Tomocube

TomoAnalysis

Version 1.5

Quick Guide

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

Table of Content

Chapter 1. Starting the Software

1.1 Introduction

TomoAnalysis is an image analysis software to analyze holotomogram data acquired by Tomocube's Holotomography systems. This quick guide explains the Viewer functions in TomoAnalysis and guide you to use its features.

Using TomoAnalysis, you can examine the holotomography data in 2D and 3D formats and understand its characteristics through image navigation and measure tools.

1.2 Installation

To install TomoAnalysis, follow the procedure.

1. Prepare a copy of TomoAnalysis installation file provided by Tomocube.
2. Run the installer and follow the installation procedure.

Note: Any previous version of TomoAnalysis installed beforehand will be removed before the installation.

1.3 License

To run TomoAnalysis, you need a license provided by Tomocube. Without a properly issued license, TomoAnalysis will terminate after warning that it cannot find a license. If you encounter a problem with a license, please contact Tomocube.

1.4 Running the software

- TomoAnalysis can be started by double-clicking the program icon for TA.
- TomoAnalysis can be started from the menu, Start > All apps > TomoAnalysis.

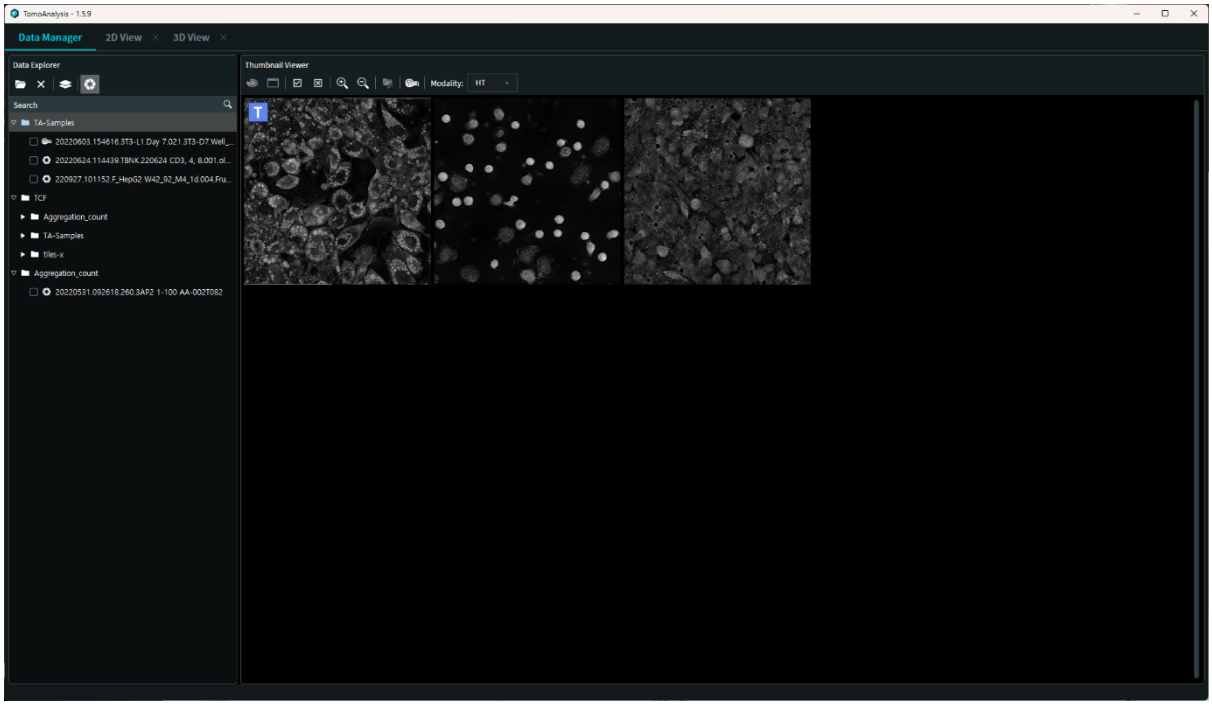
When started, the Data Manger window of TomoAnalysis appears.

1.5 File format

TomoAnalysis supports the TCF file format, which is a proprietary format for storing data acquired by Tomocube's Holotomography systems, the HT-X1 and the HT-1/2.

Chapter 2. Data Manager

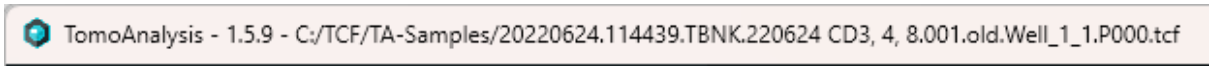
2.1 User interface



In the left **Data Explorer** panel, you can see the registered 'root' folders after opening them to load TCF files. The right **Thumbnail Viewer** panel displays thumbnails of the chosen folder.

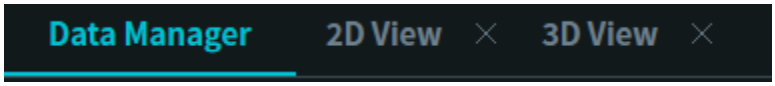
Title bar

The title bar indicates the version of TomoAnalysis as well as the information of the file if opened.



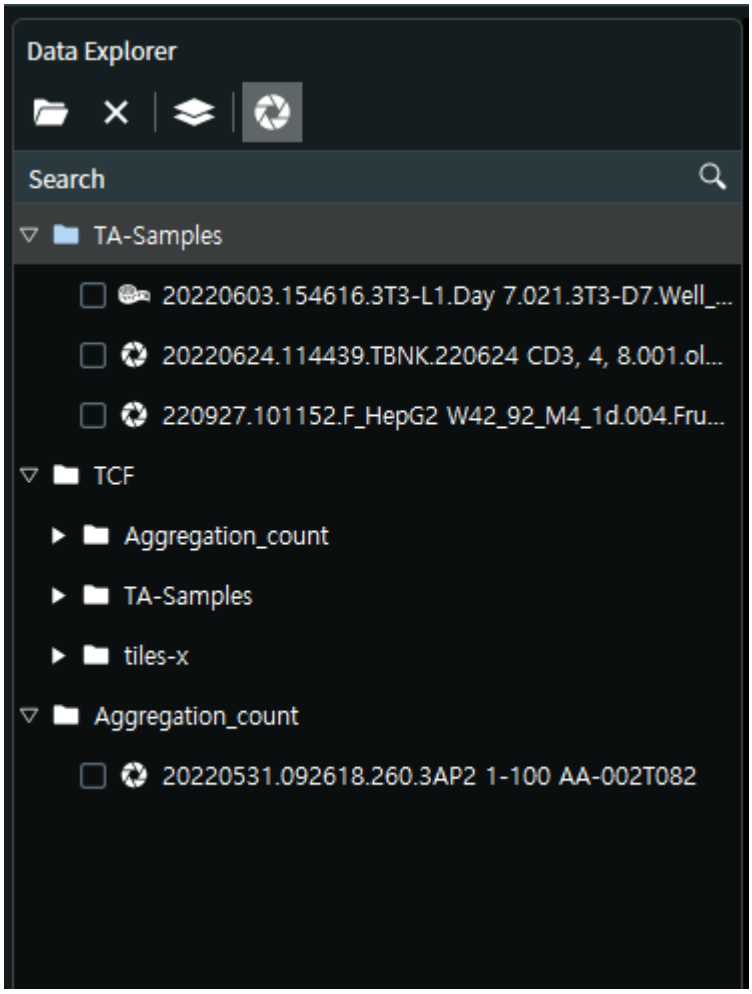
Tab bar

The tab bar shows the tabs that are opened for the analysis. The **Data Manager**, the **2D View**, and the **3D View** tabs are available.



Data Explorer panel

The panel is used to register folders that have TCF files. When folders are registered, files in their subfolders are registered simultaneously.



Parameter	Description
Open Directory	Selects a folder to load TCF files.
Remove Directory	Deregisters a selected folder from the Data Explorer.
Collapse All	Shrinks all the expanded tree of the registered folder.
Show TCF	Shows or hides all the TCF files in the folder tree in the Data Explorer.

Search field

The **Search** field is used to find or filter listed TCF files shown in the **Thumbnail**

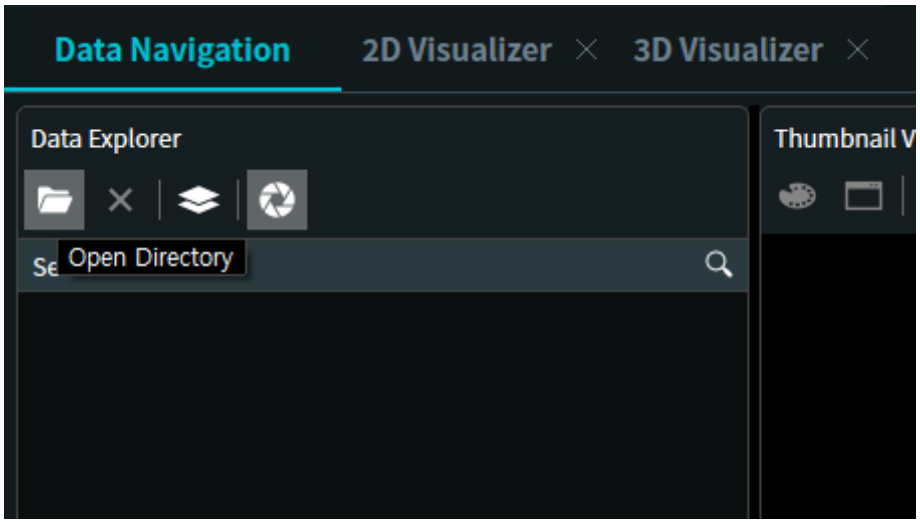
Viewer. By the keyword in the **Search** field, only those files whose name matches with the keyword appears.

Thumbnail Viewer

The panel shows thumbnails of TCF files contained in the registered folders in the Data Explorer.

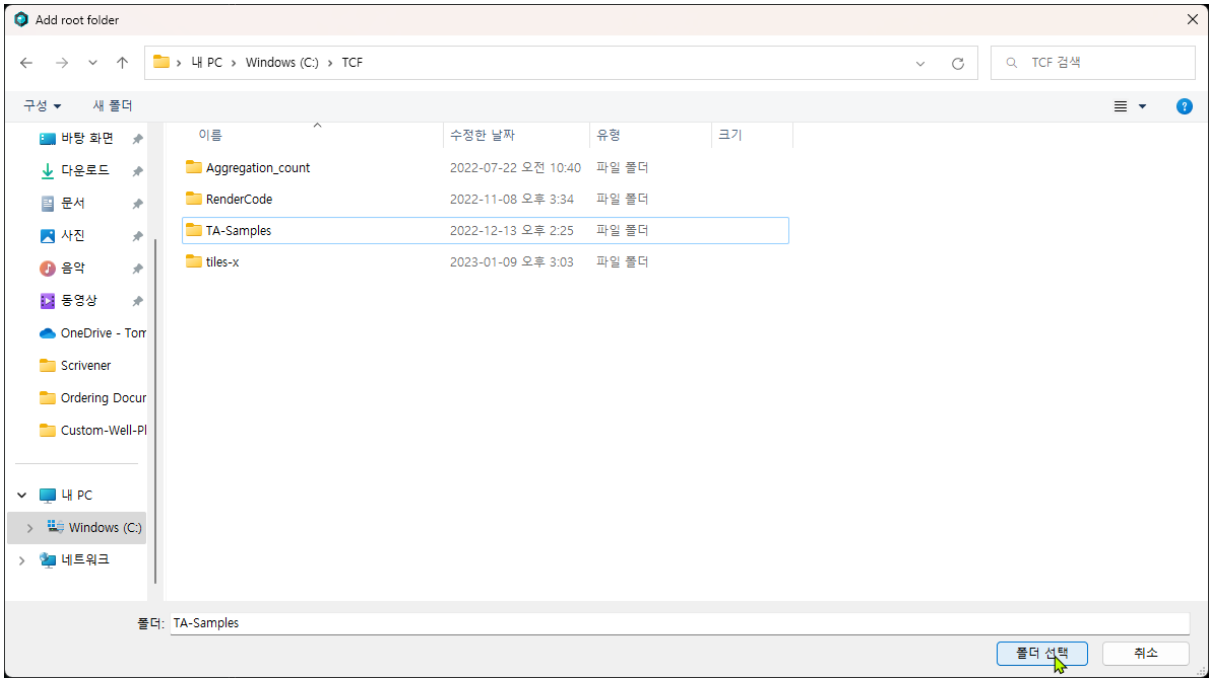
Parameter	Description
 Open with Image Viewer	Opens the selected TCF files in the 2D View tab.
 Show Details	Shows the experimental information of the TCF file.
 Select All	Selects all the TCF files shown in the Thumbnail Viewer.
 Deselect All	Deselects all the TCF files.
 Zoom in	Magnifies the size of the thumbnail images.
 Zoom out	Shrinks the size of the thumbnail images.
 Show in Explorer	Shows the selected TCF files in the system explorer.
 Timelapse play	Enables or disables timelapse image animations.
Modality	Selects the data channel to be displayed for the thumbnail image.

2.2 Adding and removing root folders



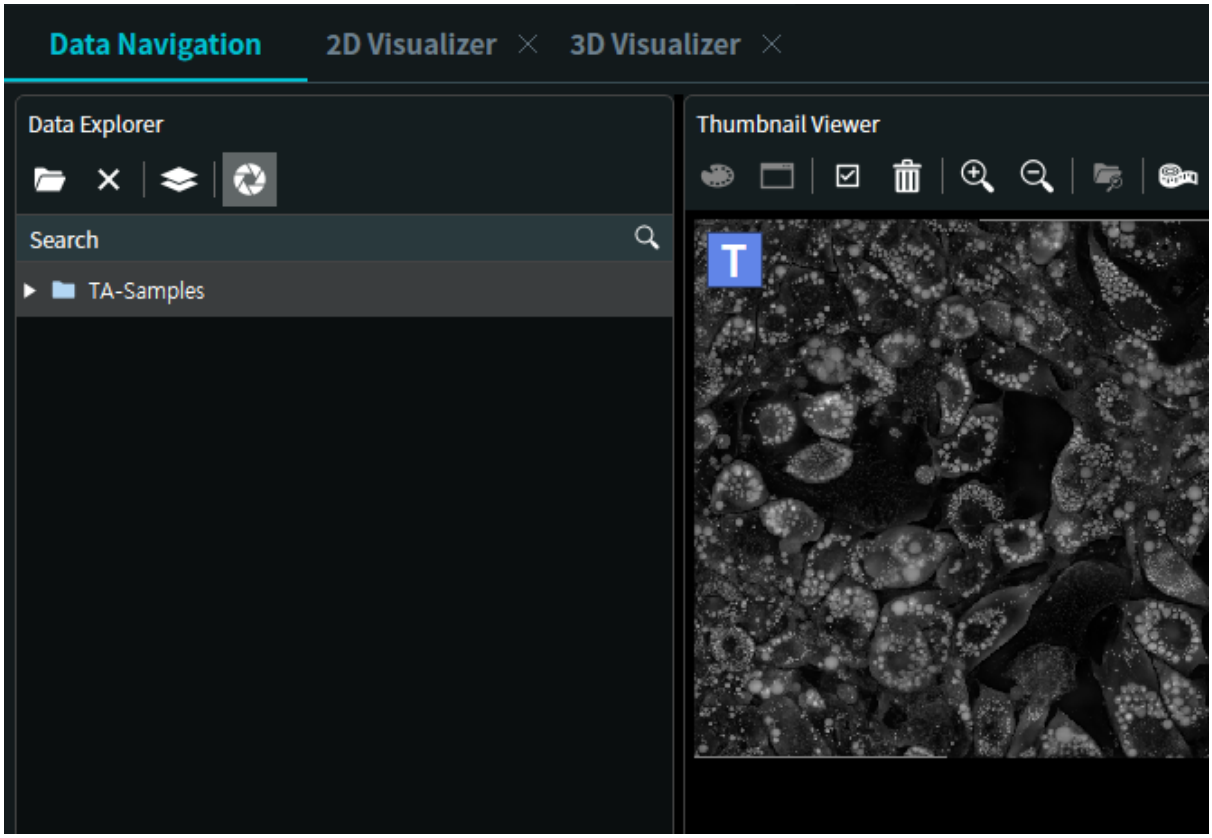
On the **Data Explorer**, click on the **Open Directory** icon  .

The window, **Add root folder**, will appear.



Select a 'root' folder that contains data files that you want to load onto TA and click **Select folder**.

Note: You can register multiple root folders by repeating the adding procedure.



The selected folder with its subfolders will be registered in the **Data Explorer** panel. All the TCF files in the folder and its subfolders will be registered in the **Thumbnail Viewer** on the right panel.

Note: It will take time to load all the thumbnails onto the Thumbnail Viewer if there are many TCF files to be loaded.

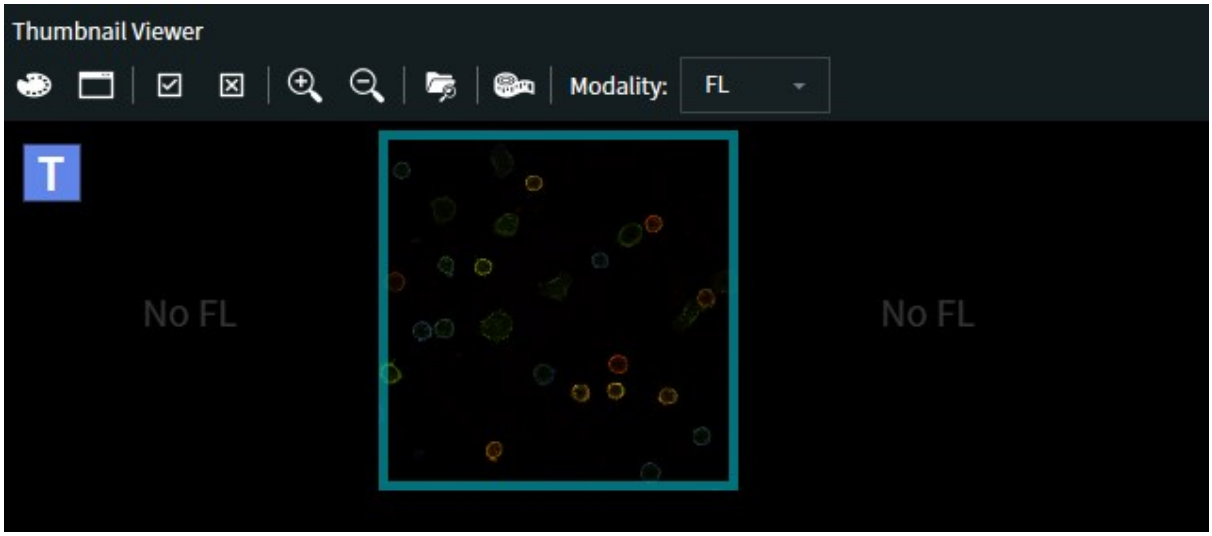
2.3 Changing the displayed data channels

The TCF files can contain multiple data channels, such as holotomography, fluorescence, or brightfield channels. In the **Thumbnail Viewer**, you can change the data channel to be displayed as thumbnails.

On the toolbar of the **Thumbnail Viewer**, click the drop-down menu saying **Modality**.

Select one of the listed channels, i.e., HT, FL, and BF for holotomography, fluorescence, and brightfield channels, respectively.

Note: Those files that do not have a certain data channel will display No HT, No FL, or No BF depending on the chosen data channel.



2.4 Adjusting the size of the thumbnail

You can change the size of the thumbnails in the **Thumbnail Viewer**.

Press the **zoom-in** or **zoom-out** buttons  to increase or decrease the size of the thumbnail.

You can also scroll the mouse wheel up or down while pressing the **Ctrl** key to increase or decrease the size of the thumbnails.

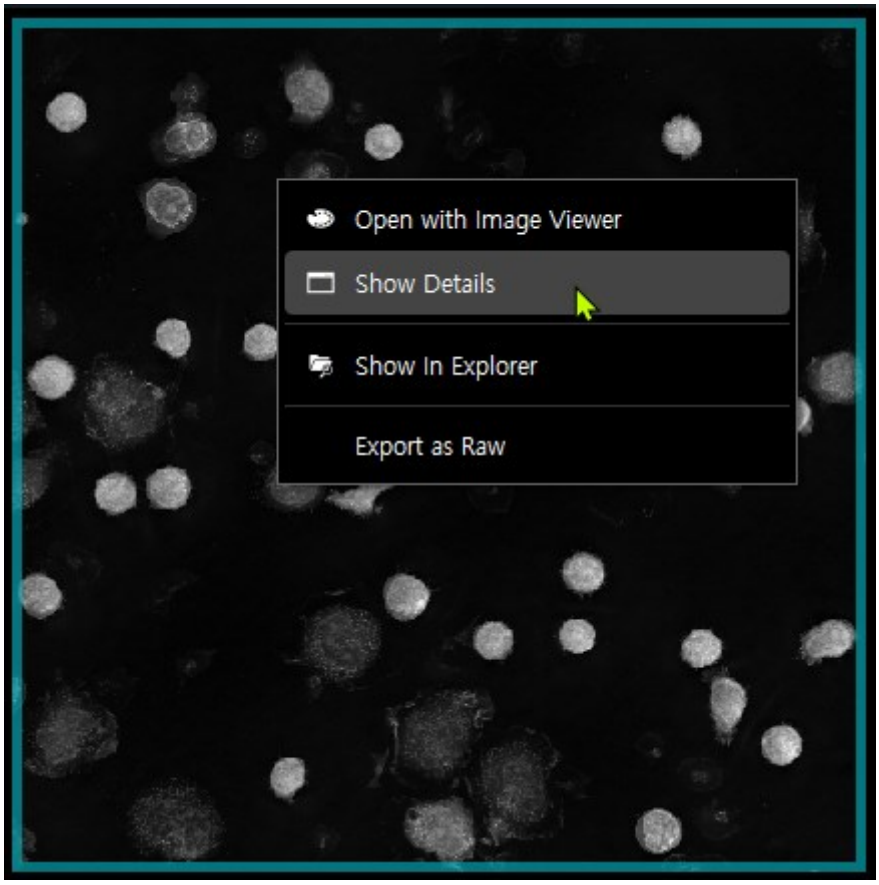
2.5 Retrieving the experimental information of TCF files

You can see the experimental information recorded in the TCF files.

Select the thumbnail of the TCF file that you want to see.

On the toolbar of the **Thumbnail Viewer** panel, click on the **Show Details** button  .

Or you can click the right button of the mouse on the selected thumbnail, where you can select the Show Details menu.



Or you can even double click on any TCF file to see the experimental information.

Then, the experimental information will appear in a new window.

2.6 Selecting and deselecting multiple TCF files

To view in the **2D View** tab, you need to select the TCF files and run the viewer.

In the **Thumbnail Viewer**, you can click TCF files that you want to select while pressing the **Ctrl** key or the **Shift** key. If you press the **Ctrl** key, only the clicked files will be selected. If you press the **Shift** key, the listed files between what you just clicked and what you clicked before will be selected. You can select TCF files across multiple 'root' folders registered in the **Data Explorer**.

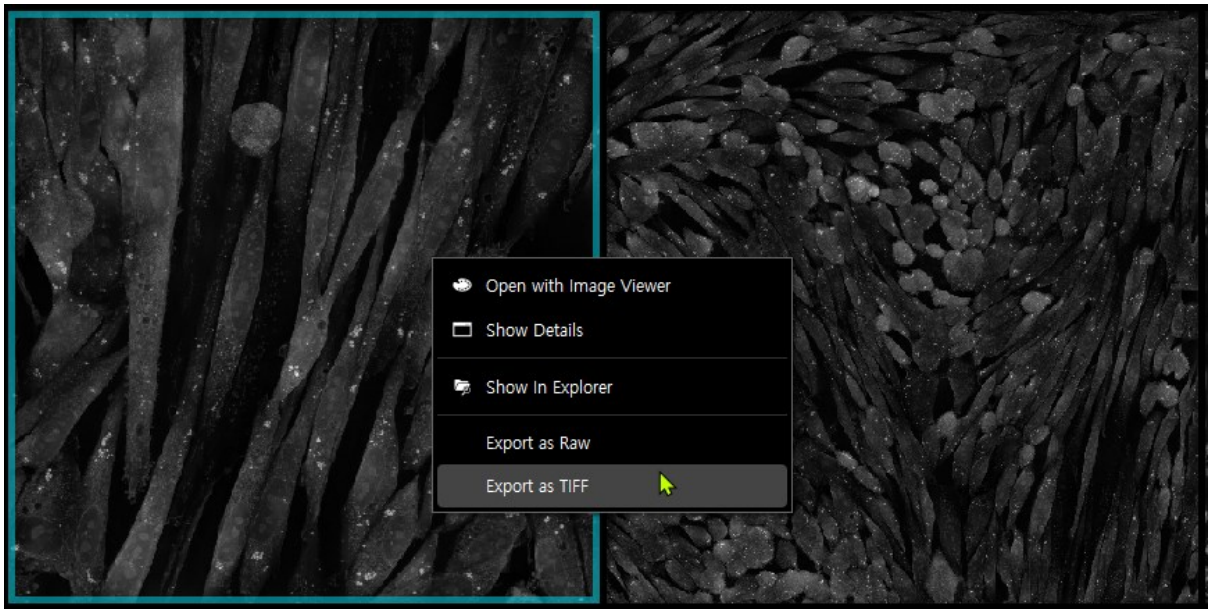
Likewise, you can deselect TCF files by click on those TCF files while pressing the **Ctrl** key or the **Shift** key.

If you want to select all the TCF files shown in the **Thumbnail Viewer**, you can click on the **Select All** button  . If you want to deselect all the selected files, you can click on the **Deselect All** button  .

2.7 Exporting to other data formats

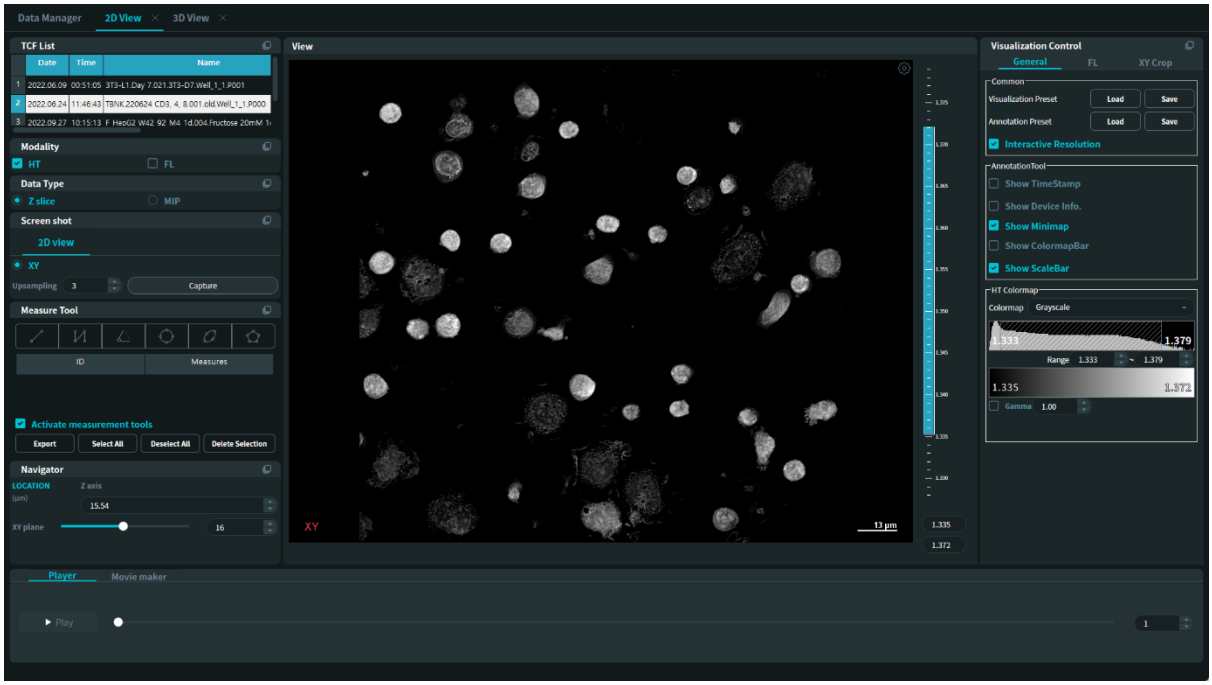
If you want to view or analyze TCF files in other image analysis software, such as ImageJ, you can export the TCF files into the RAW or the TIFF data format.

After selecting TCF files that you want, click the right button of the mouse to show the pop-up menu. Select **Export as Raw** or **Export as TIFF** to export the selected files.



Chapter 3. 2D View

3.1 User interface

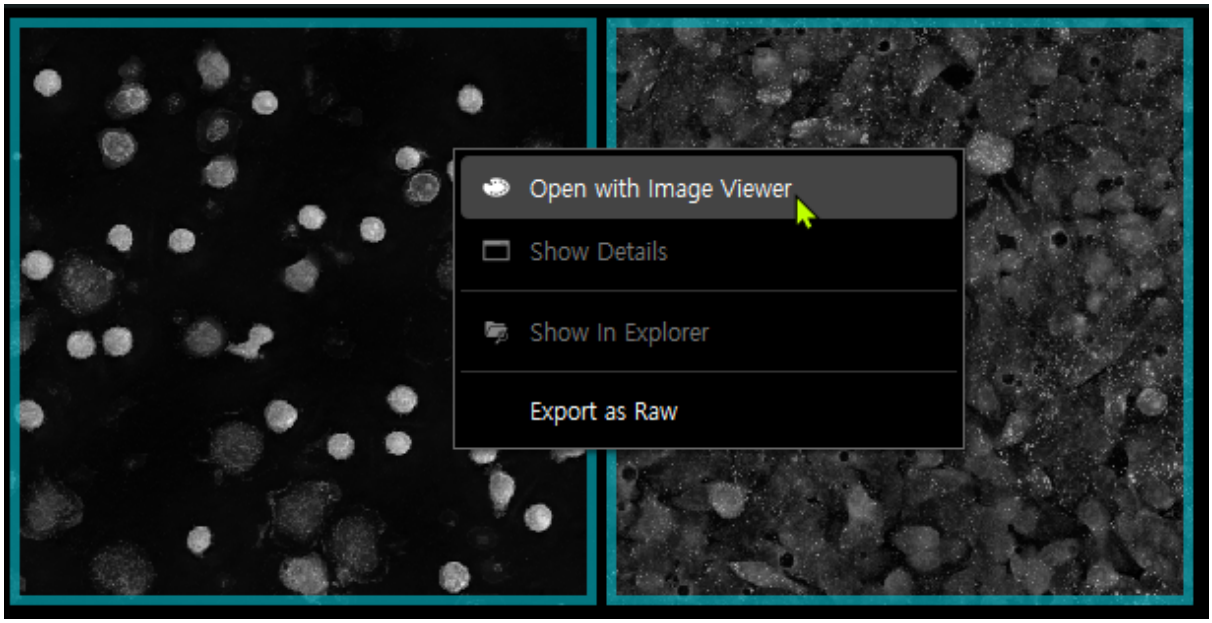


In the left **Data Control** panel, you can choose the data to view as well as the data channel, the data type, and the region of the data to be displayed. It also contains controls for image export and measuring. The center **View** panel displays the image of the selected data. In the right **Visualization Control** panel, you can manage the contrast of the image as well as the annotation items and its settings. In the bottom **Time lapse control** panel, you can change the time point or make a video file of the data if it is in time lapse.

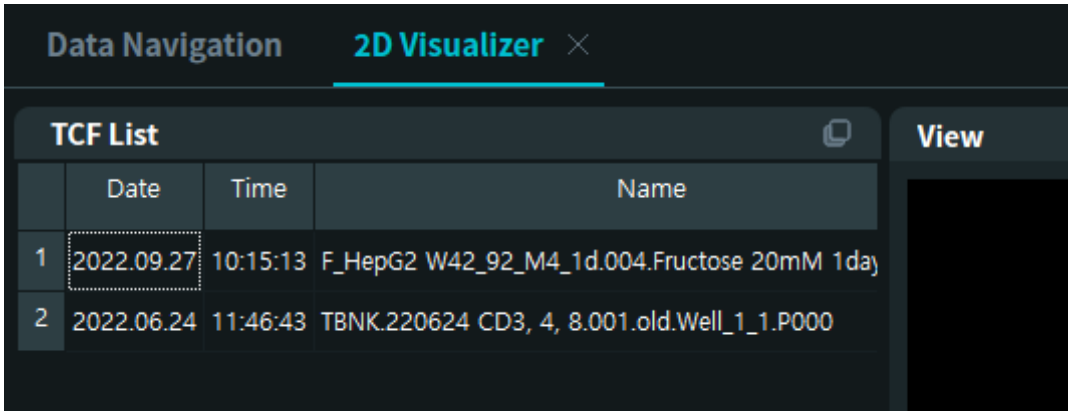
3.2 Loading TCF files in the 2D View tab

To view TCF files in the **2D View** tab, you need to load those TCF files into the worklist of the **2D View** from the **Data Manager**.

Select TCF files to view on the **Thumbnail Viewer**, and right-click on any selected file to display a pop-up menu. Select **Open with Image Viewer** to load the files into the worklist of the **2D View**. To select multiple TCF files, refer to p. 13, **Selecting and deselecting multiple TCF files**.



Then, the **2D View** tab will open and the selected files are listed on the worklist.



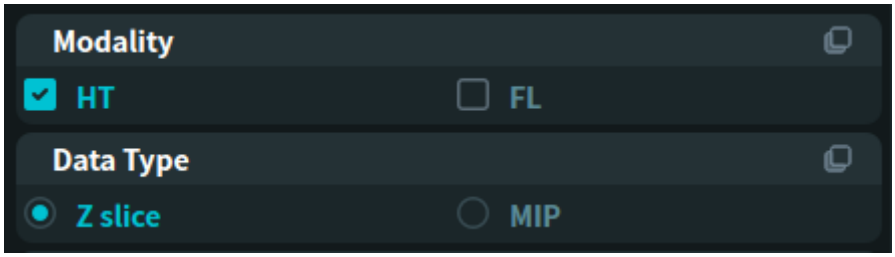
3.3 Choosing a different TCF from the worklist

Double-click on a TCF file from the worklist to see from the **View** panel.

3.4 Selecting data channels and types

Data channels

When a TCF data is opened in the **2D View**, the displayed image on the **View** panel is from the HT channel at its recorded focus plane when the data was acquired.



If an opened TCF file has data channels other than the HT channel, such as FL and BF, you can change the data type to be displayed in the **Modality** box. You can choose multiple data channels to make a blended image from them.

Note: If the BF channel is selected, all the other modality channels are deselected and only the BF image will be displayed on the **View** panel. When the BF channel is deselected, the other channels can be selectable.

Data types

In the **2D View**, the displayed image on the **View** panel, by default, is the Z slice of the opened TCF data. You can select the displayed image from the two display types, Z slice and MIP, or Maximum Intensity Projection.

3.5 Changing the location to view in space and time

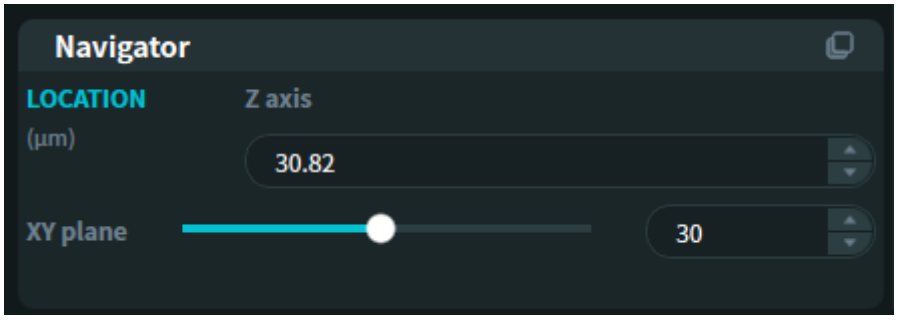
To zoom in or zoom out, you can move the mouse cursor up or down while pressing the right button of the mouse. A minimap will appear on the View panel to show the level of the zoom.

To move in the lateral direction, or the X and Y direction, you can move the mouse cursor in any direction you want to move while pressing the wheel button of the mouse. A minimap will show the location as you move.

When Z slice is chosen as the Data Type in the Data Selection panel, you can move the location of the data to be displayed in the **View** panel.

Note: When the Data Type is chosen as MIP, it does not change in the Z direction.

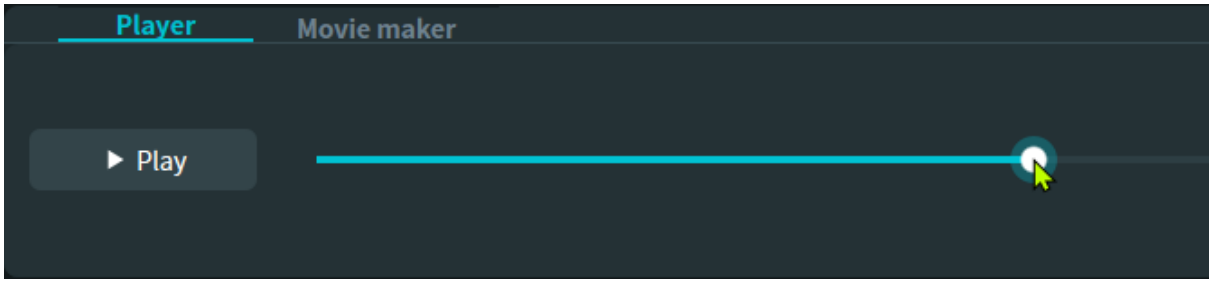
To move in the Z direction, you can use the **Navigator** box.



By keying-in a desired position in micro-meter in the Z axis number box or a desired

Z slice number in the XY plane number box, you can change the Z position to see in the **View** panel. You can also move the slider of the XY plane number box in the **Navigator** box. In addition, you can scroll the mouse wheel up or down to move the Z position.

If the TCF data is a time-lapse data file, you can change the time point. To change the time point to a desired time point, you can use the slider or the number box on the **Timelapse control** panel. You can also key in the number of the sequence of the time lapse data.



3.6 Adjusting image contrast

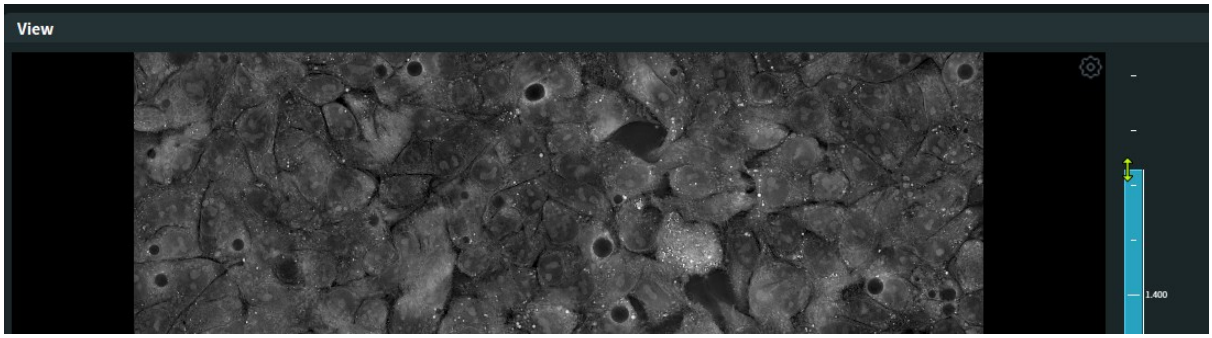
When the data is loaded, TomoAnalysis automatically finds an optimal choice for its image contrast settings for holotomography and fluorescence data.

If you are not satisfied with the automatic settings, you can adjust the contrast settings.

Holotomography (RI) data

You can adjust three parameters for the image contrast of the holotomography data, i.e., the lower and upper bound of the scalar bar and the gamma.

To change the upper and lower bound of the scalar bar, move the lower and upper bound lines located at the right side of the View panel by clicking on the lines.



Alternatively, you can key in the values for the lower and upper bounds in the number boxes below the scalar bar located at the right side of the **View** panel.

You can also adjust the gamma value to enhance the visibility of features. To enable the gamma, check **Gamma** in the **HT Colormap** box on the right **Visualization Control** panel and keying-in a desired number.

If you want to revert the image contrast of the RI data to the automatic setting, double-click on the scalar bar on the right side of the displayed image on the **View**

panel.

Fluorescence (FL) data

Prerequisite: The TCF file that you want to see has one fluorescence data channel or more and you checked the FL option on the **Modality** box of the **Data Control** panel.

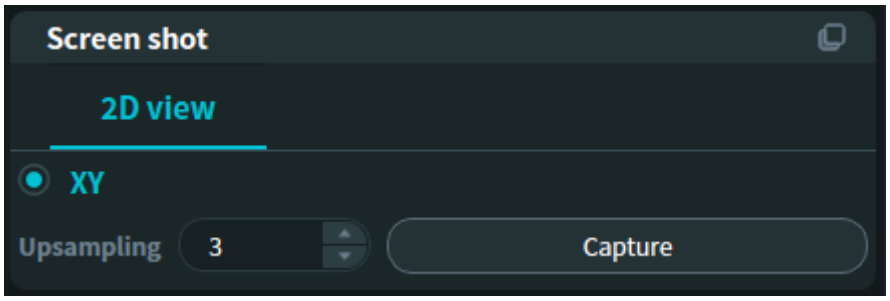
You can adjust four parameters for the image contrast of each fluorescence data channel, i.e., the lower and upper bound of the scalar bar, the gamma, and the opacity.

To change the upper and lower bound of the scalar bar, move the left or right bound lines of the striped box in the histogram of the channel you want to adjust located on the **Visualization Control** panel. You can also key in the lower and upper bound value to the **Range** number box below the histogram.

3.7 Saving a displayed TCF file to images

After adjusting the image contrast in the **View** panel, you can save the displayed image in an image format.

At the **Screen shot** box on the **Data Control** panel, press the **Capture** button to save the current image shown in the **View** panel. You can change the resolution of the saved image by changing the **Upsampling** parameter.



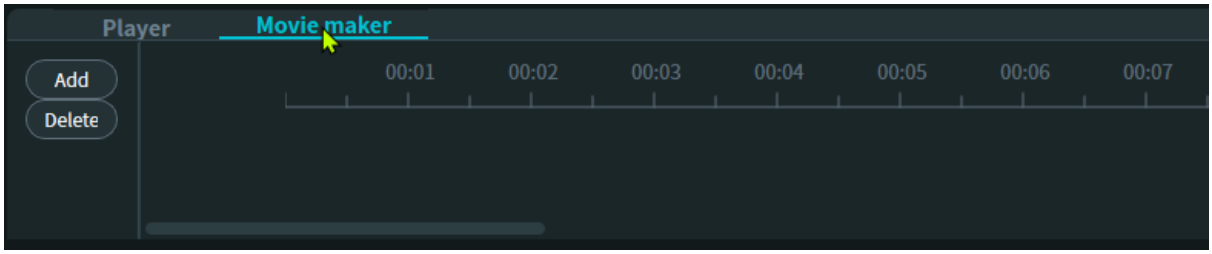
A dialog window will appear when the **Capture** button is pressed, where you can enter a file name for the image.

Note: The annotation items like the scale bar, the scalar bar, the time stamp, etc., will be included in the saved image as displayed in the View panel.

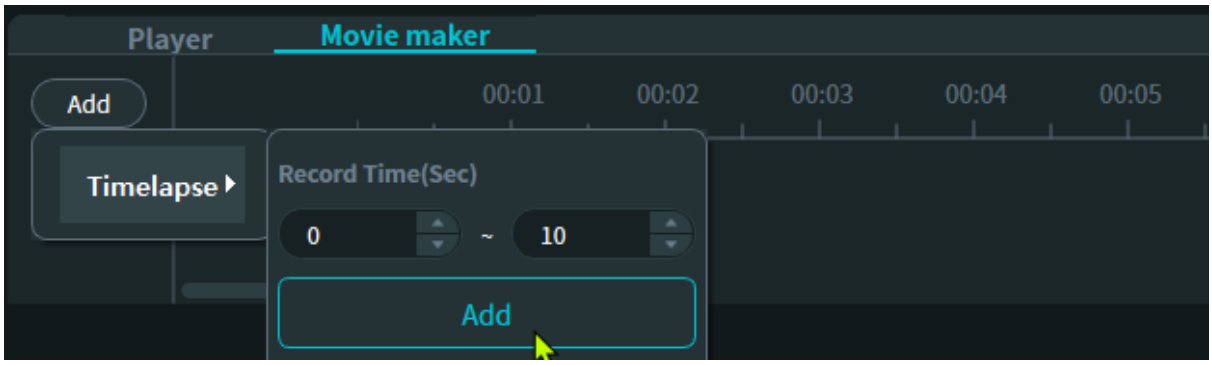
3.8 Saving a displayed TCF file to video files

If the displayed data is in time-lapse, you can save the data into a video format.

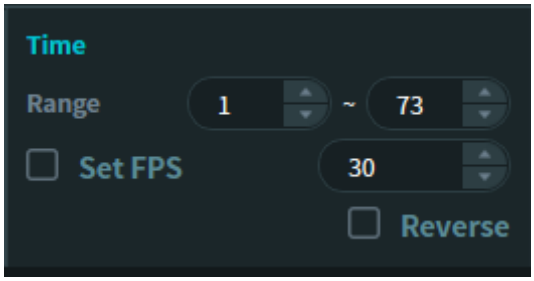
After adjusting the image contrast in the View panel, go to the **Movie maker** tab at the bottom of the window.



To add sequences to the video timetable, press the **Add** button on the left side. The **Timelapse** menu will appear, and you need to assign a time range for the video.



Press **Add** after assigning an appropriate duration for the video, then a time band will appear on the timetable according to the assigned range. On the right side of the time band, you can adjust the number of sequences you want to record with the frame rate setting.

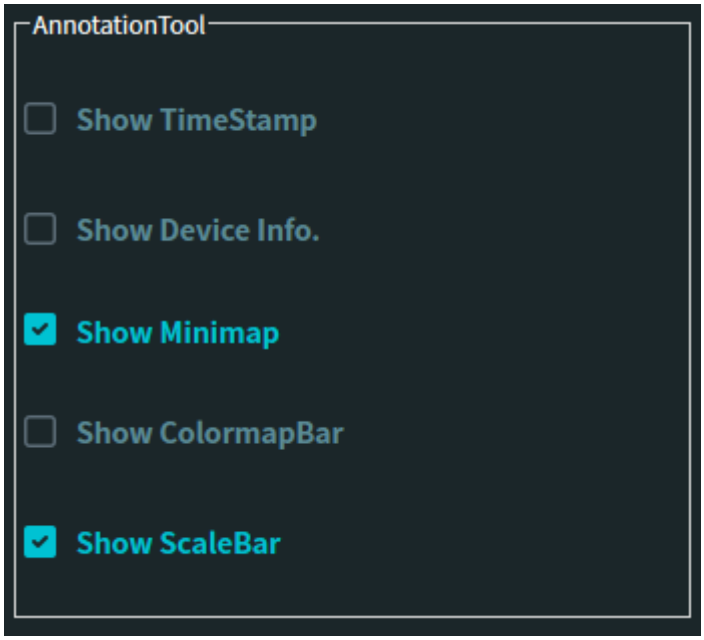


After setting the sequence number and the frame rate, press the **Record** button. If you want to see a preview, press the **Play** button.

3.9 Showing/Hiding annotations

There are annotation items that can be placed on the displayed data image on the **View** panel. Users can add or remove the annotation items on the displayed data image, such as the time stamp, the sample name, the scale bar, and the scalar bar. By default, the **View** panel displays two annotation items, i.e., the scale bar and the mini map.

To place or remove annotation items on the image, check or uncheck the **Annotation Tool** box on the **Visualization Control** panel on the right.

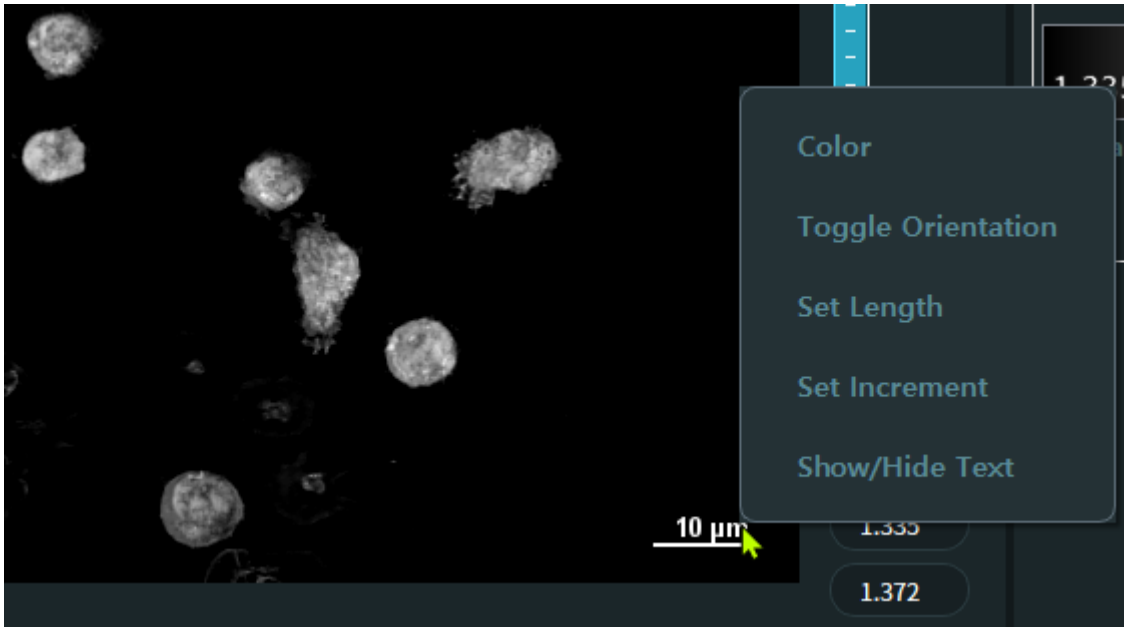


Note: The mini map only appears on the **View** panel and are not placed when the displayed image is saved regardless whether the minimap is checked or not.

3.10 Changing the shape of the scale bar

Prerequisite: You have checked the **Show Scale Bar** menu at the **Annotation Tool** box.

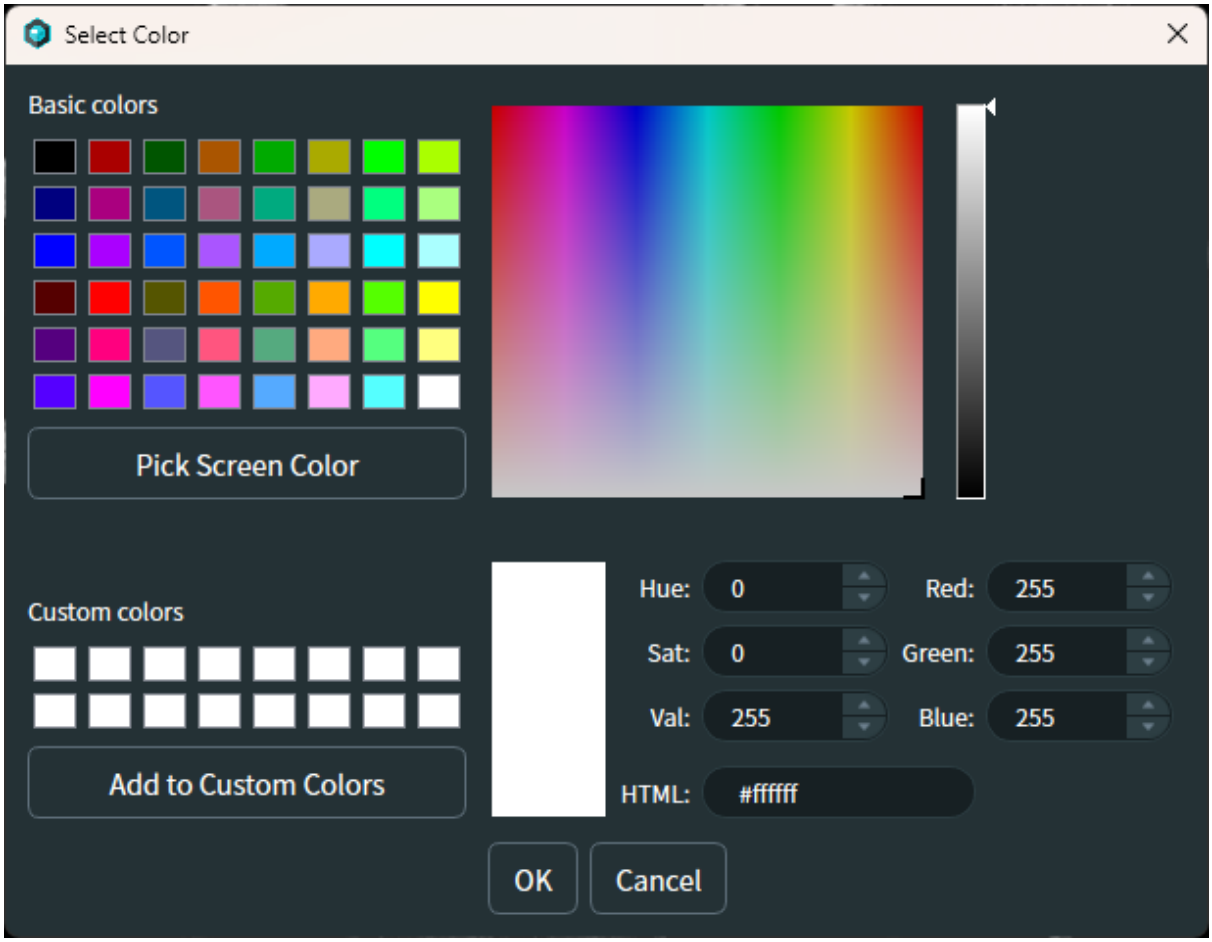
On the scale bar, right-click the mouse button to show a pop-up menu for the scale bar control.



The menu reads **Color**, **Toggle Orientation**, **Set Length**, **Set Increment**, and **Show/Hide Text** from top to bottom. If you select one of them, you can do as follows:

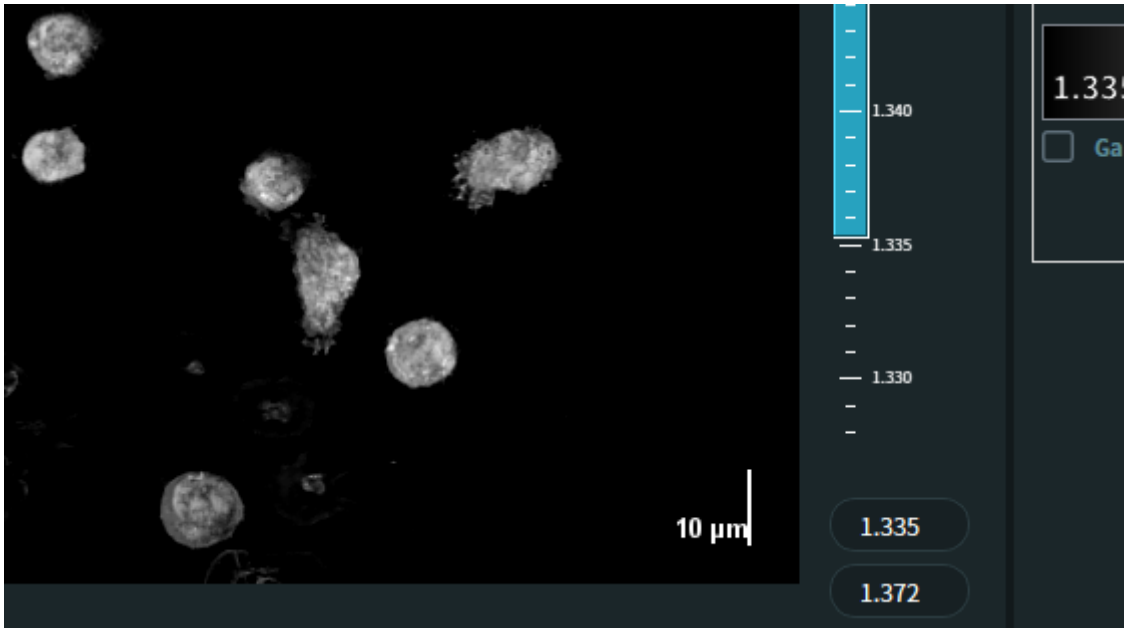
Color

The **Select Color** window will appear, where you can change the color of the scale bar.



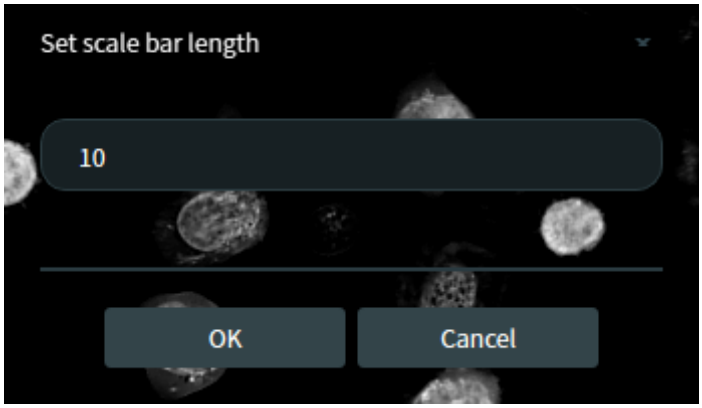
Toggle Orientation

The direction of the scale bar changes from the horizontal direction to the vertical direction or vice versa.



Set Length

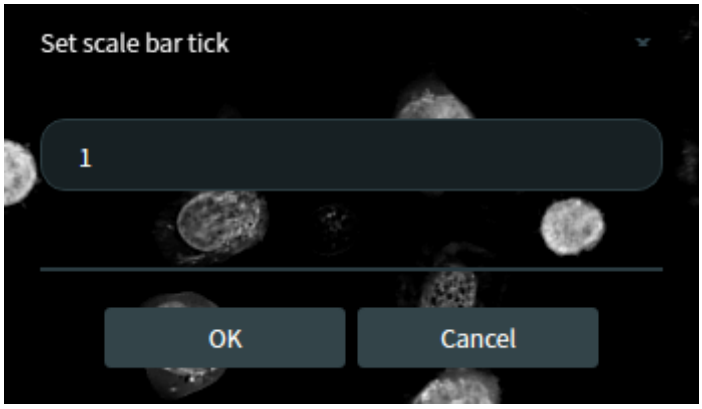
The **Set scale bar length** window appears, where you set the length of the scale bar as you choose.



Note: The length should be larger than the value given by the increment of the scale bar. If not, the scale length is set to the increment. See the **Set Increment** menu for setting the increment value.

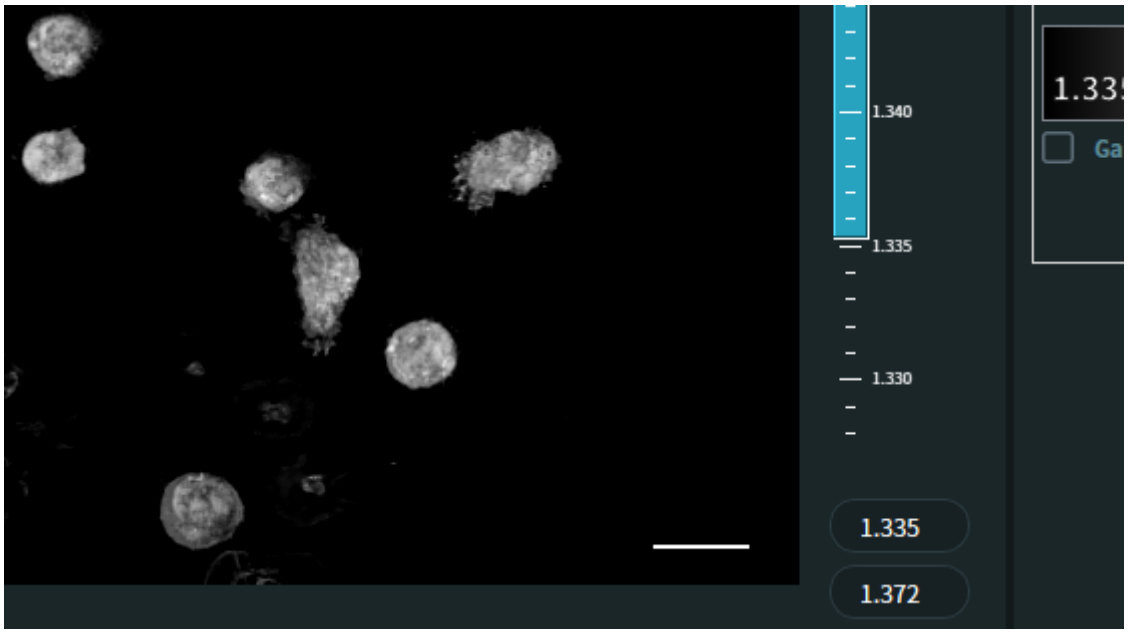
Set Increment

The **Set scale bar tick** window appears, where you set the increment of the length change of the scale bar.



Show/Hide Text

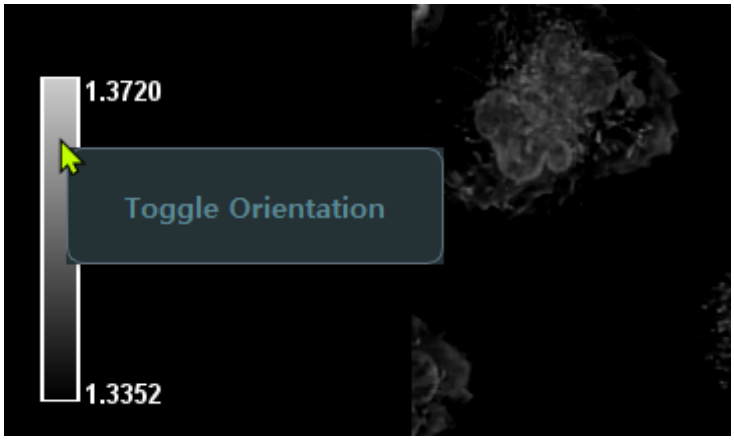
The number of the scale bar appears or disappears.



3.11 Changing the scalar bar settings

Prerequisite: You have checked the **Show Colormap Bar** menu at the **Annotation Tool** box.

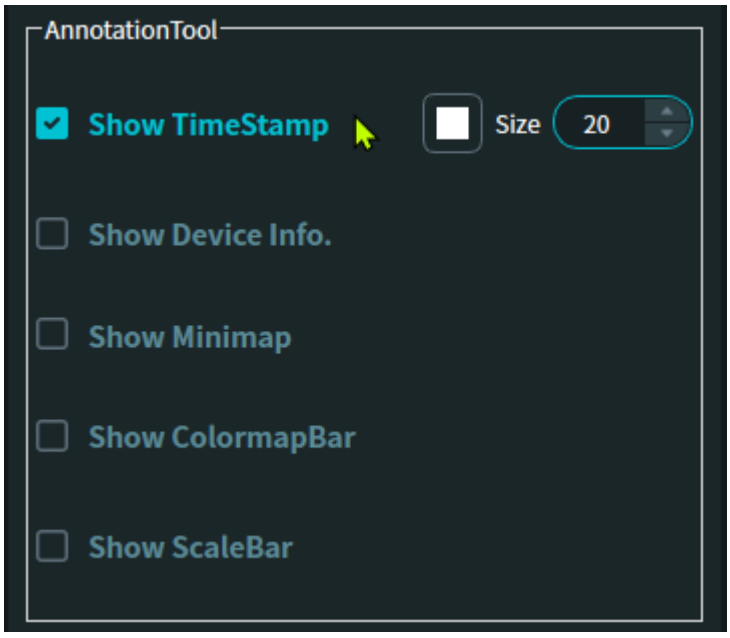
On the scalar bar, right-click the mouse button to show a pop-up menu for the scalar bar control as below. The menu shows the **Toggle Orientation** menu, which changes the direction of the scalar bar from the vertical direction to the horizontal one or vice versa.



3.12 Changing the time stamp settings

Prerequisite: You have checked the **Show Timestamp** menu at the **Annotation Tool** box.

At the **Show TimeStamp** menu on the **Annotation Tool** box, you can find the color indicating rectangle and the size of the text for the time stamp.



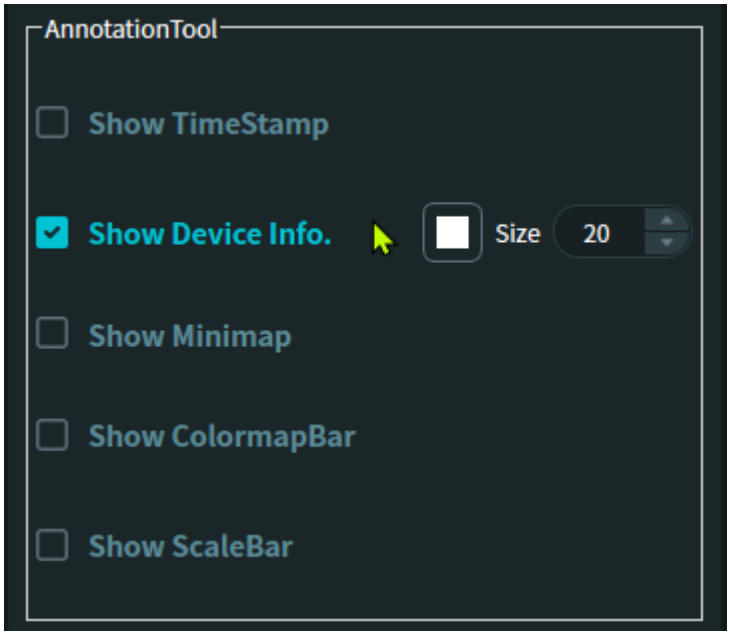
If you click on the rectangle, shown as white in the figure above, the **Select Color** window appears, where you can change the color of the time stamp.

If you change the number on the right text box, the size of the time stamp text will change accordingly.

3.13 Changing the sample information settings

Prerequisite: You have checked the **Show Device Info.** menu at the **Annotation Tool** box.

At the **Show Device Info.** menu on the **Annotation Tool** box, you can find the color indicating rectangle and the size of the text for the sample information.



If you click on the rectangle, shown as white in the figure above, the **Select Color** window appears, where you can change the color of the sample information.

If you change the number on the right text box, the size of the sample information text will change accordingly.

3.14 Changing the location of annotation items

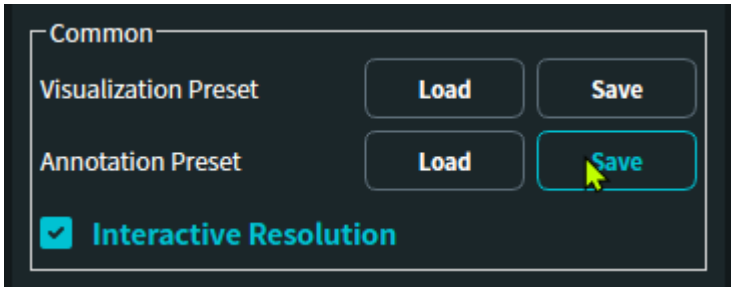
Prerequisite: You have checked relevant annotation items at the **Annotation Tool** box.

The location of the displayed annotation items can be adjusted by the user. Movable items are the scale bar, the scalar bar, and the time stamp. To move an item, drag-and-drop the item to the desired location while holding the wheel button of the mouse.

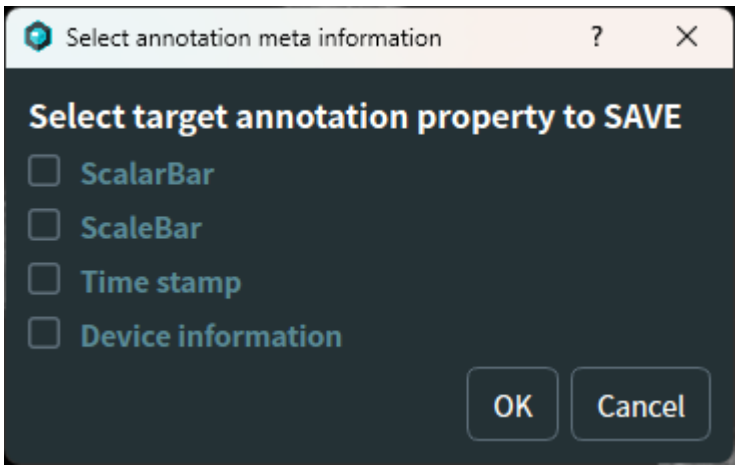
3.15 Saving/Loading annotation settings

You can save the settings for the annotation items as a file to apply them to other data images.

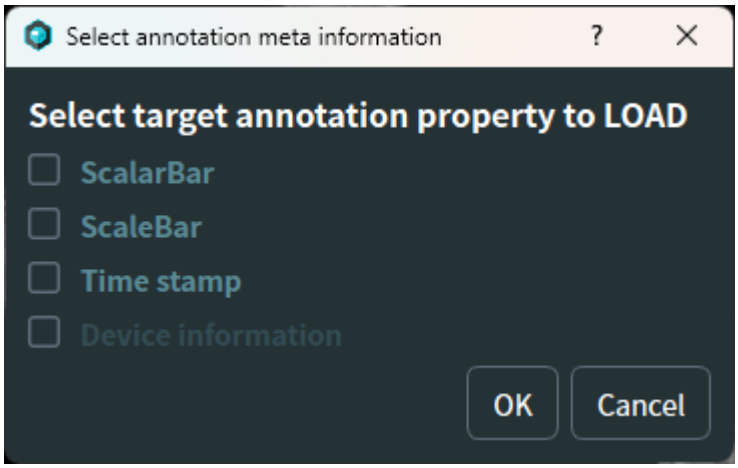
On the **Common** box of the **Visualization Control** panel, you can find two menus for saving and loading the settings.



To save the setting, press the **Save** button. A window appears as below, where you can choose the settings to be saved. After selecting desired items, press OK to designate the name and the location of the file to be saved.



To load a saved setting file, press the **Load** button to open the file. When the setting file is opened, you can choose settings for the annotation items to apply to the current displayed image. To apply the setting, press OK. If the setting file does not include all the annotation settings, those unavailable item settings are shown dark and not selectable.



3.16 Using measure tools

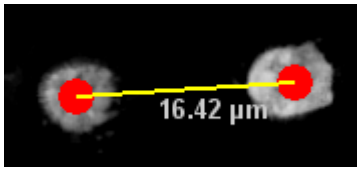
You can get simple measurement results using the **Measure Tools**.

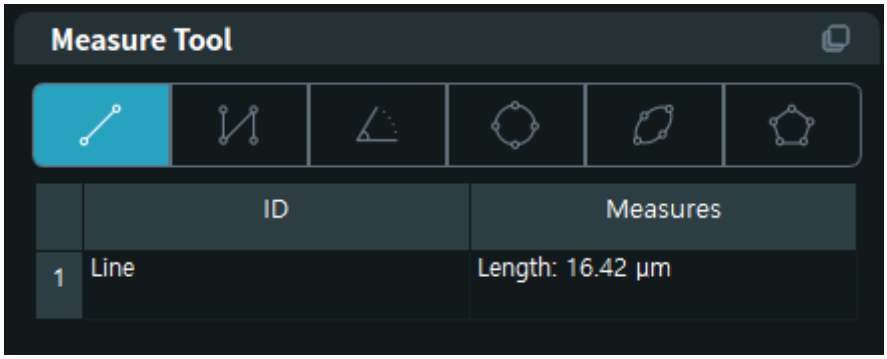
There are six measure elements in total, i.e., line, segmented line, angle, circle,

ellipse, and polygon. By drawing the measure elements on the displayed image, you can get simple geometric information about the sample.

Line

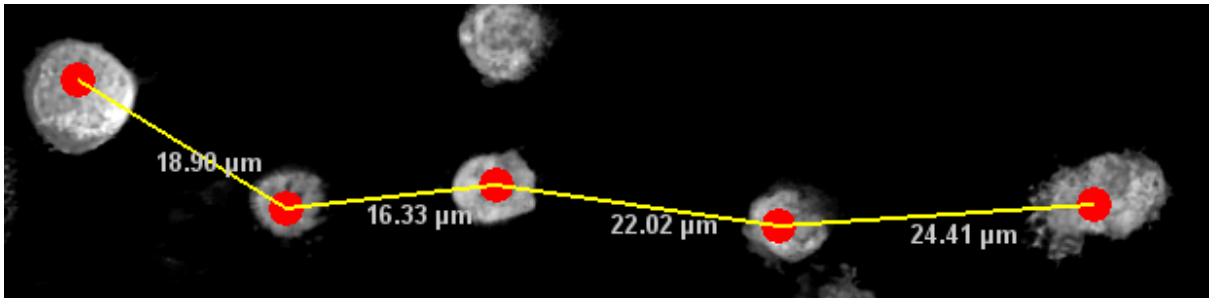
You can select two points on the image to get the distance between the two points. When drawn, the results are listed on the **Measure Tool** box.

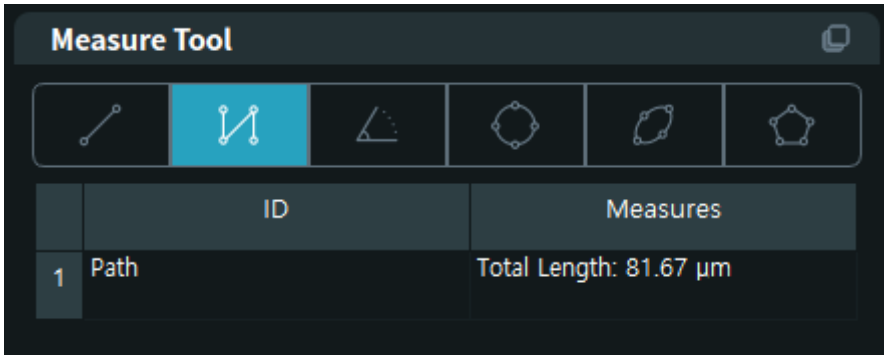




Segmented line

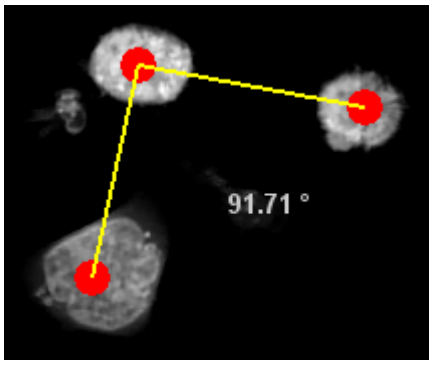
You can select multiple points on the image to get the distance between successive points in the line. For the last point, you need to double-click on the image instead of single-click. When drawn, the results are listed on the **Measure Tool** box.





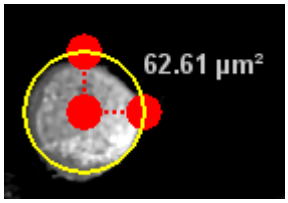
Angle

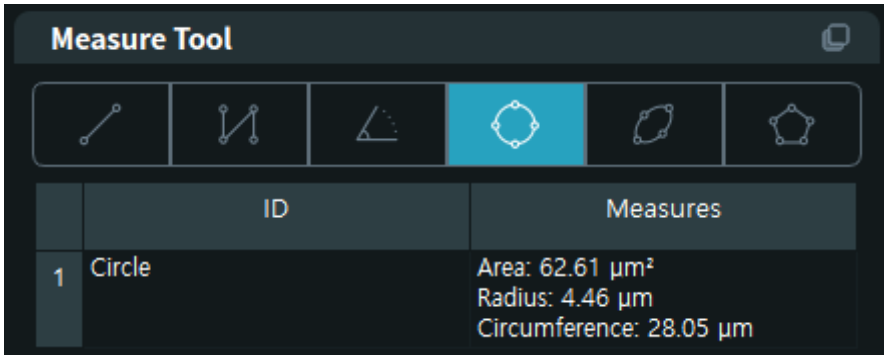
You can select three points on the image to get the angle made with two lines, where the angles are measured at the second point. When drawn, the results are listed on the **Measure Tool** box.



Circle

By drag-and-draw on the image, you can draw a circle. When drawn, the results are listed on the **Measure Tool** box.

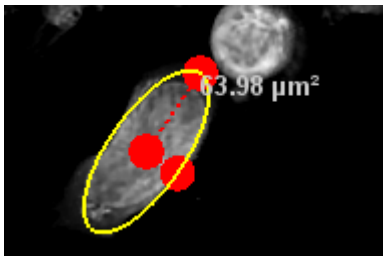


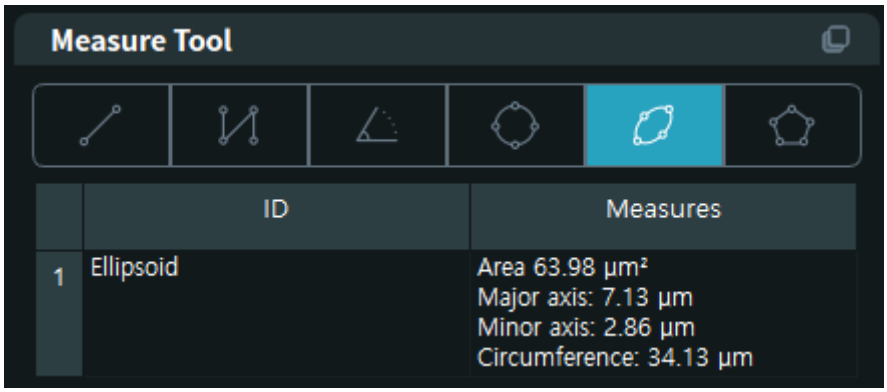


Ellipse

By drag-and-draw on the image, you can draw an ellipse, whose long and short axis

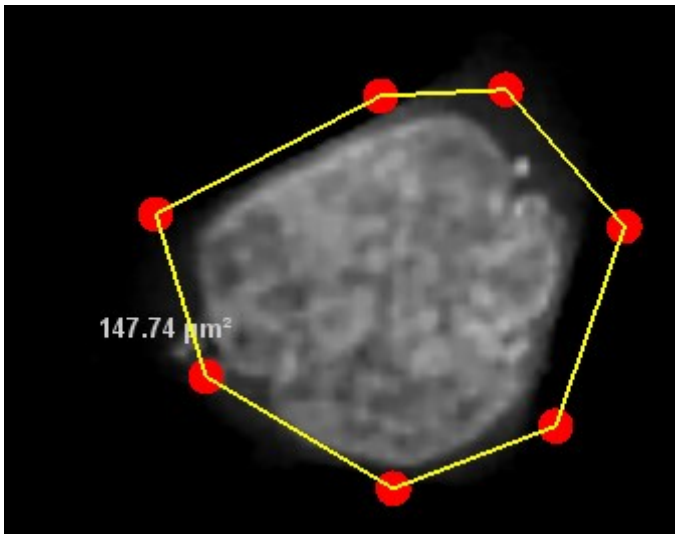
are parallel to the x and y directions, respectively. By drag-and-drop one of the two points on the rim, you can adjust the orientation of the ellipse to match the desired shape. When drawn, the results are listed on the **Measure Tool** box.





Polygon

Like the Segmented line, you can select multiple points on the image to get the distance between successive points in the line. For the last point, you need to double-click on the image instead of single-click to make it closed. When drawn, the results are listed on the **Measure Tool** box.



3.17 Deleting placed measure elements

When you want to delete undesired elements, you can delete those elements from the **Measure Tool** box.

From the list of the drawn measure elements, select those elements you want to delete by clicking the mouse cursor. To select multiple elements, hold the **Ctrl** button. Or you can press the **Select All** button to select all the drawn items.

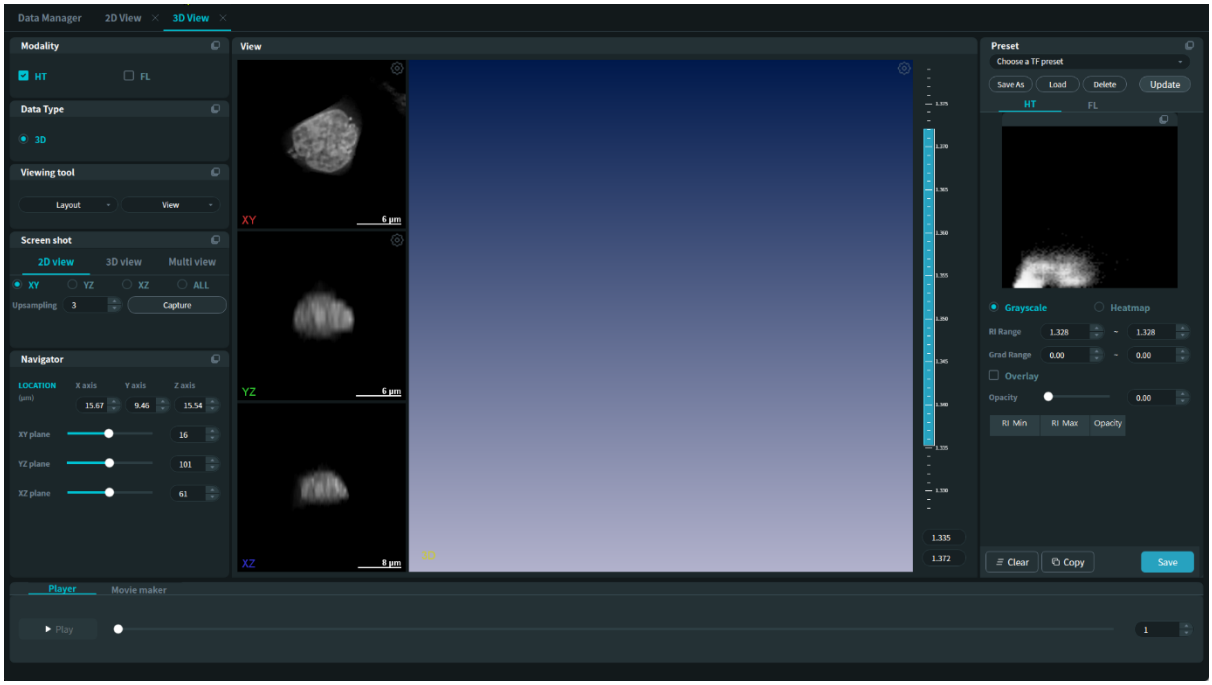
After finishing the selection, press the **Delete Selection** button.

3.18 Saving measurement results

After the measure elements are drawn, you can save the results in the CSV file format. Click the **Export** button at the bottom of the **Measure Tool** box to open a dialog window. All the listed results are saved regardless whether they are selected or not.

Chapter 4. 3D View

4.1 User Interface

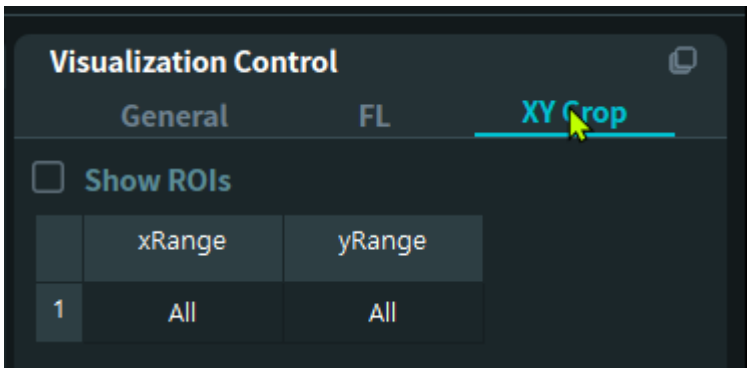


The **3D View** tab is divided into four main areas. In the left data control area, you can select the data channel and the data type as well as the position of the displayed region. The center View panel is used to display the data in various representations, i.e., the XY, YZ, XZ planes as well as the 3D volume rendered image. The right contrast control area you can define the region to be displayed on the 3D volume rendering.

4.2 Selecting Regions of Interest for 3D View

To view the TCF data in 3D, you need to define the ROI in the XY Crop menu for the 3D volume rendering.

To define the ROI, go to the XY Crop menu at the **Visualization Control** panel of the 2D View tab.



By default, the full area is registered as FOV unless the data is too big, i.e., less than

2000 × 2000 pixels in size.

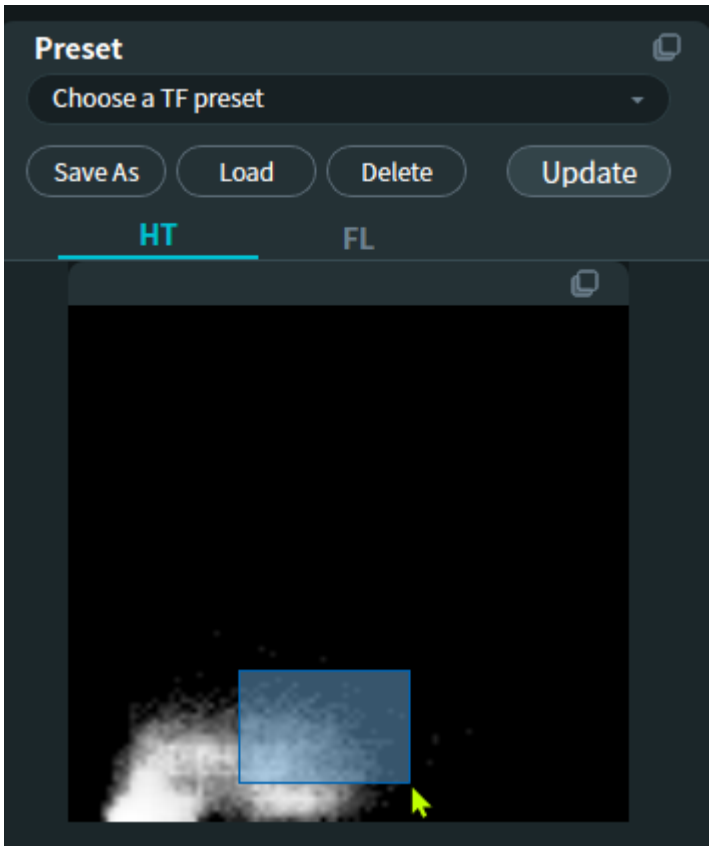
In case either the image is too large or you want to view a part of the data, you can designate a ROI for 3D rendering. To designate a ROI, press the **Add** button at the bottom of the XY Crop menu on the **Visualization Control** panel. When pressed, you can draw a rectangle on the image by drag-and-drop the mouse cursor. You can add multiple ROIs.

To view in 3D, select one of the registered ROIs and press the **Show ROI in 3D Visualizer** button at the bottom. Then, the **3D View** tab will open.

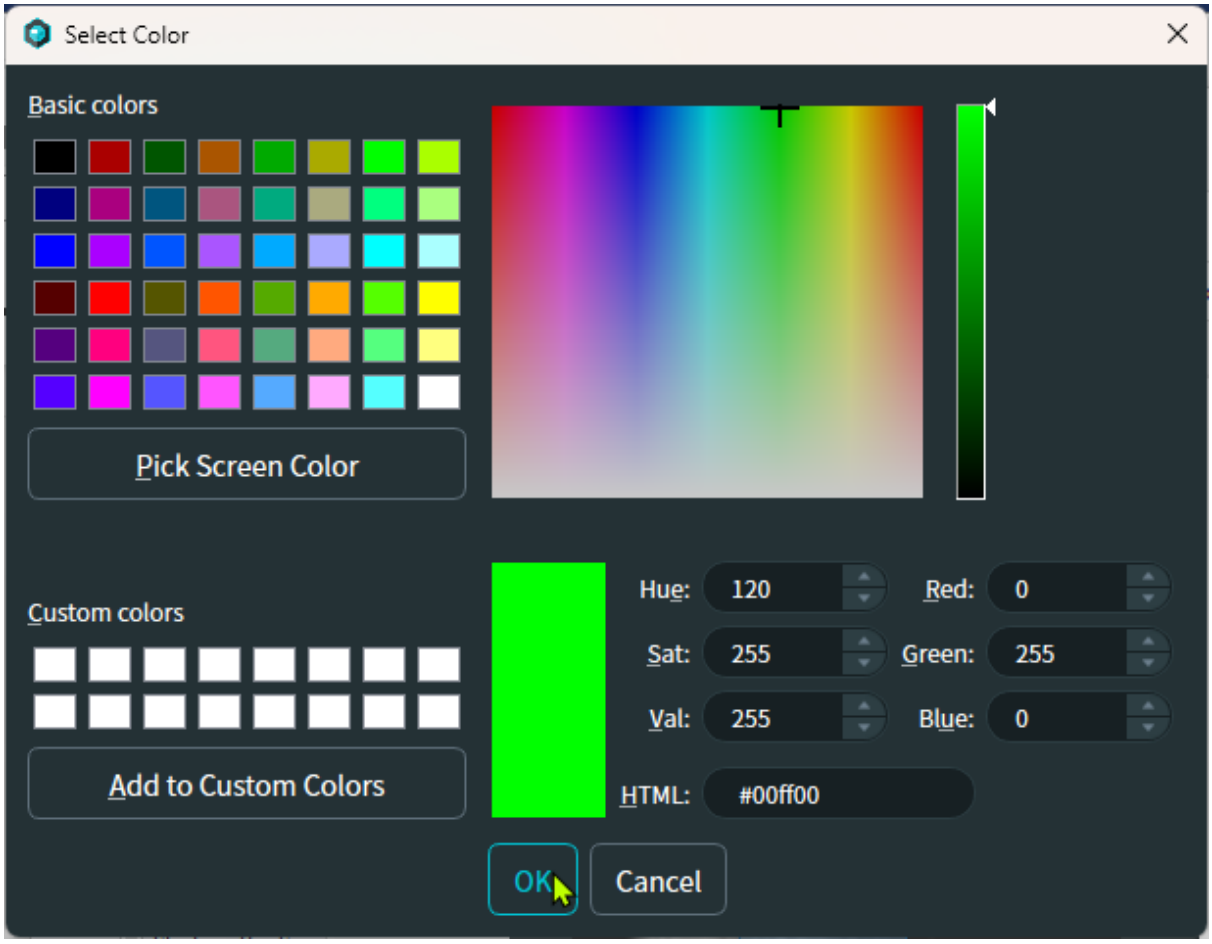
4.3 Making the initial 3D volume rendering

When a TCF data is opened in the **3D View** tab, the 3D rendering area in the **View** panel does not show anything but the 2D slice view from the XY, YZ, and XZ plane.

To make the data visible in the 3D rendering area, you need to choose voxels to be displayed via the **RI transfer function map** located on the right **Preset** area.



Draw a rectangle to define voxels to be displayed. The rectangle defines the range of the RI and the gradient of RI for those voxels. When the rectangle is drawn, the **Select Color** window appear.



Choose an appropriate color for the voxels and press OK. Corresponding voxels to the selected range will be visible on the **View** panel.

You can repeat the steps above to add multiple colors to the 3D rendering.

4.4 Changing the viewpoint in 3D

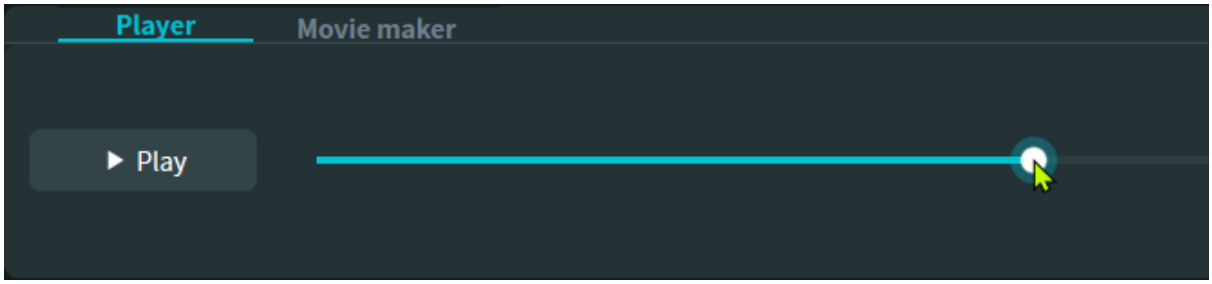
To zoom in or zoom out, you can scroll the mouse wheel down or up on the 3D rendering area of the **View** panel.

To move the viewpoint to a different angle, you can hold the left button of the mouse to change the viewpoint as you wish to.

To move the viewpoint in the parallel direction to the current viewpoint, you can hold the wheel button of the mouse to change the viewpoint.

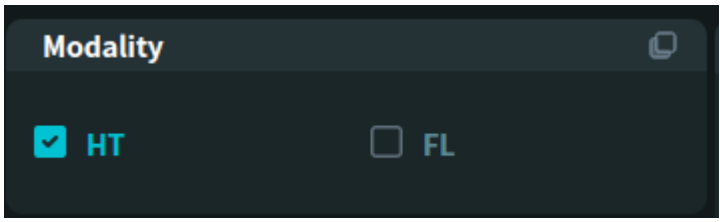
4.5 Changing the time point to view

If the TCF data is a time-lapse data file, you can change the time point. To change the time point to a desired time point, you can use the slider or the number box on the Timelapse control panel. You can also key in the number of the sequence of the time lapse data.



4.6 Selecting data channels

When a TCF data is opened in the 3D View, the displayed image on the View panel is from the HT channel.

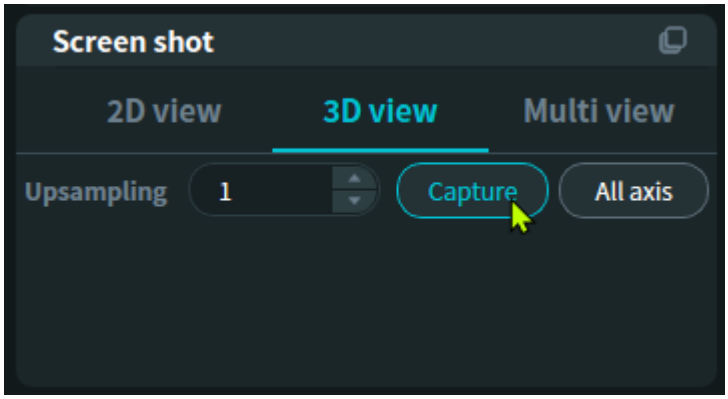


If an opened TCF file has data channels other than the HT channel, such as FL and BF, you can change the data type to be displayed in the **Modality** box. You can choose multiple data channels to make a blended image from them.

4.7 Saving displayed TCF file to images

After adjusting the image contrast in the View panel, you can save the displayed image in an image format.

At the **Screen shot** box on the **Data Control** panel, choose the **3D view** tab and press the **Capture** button to save the current 3D volume rendered image shown in the **View** panel. You can change the resolution of the saved image by changing the **Upsampling** parameter.



A dialog window will appear when the **Capture** button is pressed, where you can enter a file name for the image.

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