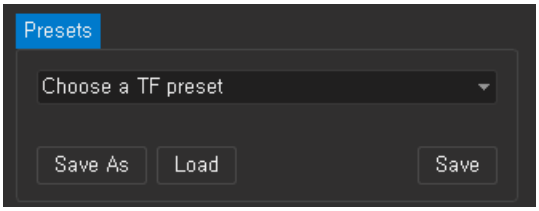
2. Then the panel will be activated.

3. Depending on system specifications and data size, it can take up to 30 seconds to prepare a rendered image.

3.4.2 Preset Selection

TomoStudio provides transfer function presets including Red Blood Cell, Eukaryotic Cell, and White Blood cell. A user can also create new transfer functions and save them for later use. The presets we offer can be a good starting point for customizing a new transfer function.

1. Click on the **Preset** tab.



2. Click on the drop-down list titled "**Choose a TF preset**," and then select one in the list.

Alternatively, the user can also load a transfer function preset from another file.

1. Click on the **Preset** tab.

2. Click on the **Load** button, and then a file dialog will pop up

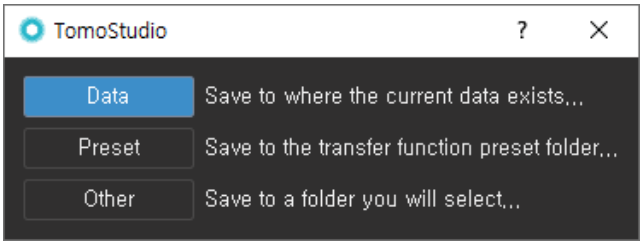
3. Select a preset file (*.xml) and then click **Open** button to use it.

3.4.3 Save Transfer Function

Once a transfer function is customized, the user can save it for later use.

1. Click on the **Save** button if the user would like to overwrite a preexisting preset.

2. Click on the **Save As** button if the user prefers to save the customized preset in another location, which can be selected from the pop-up dialog box.

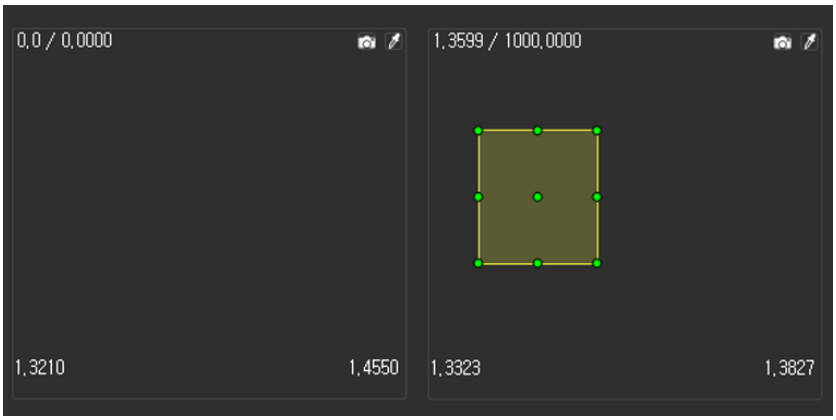


3.4.4 Customize Transfer Function

On the RI canvas, the user can interactively create a customized transfer function.

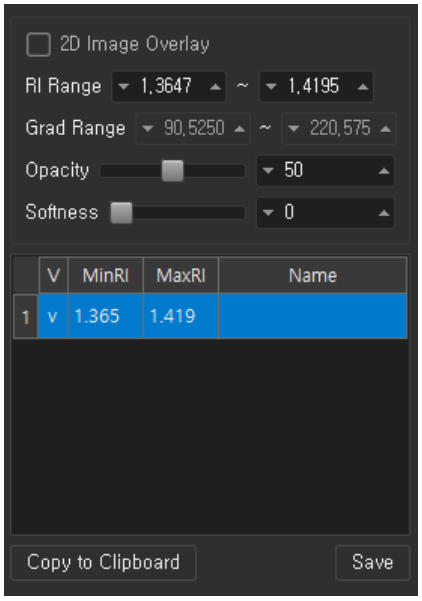
Transfer Function Canvas

In the below image, the left side shows an empty RI canvas, and the right side shows a user-generated transfer function box with a specific RI and a gradient range.



The X-axis of the canvas means RI value, and the Y-axis is the normalized gradient of RI which ranges from 0 to 1000. The gradient of RI represents how much the RI value at each position differs from its neighbors.

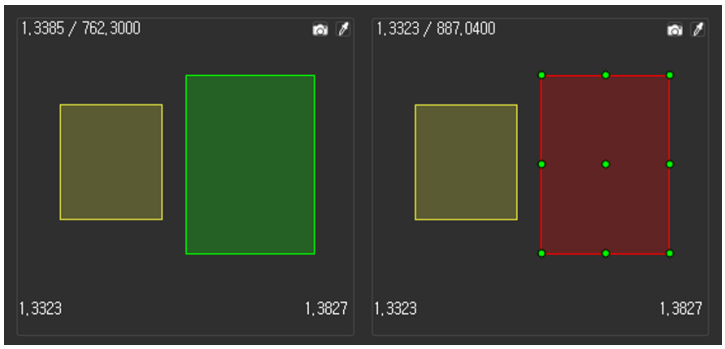
Each transfer function has five properties - RI range, gradient range, color, opacity, and softness - as seen below:



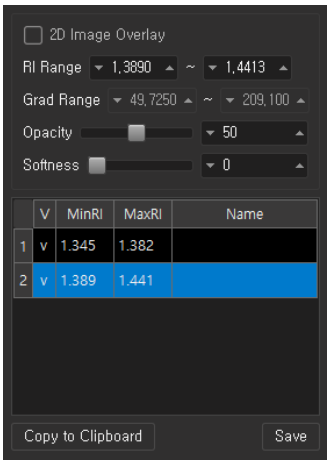
All voxels specified by RI and gradient ranges are visualized with different color, opacity, and softness.

Create a Transfer Function Box

To create a transfer function, draw a box while the left mouse button is pressed and then release it. The color of the new transfer function is assigned automatically, but the user can change it by double-clicking the box with the left mouse button.



The RI and gradient ranges of each transfer function are displayed on the table as below.

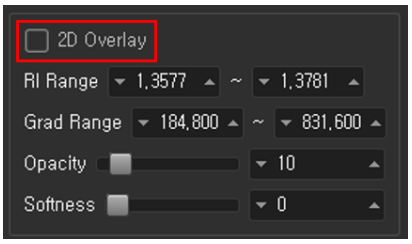


Modify Transfer Function

Once a transfer function box is selected by left clicking it, multiple control points will be shown. The size of the box can be changed by dragging each control point with the left mouse button. The box can be moved by dragging the control point at the center. Also, right-clicking the box will remove the it from the display panel.

Image Overlay

To overlay transfer function colors on the 2D slice image, click the **2D Image Overlay** checkbox.



3.5 Fluorescence Volume Visualization

If fluorescence images were also taken, the fluorescence volume visualization tab, **FL**, would be activated. Range, gamma, and visibility of each channel can be adjusted. Opacity control is also available.

3.5.1 Range Control

The intensity range can be adjusted by moving the handles of the slider. Voxels with intensity lower than the minimum value are not visualized. Voxels with intensity higher than the maximum value are visualized as same as the maximum intensity no matter what actual values they have.

3.5.2 Gamma Control

The intensity values of voxels are linearly expanded from the lowest to the highest as default if those values are in the range. The signal of interest can be amplified by using the gamma control slider.

3.5.3 Visibility Control

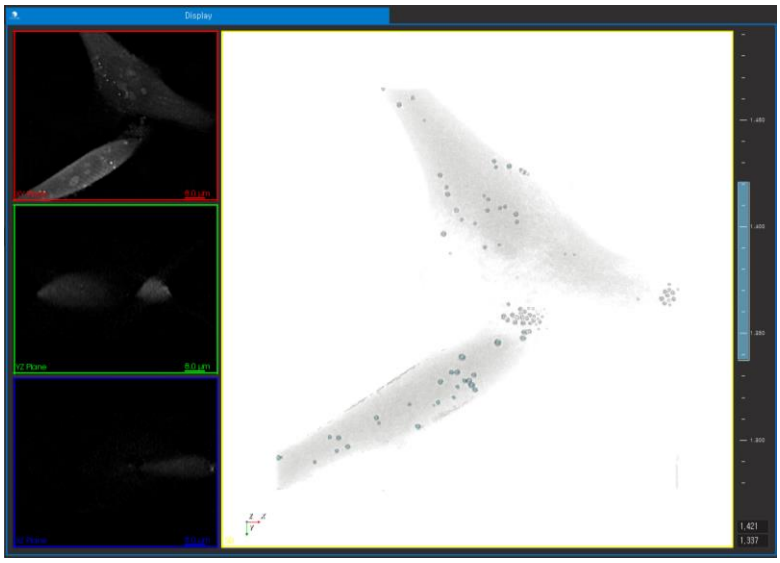
Each channel's visibility can be turned on/off by checking/unchecking the checkbox **Visible**. The visibility of all channels can be simultaneously controlled with two buttons; **Show All** and **Hide All**.

3.5.4 Opacity Control

The opacity of each channel can be controlled by moving the handles of the slider.

3.6 Display

The display panel consists of three 2D and one 3D windows. Each 2D window displays an anatomical slice of the tomogram. In the default layout, the red, green, and blue boxes show the XY, YZ, and XZ windows, respectively. The yellow box shows the 3D window.



3.6.1 3D Window Control

Unlike 2D windows, the user can navigate the 3D image by using mouse and keyboard.

Functionality	Description
Rotate	Move the mouse pointer while the left mouse button is pressed
Zoom	Move the mouse pointer up/down while the right mouse button is pressed or Use a scroll wheel
Pan	Move a mouse pointer while the left mouse button or Shift key is pressed

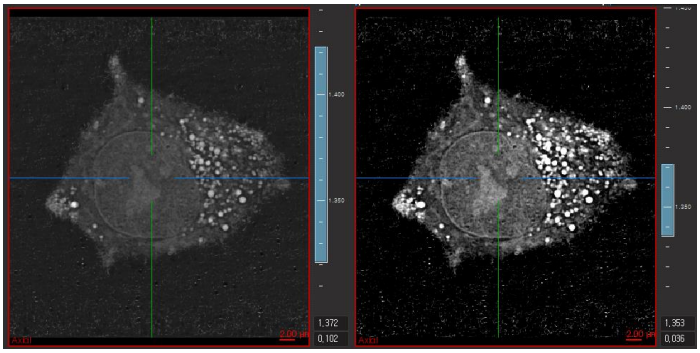
3.6.2 Hover Menu

There is a hover menu at the top-right corner of each window. It consists of three menu icons. The first two icons are the same for the 2D and 3D windows, but the third icon of the 3D window has more functionalities than that of 2D windows.

Icon	Submenu	Description
	Reset view	Reset view status
	Show crosshair	Show/hide a crosshair
	Crosshair rotation	4 options for the crosshair rotation are offered – No crosshair rotation (default), Crosshair rotation, Coupled crosshair rotation, and Swivel mode
	-	Enlarge the selected window or restore to the previous window layout
	Layout	One of 8 window layouts can be selected here
	Background (3D only)	The background of a 3D view can be selected from three options - gradient, black, and white (default).
	View	Show/hide the orientation marker, axis grid, and color bar on a 3D view. Adjust length and location of the scale bar on a 2D view. Show/hide the timestamp and title (both 2D and 3D)

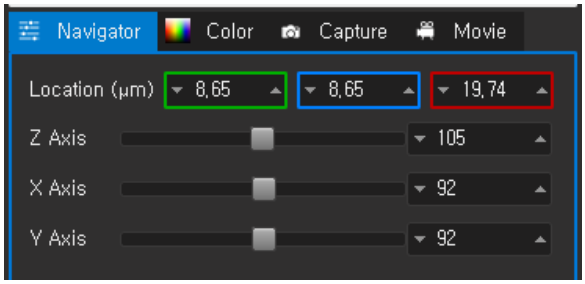
3.6.3 Level Window

The level window for adjusting the RI range is located on the right side of the display panel. It is a tool for adjusting the contrast of the 2D slices.



3.7 Image Navigator

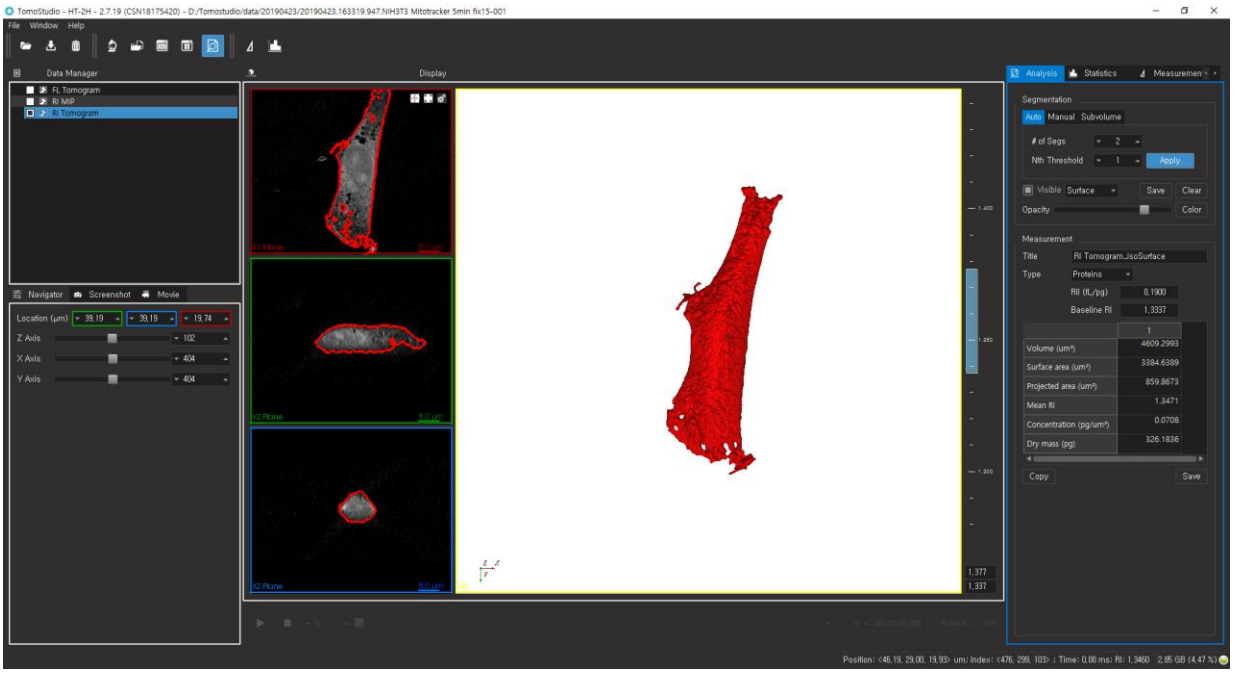
Fast navigation through the available tomogram can be achieved by using **Image Navigator**. Users can move to another 2D slices by dragging sliders or entering slice numbers. Numbers in the color boxes are in micrometers and the numbers in boxes next to sliders represent the frame numbers.



Analysis

4.1 Overview

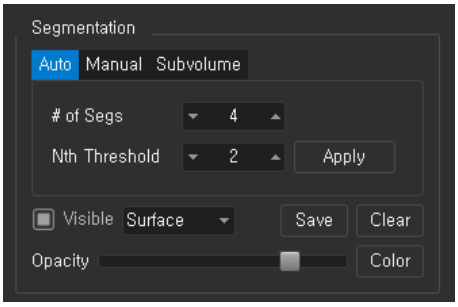
The **Analysis** perspective is very similar to the research perspective except the panel on the right side; there is an analysis panel instead of the volume visualization panel. This perspective provides quantitative information of the imaged cell such as volume, dry mass, etc.



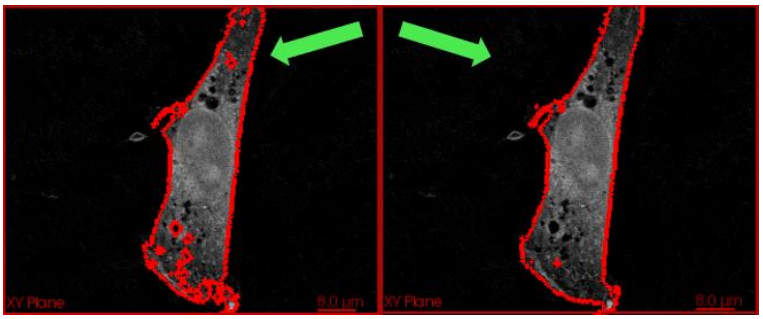
4.2 Segmentation

TomoStudio is able to create an isosurface of the image. The quantitative information like volume, surface area, and the dry mass is calculated based on the isosurface.

Once data is selected from data manager panel, **Apply** button is activated in **Auto** tab. To create an isosurface of the selected data, press the **Apply** button.

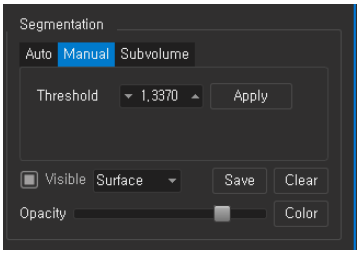


Basically, TomoStudio uses Otsu threshold method for deciding a threshold which is used to divide RI values in two parts. However, it is not proper for some cases. Now the user can decide how many parts to be divided into and which threshold value will be used.



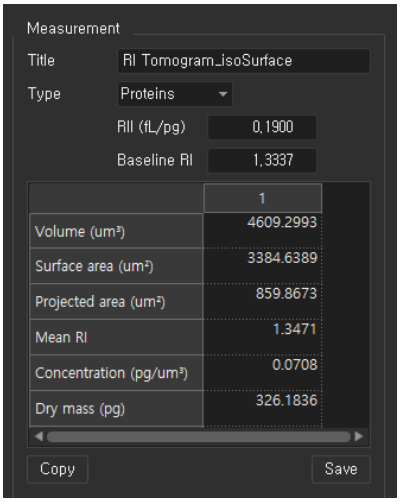
In the image above, left is segmented with default parameters (# of Segs = 2, Nth Threshold = 1); right is segmented with user-defined parameters (# of Segs = 4, Nth Threshold = 2).

Open **Manual** tab, enter threshold value, and click **Apply** button to change RI threshold manually.

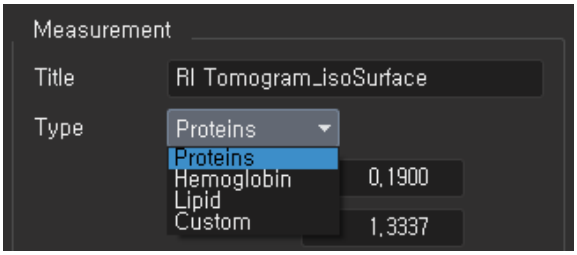


4.3 Quantification

After segmentation is finished, the quantitative information of the isosurface will be displayed.



TomoStudio provides various parameters such as volume, surface area, projected area, protein concentration, dry mass, and sphericity. When dry mass is measured, the type of materials should be specified. There are three pre-defined RII (refractive index increment, fL/pg) values; Proteins (0.1900), Hemoglobin (0.1500), and Lipids (0.1350). These values were measured with water, so the baseline RI is that of water, not that of a medium.



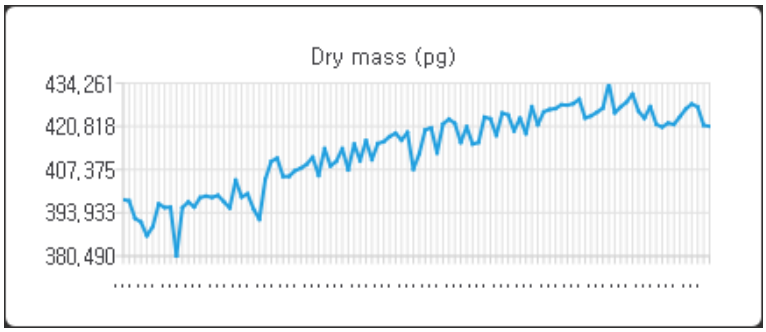
Barer's relation

$$n = n_m + \alpha C$$

states that the refractive index of biological samples, n, is linearly proportional to the protein concentration, C, where n_m is the refractive index value of surrounding medium and a (or dn/dc) is the refractive index increment of protein.

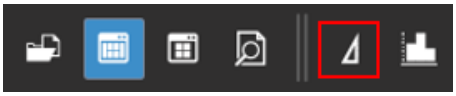
There are two methods for exporting quantitative data. The first method is to **copy** the table into the clipboard. The copied data can be pasted into any other text editors or spreadsheet software such as Microsoft Excel. The second method is to **save** the data into a CSV file which can be opened in Microsoft Excel directly.

In the case of time-lapse data, how those parameters change with time will be displayed as the graph shown below.

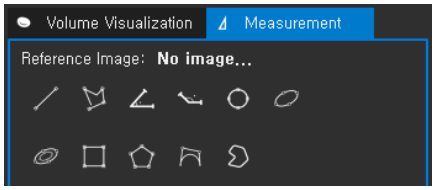


4.4 Measurements

Click on Measurements icon to activate the measurements tab.



Tools in Measurements view help the user measure geometric figures on a 2D image or a single slice of a 3D image.

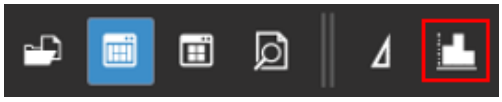


After drawing figures with the selected tool in the 2D window, measured data is displayed on the **Measurement** view.

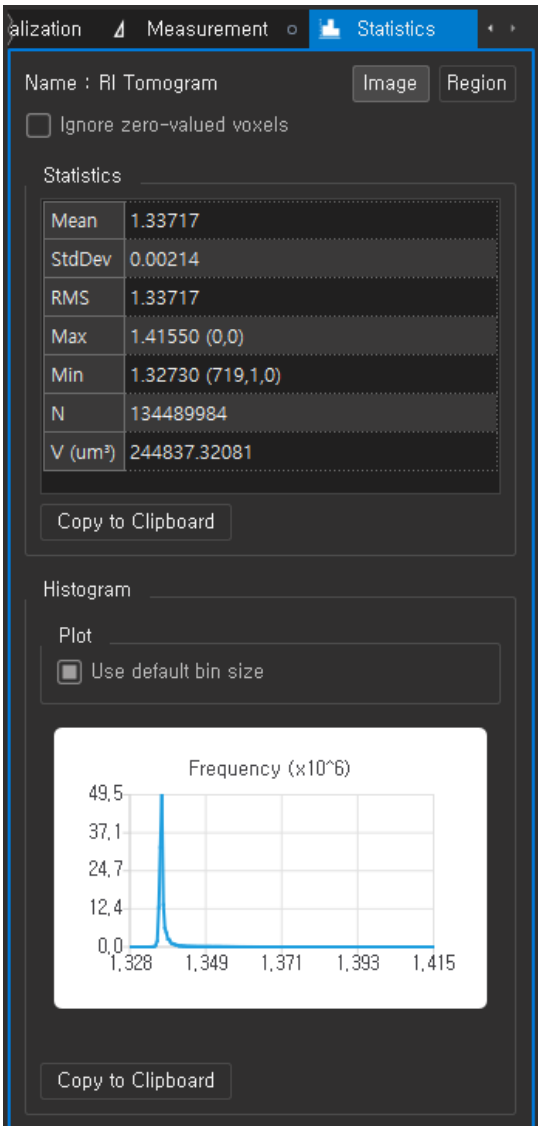
Icon	Title	Parameter(s) to be measured
	Line	Length between two points
	Path	Total length of the path
	Angle	Angle
	Four Point Angle	Inner angle at the intersection between two lines
	Circle	Radius and diameter, Area
	Ellipse	Major axis length, Minor axis length
	Double Ellipse	Major and minor axis lengths Distance between two ellipses
	Rectangle	Circumference, Area
	Polygon	Circumference, Area
	Bezier Curve	Length of the curve
	Subdivision Polygon	Circumference, Area

4.5 Statistics

Click on **Statistics** icon to activate the statistics tab.

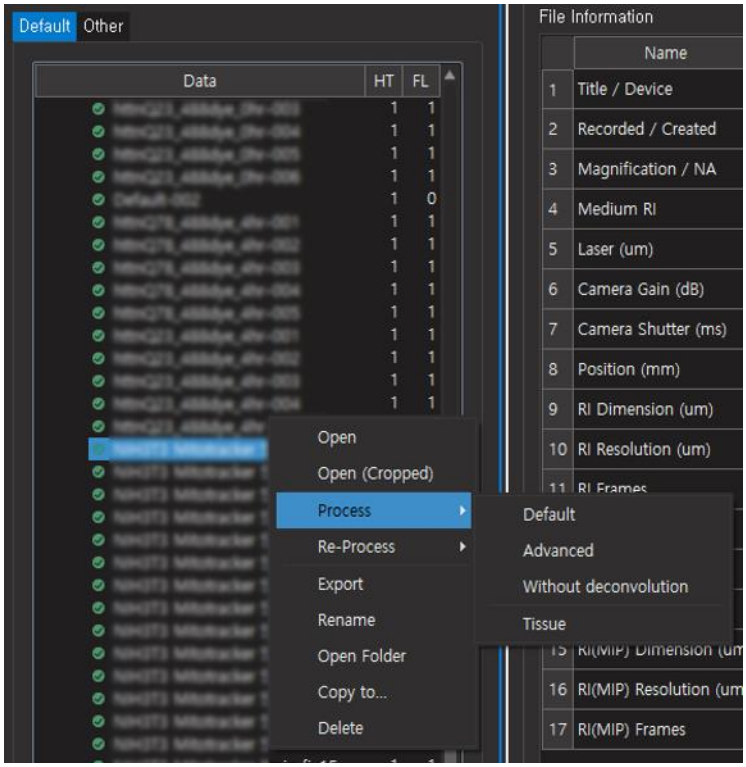


The **Statistics** view provides a functionality for computing statistic features of a whole image or a region selected by using several measurement tools. There are two sections in this view – Statistics and Histogram. When the user selects an image or a measured item on the data manager panel, statistical values and RI histogram are updated automatically. These values can be copied to the clipboard and then pasted to other spreadsheet programs.



Data Export

The **Data Navigation** shows a list of acquired image folders. Each image folder is in a date folder. By clicking the right mouse button on an image folder, the user will see the pop-up menu below.

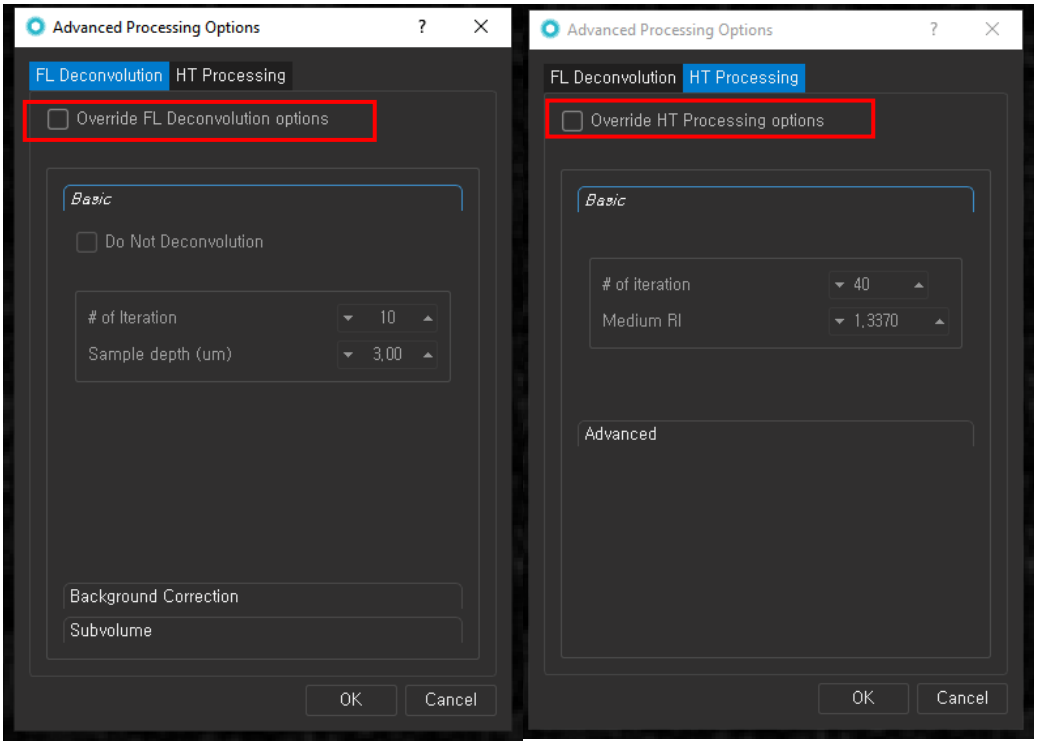


Below is the description of each menu.

Menu	Description
Process	Processes selected image folder(s) to generate a TCF file(s). Once data is processed, the color of the check-shaped icon will change to green. Multiple selections are supported. Process is skipped if there are already existing TCF file(s).
Re-process	Processes all folders selected, including ones with already existing TCF file(s)
Export	Exports the data in a chosen image type and file format
Rename	Rename selected image folder
Open Folder	Opens a folder using Windows Explorer.
Copy to…	Copy the image folder(s) to where the user selects. Multiple selections are supported.
Delete	Delete selected image folder(s). The deleted folder cannot be recovered.

There are multiple options for processing: Default, Advanced and Without deconvolution. Default automatically processes the data with 40 iterations for HT processing, and 10 iterations for FL deconvolution. Without deconvolution processes the data without any iterations.

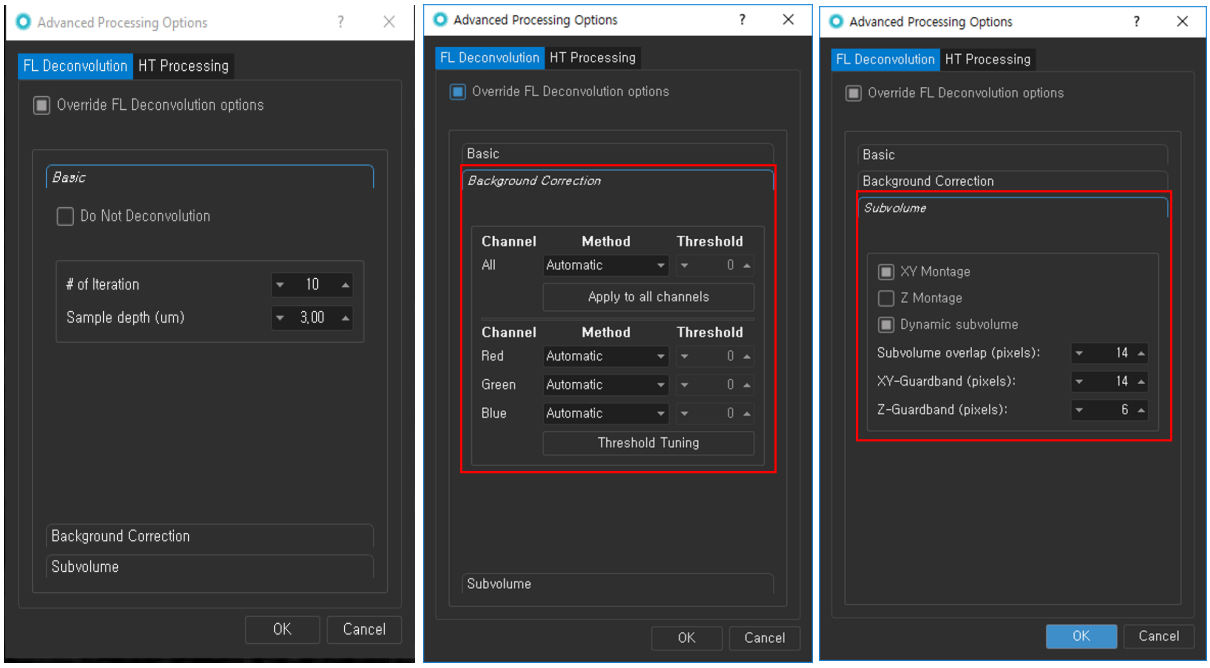
Choosing **Advanced** option will show processing options for FL deconvolution and HT processing.



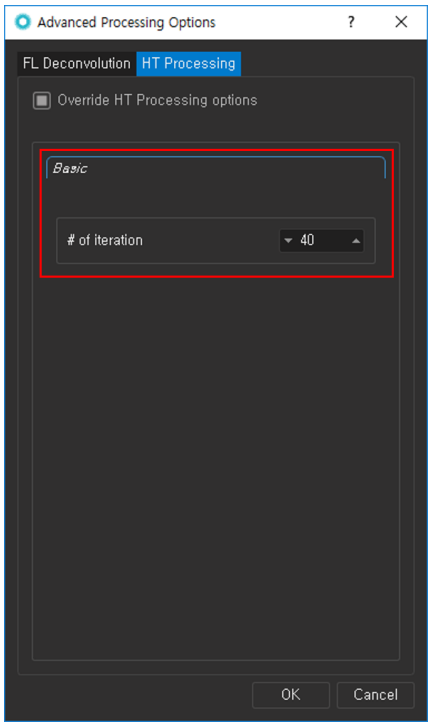
To manually adjust number of iterations, click '**Override FL deconvolution options**' or '**Override HT processing options**.'

In FL deconvolution, there are three tabs; Basic, Background Correction, and Subvolume. In **Basic** tab, the user can change the number of iteration and the sample depth. For time-lapse images, the setup value of the first frame would be automatically applied to the last frame. In **Background Correction** tab, the user can choose whether to use pre-defined (automatic) setup value or manually define own value for the background threshold of all 3 channels. By clicking Threshold Tuning button, more detailed adjustment can be made.

In **Subvolume** tab, deconvolution process details can be adjusted. By checking or unchecking XY Montage, Z Montage, and Dynamic subvolume, the RAM usage can be efficiently adjusted. If the user checked XY and/or Z Montage, adjusting Subvolume overlap, XY-Guardband, and Z-Guardband pixels numbers would affect the overall processing time as well as the quality of deconvoluted images.



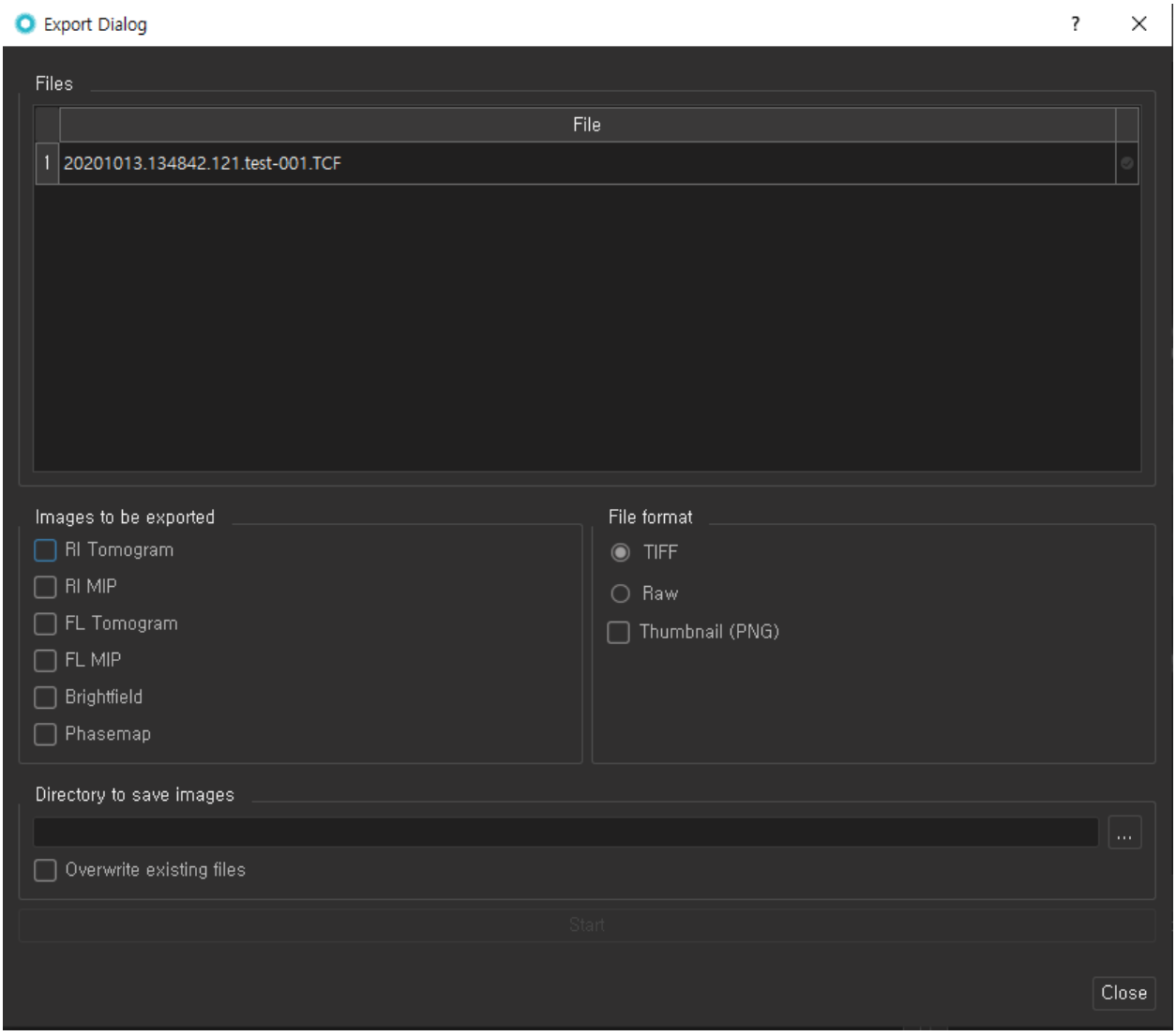
Default number of iterations for FL deconvolution is 10. To process the fluorescence image without deconvolution, choose **[Process]** - **[Without deconvolution]**. This will produce epi-fluorescence like image.



About **Advanced processing for HT images**, the number of iterations can be changed. The default iteration number is 40. Decreasing the iteration below 40 will result in z-axis aberration. On the other hand, increasing the iteration above 40 will leave only the strong signals, such as organelles with high RI values.

5.1 Batch Export

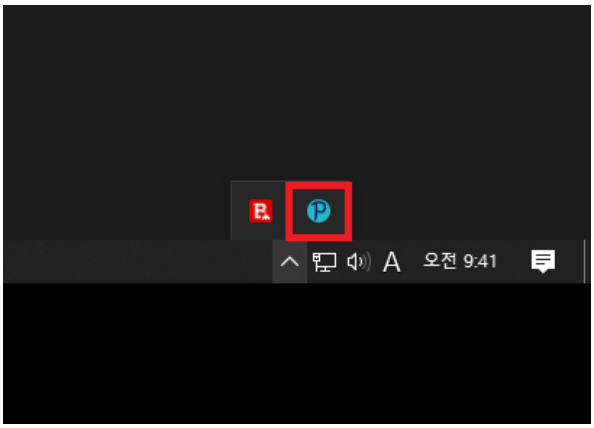
Clicking **Export** will display a dialog shown below:



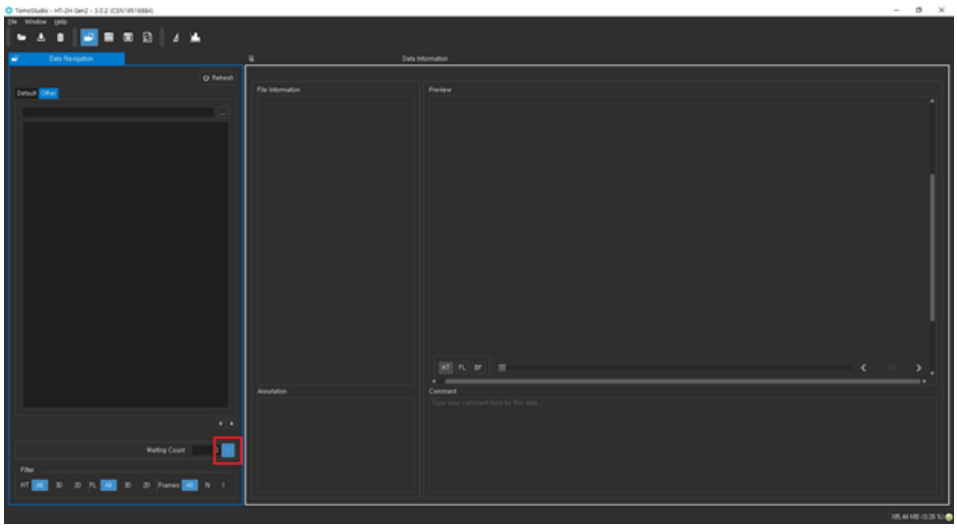
The images to be exported can be multi-selected, and the file format, either TIFF or Raw, also need to be selected. Once the Directory to save images is selected, ‘Start’ button will be activated. Clicking ‘Start’ will save the images into the chosen directory.

5.2 TomoProcessing Server

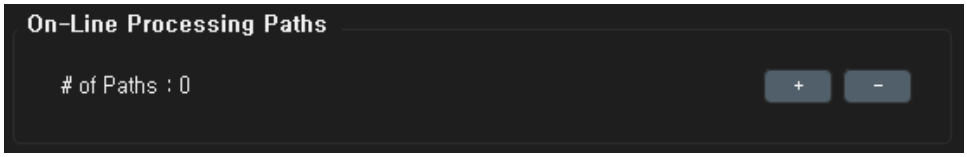
The TomoProcessing Server operates separately from TomoStudio which means the user can simultaneously process the acquired images and take new images. In order to see TomoProcessing Server, click on ‘show-hidden-icons’ in the taskbar and select TomoProcessing Server icon.



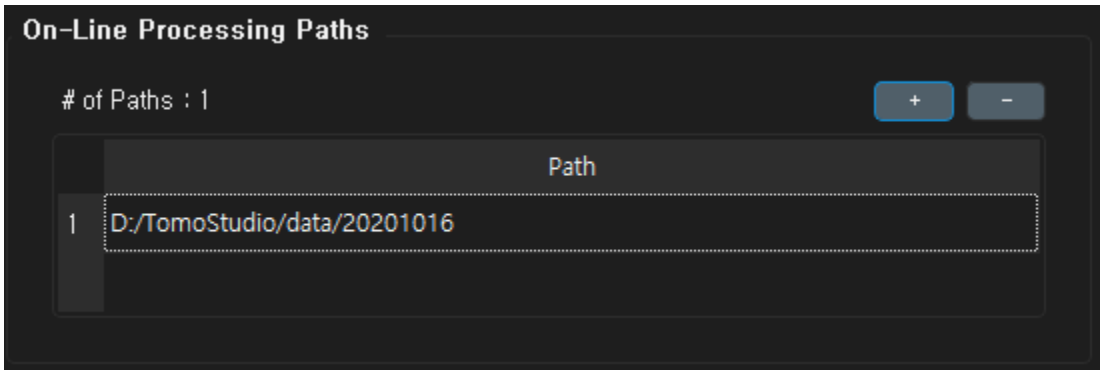
The user can also open the Processing Server in TomoStudio in the Data Navigation tab by clicking the menu icon next to Waiting Count.



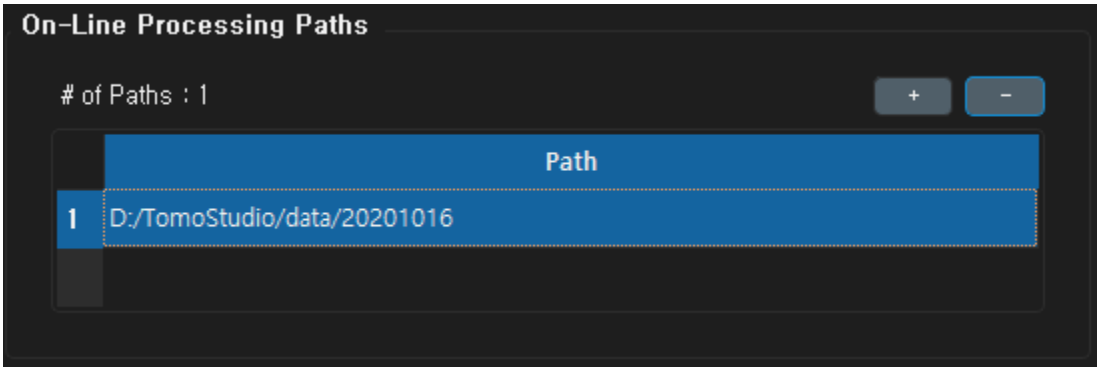
On-line Processing path can be set. The On-line Processing function searches inside the configured path and processes data. When acquiring long term time-lapse imaging data, the acquired data is processed even if acquisition has not been completed.



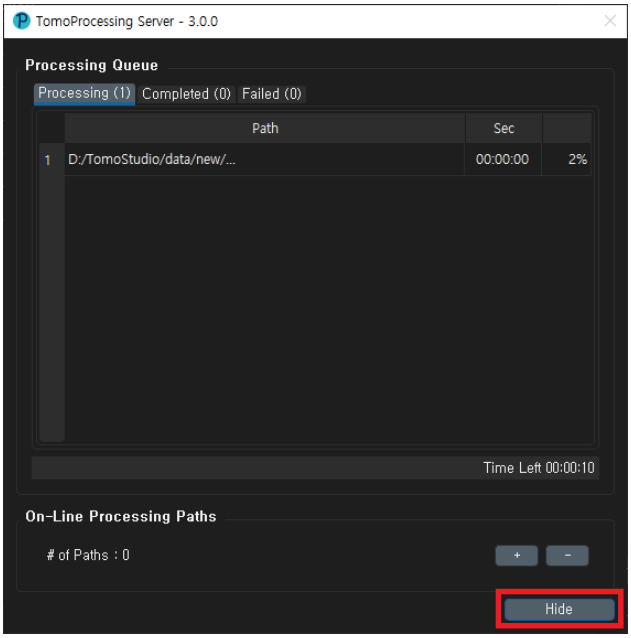
Click the ‘+’ button to set the path you wish to process. The added path is searched and a list is created in the Processing Queue.



You can also select and remove a path by clicking the ‘-’ button. When the path is removed, files inside the removed path are no longer searched.

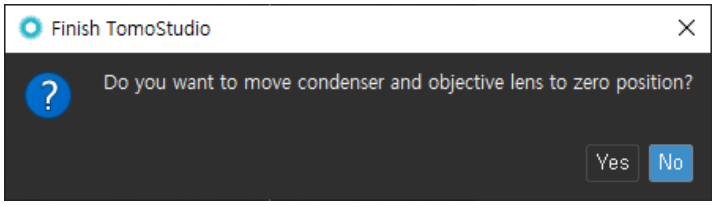


The user can also choose the working folder. Clicking the **Hide** button will hide TomoProcessing Server window.



5.3 Exit TomoStudio

The user can exit TomoStudio by clicking the close button at the top right on the screen. When clicking that button, a pop-up window will show up as below.

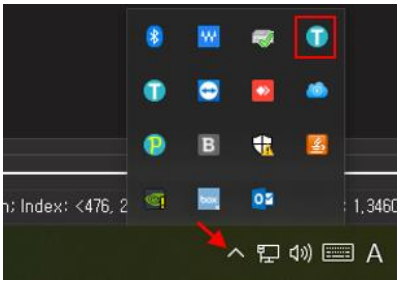


In this window, the user can change whether to move the condenser and objective to their zero positions. Once clicking Yes, the axial positions of both condenser and objective will move to the initial ‘zero’ value and horizontal position of the stage will be reset next time the user starts TomoStudio. If the user clicks No, the axial and horizontal positions of lenses and stage will not move even next time TomoStudio turns on.

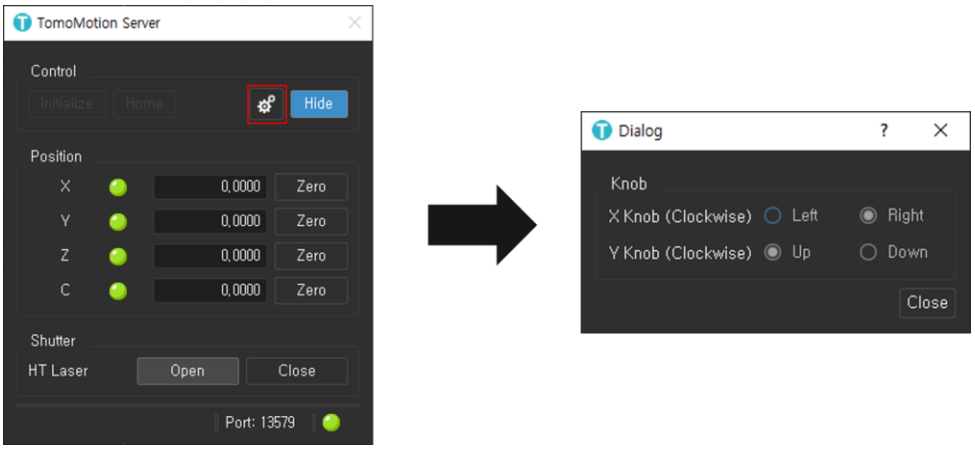
Appendix

TomoMotion Server

TomoMotion Server is a separately operating server in which the user can check and control the lenses, stage, and camera shutter. To see TomoMotion Server, click on ‘show-hidden-icons’ on the taskbar and select TomoMotion Server icon.

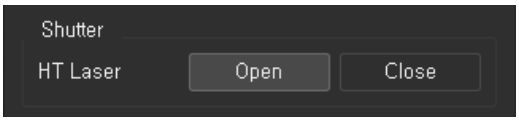


By clicking the setup icon in the **Control** session, the user can change the directions or X and Y knob.



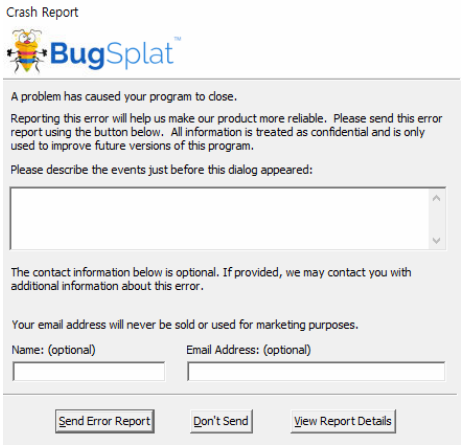
The **Position** session shows current positions of the stage, objective, and condenser and the user can move them to ‘zero’ positions by clicking Zero button.

In the **Shutter** session, the user can open and close the camera shutter.



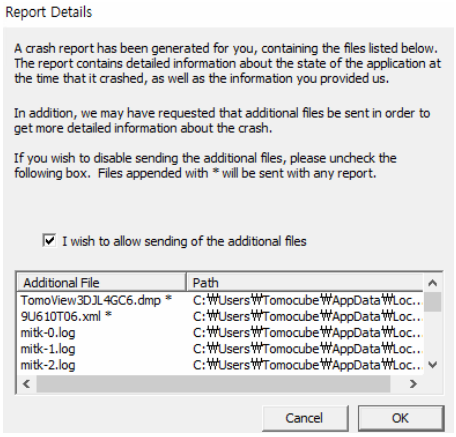
Error report

If TomoStudio crashes unexpectedly, the crash reporting module starts collecting information about the causes of the crash. However, Tomocube never collects any information unless the user agrees to send information to our server. The crash reporting module shows the window like below after it is ready to send information:



If you agree to send a report to the server, please click on **Send Error Report** button. (Optional) For users who enters the contact information, Tomocube will update the progress for resolving reported error(s).

The user can check the detail information in a report by clicking on **View Report Details** button.



Troubleshooting

1. Processing speed is slow
   Possible cause: If the computer has been left turned on for a long time or if there are too many software being used at the moment, the computer can become slow
   - Reboot the computer and check again.
   - Update Nvidia driver to a newest version
   - Reinstall TomoStudio software
2. Processing does not work
   - Check if Windows update is in progress
   - If the update is in progress, wait for the updates to finish, reboot and try again
   - If the computer is not updating, reboot and try again
   - If there are less than 10GB of free space in the C drive, delete unnecessary files and try again
3. TomoStudio crashes after updating to a new version
   - Reboot and try again
4. RI tomogram does not appear after loading
   - Use ‘Open (Cropped)’ mode to open the data. This reduces the size of the data
   - Alternatively, when acquiring time-lapse images, set the number of images to below 100 counts
5. Different cells are selected when running Basic analysis.
   Possible cause: Basic analysis assumes only one cell is within the FOV
   - Use ‘Open (Cropped)’ mode to select certain cellular area and re-run basic analysis again.
6. TomoStudio stops or slows when running processing for a long time (ex: overnight)
   Possible cause: The computer may be on power saving mode
   - Open **Power Options** in the Control Panel and change plan setting next to your current power plan.
   - Change “Put the computer sleep” to never.
   - You may also need to check Additional power settings and change the power plan to “High performance”.