Tomocube

TomoAnalysis

Version 2.1

User Manual

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Chapter 1. Introduction

1.1 About TomoAnalysis

TomoAnalysis is advanced software optimized for the HT series, designed specifically for the analysis of refractive index (RI) tomograms obtained from Tomocube's Holotomography (HT) systems. With customizable analysis pipelines constructed from various processes, the software enables flexible and tailored analysis to meet diverse research needs. It provides precise, automated analysis of cellular and subcellular structures, ensuring accurate quantification of critical biophysical metrics.

Key features:

- **AI-Driven Segmentation:** Automates the segmentation of cellular structures, utilizing AI models for enhanced precision.
- **Refractive Index-Based Thresholding:** Accurately segments objects such as cells, organelles, and organoids based on RI values.
- **Morphological and Quantitative Analysis:** Measures key metrics like volume, surface area, length, dry mass, and concentration.
- **Batch Processing:** Allows efficient analysis of multiple datasets simultaneously.
- **3D Visualization:** Provides comprehensive 3D visual interpretation of structures.
- **Fluorescence-Integrated Analysis:** Enhances analysis by combining 3D fluorescence data with HT for broader application.

1.2 Installing TomoAnalysis

To install **TomoAnalysis** on a Windows PC, follow these steps.

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

NOTE: Any previously installed version of **TomoAnalysis** will be removed during the installation process.

1.3 Installing TomoAnalysis Extensions

TomoAnalysis uses a modular approach for data analysis, allowing the software to accommodate extended functions through the installation of **TomoAnalysis Extensions.**

Most of these extensions are related to GPU-based processing and AI analysis methods, and the list of available extensions will continue to grow as **TomoAnalysis** evolves.

To install one of the extensions provided by Tomocube, follow these steps:

1. Obtain the **TomoAnalysis Extension** installer provided by Tomocube.
2. Run the installer and follow the installation procedure.

NOTE: Install extensions on a drive other than the OS drive.

NOTE: The installation location of the **Extension** may be different from that of the **TomoAnalysis** executables.

1.4 Hardware Requirements

To run the **TomoAnalysis** properly, the workstation should meet the following system requirements.

Recommended specifications

- CPU: Intel i7-Series
- RAM: 64 GB
- Graphic card: Nvidia GeForce RTX 3070
- Operating system: Microsoft® Windows 10 or later (64-bit version)
- Screen: QHD (2560 × 1440)

Minimum specifications

- CPU: Multi-core CPU
- RAM: 32 GB
- Graphic card: Nvidia GeForce RTX 1070
- Operating system: Microsoft® Windows 7 (64-bit version)
- Screen: FHD (1920 × 1080)

* Graphic cards with a Radeon GPU or an internal GPU do not work properly

1.5 License

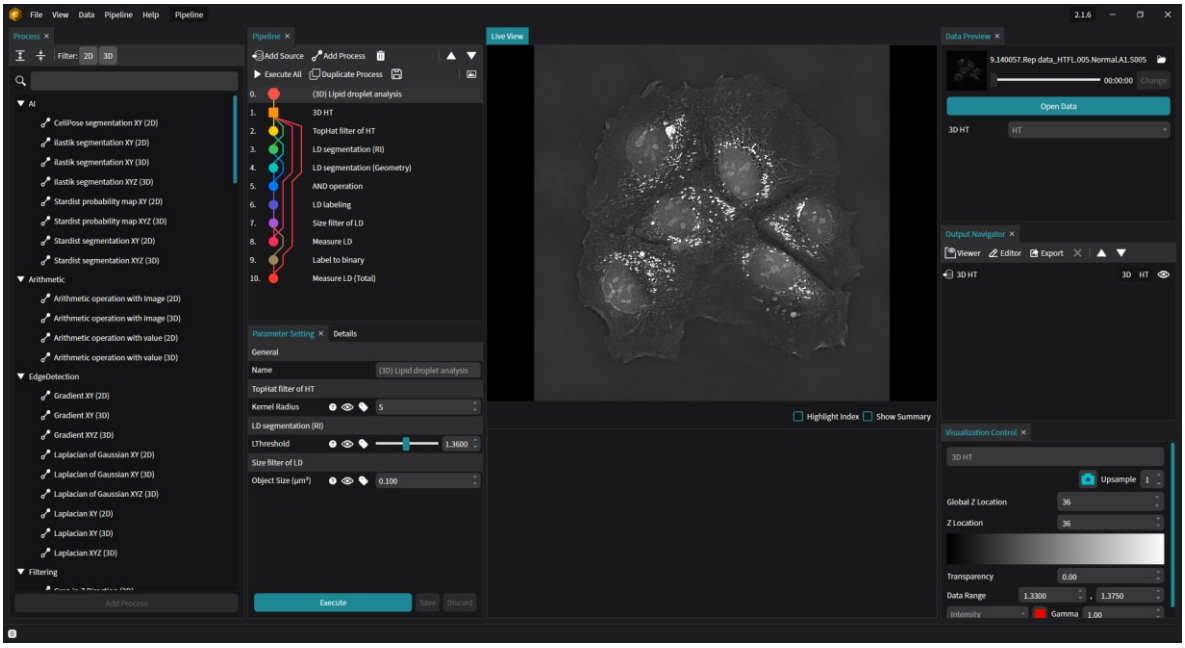
TomoAnalysis requires a license provided by Tomocube to run. Without a properly issued license, **TomoAnalysis** will terminate after displaying a warning indicating that no valid license was found. For any issues related to the license, please contact Tomocube.

NOTE: Before installing **TomoAnalysis**, if there are multiple Windows accounts on a single PC, be sure to select the desired user account (or administrator account) for both installation and license activation. If installation or activation occurs on the wrong account by mistake, please contact Tomocube for assistance.

Chapter 2. User Interface

2.1 Introduction

TomoAnalysis provides a flexible user interface that displays details of the analysis procedure, the image under analysis, and settings for processing and visualization. While the layout of the user interface may vary depending on the analysis perspective, its primary structure, including its panels, remains consistent across different perspectives.



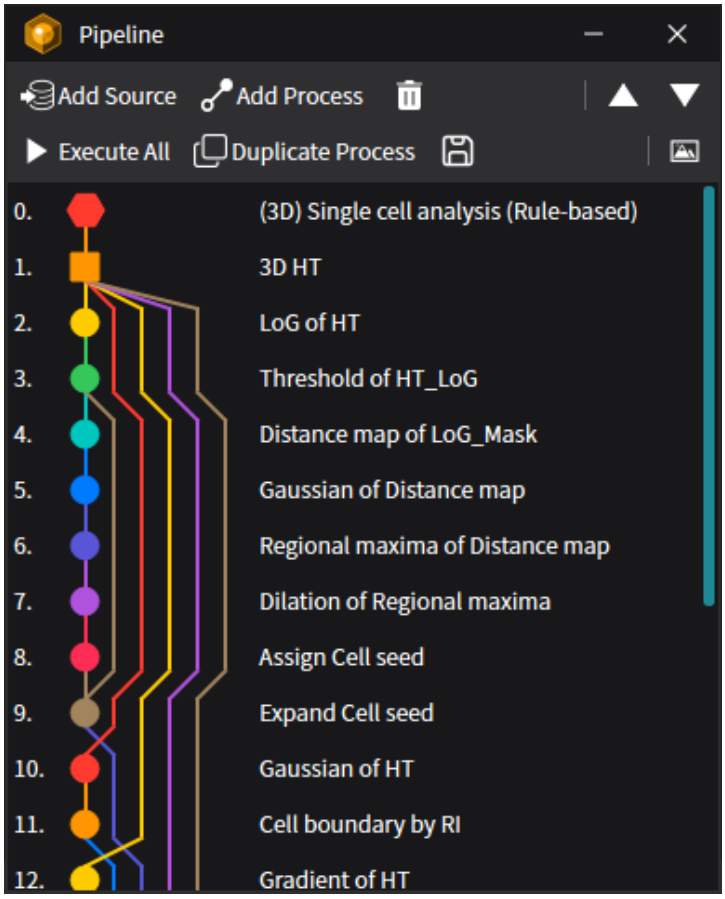
2.2 Panels

2.2.1. Pipeline Panel

The **Analysis Pipeline** is a series of analysis procedures used in **TomoAnalysis** for image analysis, where each procedure is modularized as a "Process." The **Pipeline** panel is available in both the open and create pipeline project.

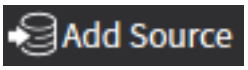
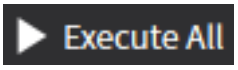
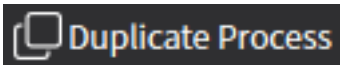
The **Pipeline** panel shows the structure of the pipeline used for the analysis. In this panel, users can identify and control each process used in the pipeline. When an analysis pipeline is loaded, the name of the pipeline and its processes are listed according to their execution order. The colored lines in the pipeline indicate the data connections between preceding and following processes.

If the user selects a process in the panel, its corresponding parameters are shown in the **Parameter Setting** panel.

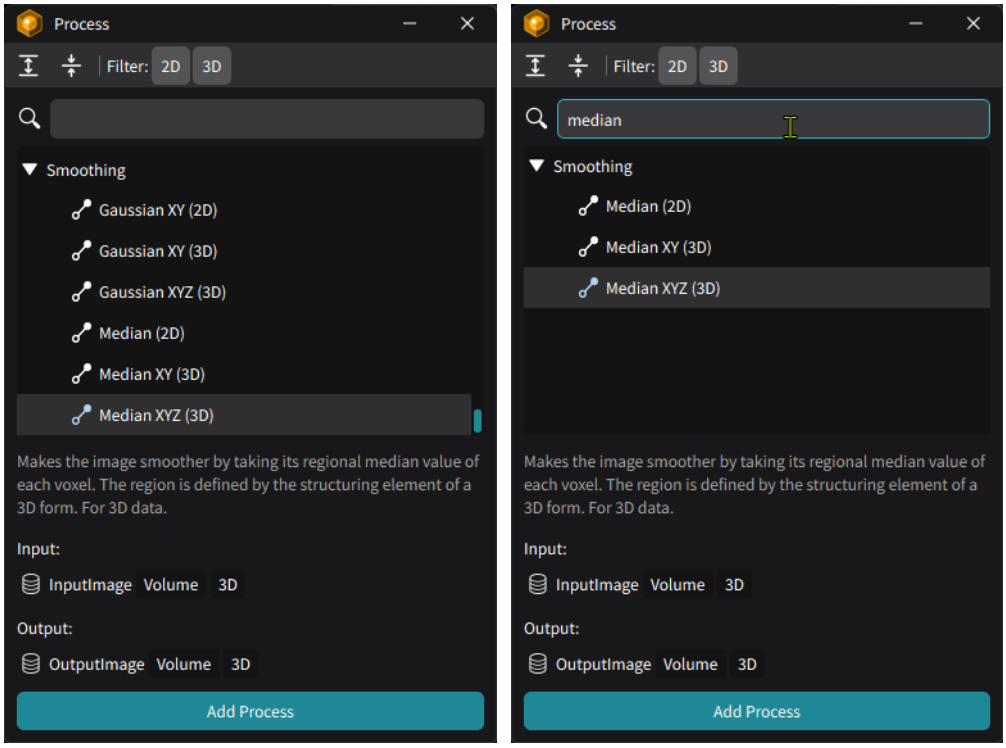


Editing Pipelines

In the **Pipeline Editor** mode, the user can edit the pipeline by adding or removing data sources and processes using the buttons at the top of the panel:

Features	**Icon**	**Description**
Add Source		Add an image channel to the pipeline
Add Process		Open the Process panel to add processes to the pipeline
Remove		Remove a selected source or process from the pipeline.
Move Up/Down		Change the execution order of a selected process.
Execute all		Execute all processes.
Duplicate process		Duplicate selected process.
Save pipeline		Save all the changes of pipeline.
Toggle diagram		Toggle diagram/icon view.

2.2.2. Process Panel



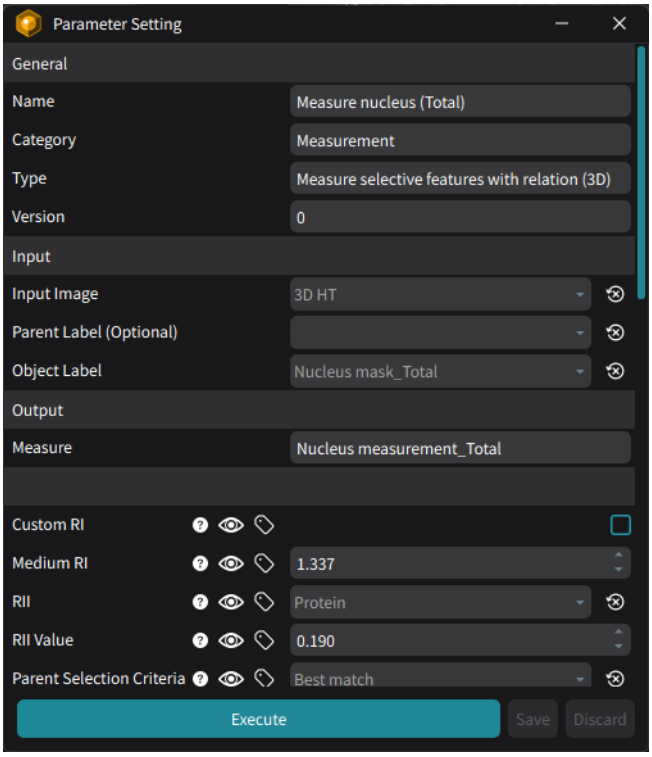
In **Pipeline Editor** mode, the **Process** panel opens when the **Add Process** button is selected in the **Pipeline** panel. The available processes are listed in the middle of the panel and can be filtered using the function at the top of the panel or searched by keyword at the bottom.

If a process is selected, its description is displayed at the bottom of the list, including information about input and output types as well as dimensions.

To add a process, click the **Add Process** button at the bottom after selecting a process from the list. The process will then appear in the **Pipeline** panel.

Options	**Icon**	**Function**
Expand/Collapse		Expand/Collapse Process group tree.
Filter 2D/3D		On/off filtering of Process list for 2D and 3D.

2.2.3. Parameter Setting Panel



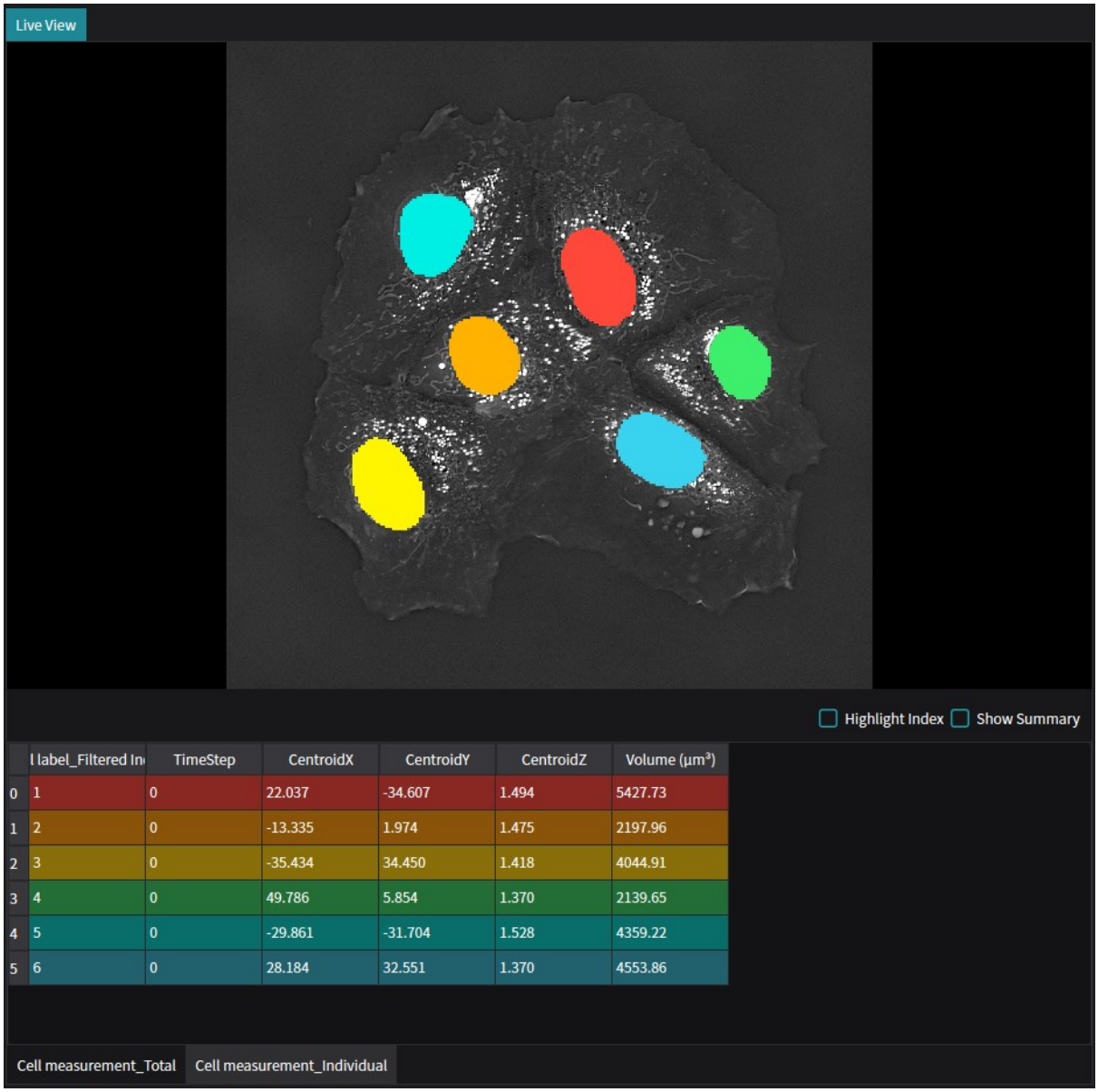
The **Parameter Setting** panel displays information about the selected process in the **Pipeline** panel, including the name and type of the process, the input and output data, and the parameters used for the process.

In the General section, information about the process itself, such as name, process category, and version, is displayed.

In the Input and Output sections, information about the input and output data is shown. Users can select or change the input data by choosing from the dropdown menu for each input selector. Depending on the process, the number of input data may vary. In the output section, the user can assign a name to the output data to be used by other processes in the pipeline.

In the subsequent sections, the parameters for executing the process are listed. Users can adjust each parameter to achieve optimal results. Refer to **Chapter 8. Processes** for more details on how they work and how to control the parameters.

2.2.4. Live View Panel



Outputs generated by processes in **TomoAnalysis** are categorized as Image, Mask, Label, or Measurement data. The first three data types can be visually reviewed in the top image viewer section of the **Live View** panel, while measurement table is displayed in the bottom section of the panel.

Image-Type Data

When a process outputs Image, Mask, or Label data, the result is loaded into the **Live View** panel and registered in the **Output Navigator** by its output name. Image contrast can be adjusted using the **Output Navigator** and **Visualization Control** panels, while image navigation within the **Live View** panel can be done using the following mouse actions:

Navigation	**Mouse Action**
Panning	Move the cursor while pressing the scroll (middle) button.
Zooming in/out	Move the cursor up/down while pressing the right-click button.
Move in Z direction	Scroll the mouse wheel up or down to move in the Z direction (for 3D data).
Reset view	Double-click the right button.

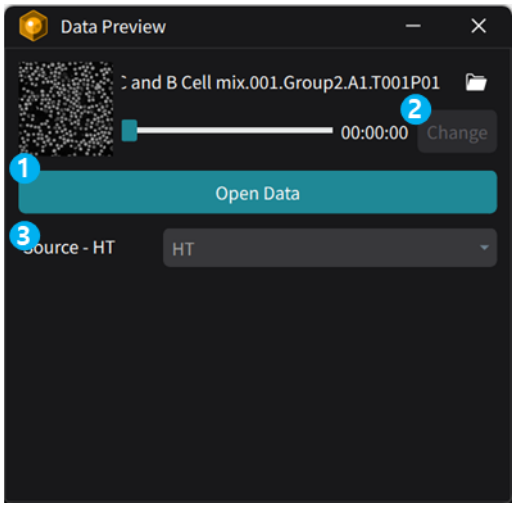
Measurement-Type Data

When a process produces a Measurement data output, it is displayed in a tabular format in the bottom section of the **Live View** panel and registered in the **Output Navigator** by its output name. If there are multiple Measurement data outputs, users can switch between them by clicking on the output names listed in the tab.

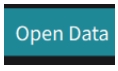
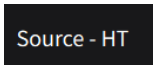
The Measurement data table can be sorted in ascending or descending order by clicking a column header. Each row of the Measurement data corresponds to the object label from which the measurement data was acquired. If the **Highlight Index** option is enabled, the corresponding label is highlighted in the viewer when the corresponding row in the table is selected, and vice versa.

When the **Show Summary** option is enabled, the table shows statistics such as sum, average, standard deviation, and median values. Users can uncheck this option to view results by label index.

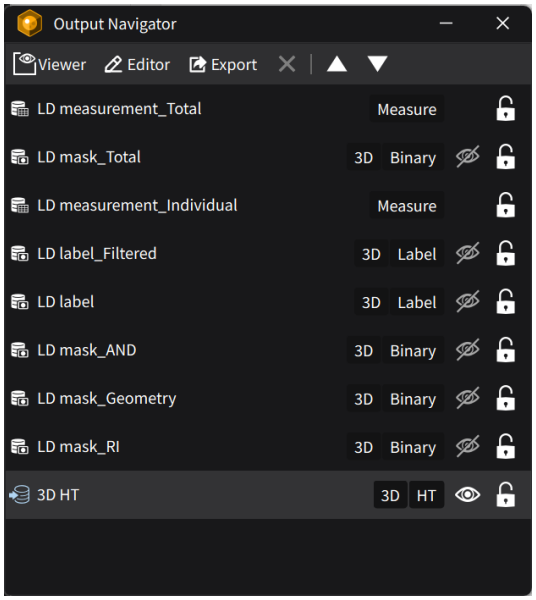
2.2.5. Data Preview Panel



The **Data Preview** panel is used to load a TCF file and select source channels for testing the pipeline and its processes during pipeline editing.

#	Features	Description
1		Open and load a TCF file.
2		Change the timepoint, in case the loaded TCF file is a timelapse.
3		Select a channel for the source.

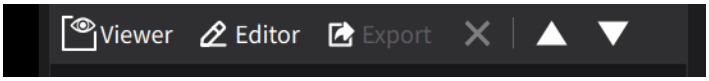
2.2.6. Output Navigator Panel



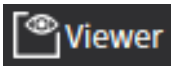
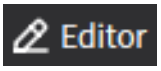
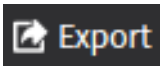
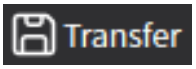
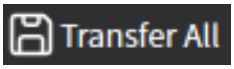
When a process in the pipeline is successfully executed, the generated output data is registered in the **Output Navigator** panel, along with the Source data of the pipeline. If the output data has not yet been registered, its name appears at the top of the list.

Once registered, the **Output Navigator** displays basic information about the data with icons and labels. Each row corresponds to one of the outputs produced by the processes in the pipeline, and the status of each can be identified from icons and tags in the same row.

Category	Icon, Tags	Description
Output Class		Image data (from TCF)
		Image data (from Process, Loaded from saved results)
		Mask/label data (from Process, Loaded from saved results)
		Mask/label data (from Process, Reprocessed)
		Measurement data (from Process, Loaded from saved results)
		Invalid data
Output Tag	**HT**	Data type is Holotomography
	FL	Data type is Fluorescence
	3D	Data dimension is 3D.
	2D	Data dimension is 2D.
	Binary	Data type is a binary mask.
	Label	Data type is a label mask.
	Measure	Data type is a measurement metric.
Output visibility		Data is visible on the Live View panel.
		Data is invisible on the Live View panel.
Output lock		Data is unlocked and can be reprocessed.
		Data is locked and ignored when the process is executed.



Additional data control

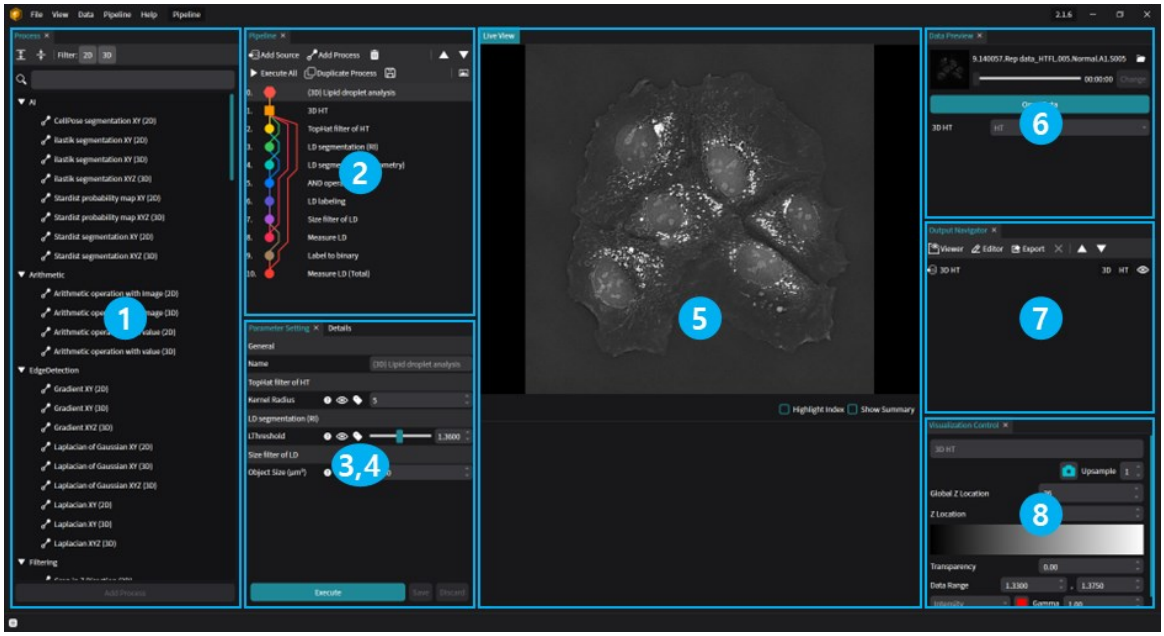
Features	Icon	Description
Viewer		Open a new viewer panel to render in a different viewing mode.
Editor		Open the Mask Editor to edit the selected binary/label mask data.
Export		Export the selected data.
Transfer		Transfer a selected mask data into the Output Manager after editing the mask data (Mask Editing mode only).
Transfer All		Transfer all the edited mask data into the Output Manager after editing (Mask Editing mode only).
Remove		Remove the Output from the panel.
Move up/down		Change the stacked order of the selected data up/downward.

2.2.7. Visualization Control Panel

The **Visualization Control** panel adjusts the contrast and transparency of selected output data in the **Output Navigator** panel. The panel layout changes according to the selected data output.

#	Option	Description
1	Capture	Capture a screenshot of the Live View panel.
2	Upsample	Set the scaling ratio of the screenshot.
3	Global Z Location	Set the absolute Z location of the displayed layer.
4	Z Location	Set the Z location of the selected output relative to itself.
5	Transparency	Adjust the transparency level of the selected output.
6	Data Range	Set the minimum and maximum intensity to be displayed.
7	Colormap	Set the colormap (Look up table) options.
8	Color	Set the color for Single Tone colormap.
9	Gamma	Adjust gamma for intensity control.

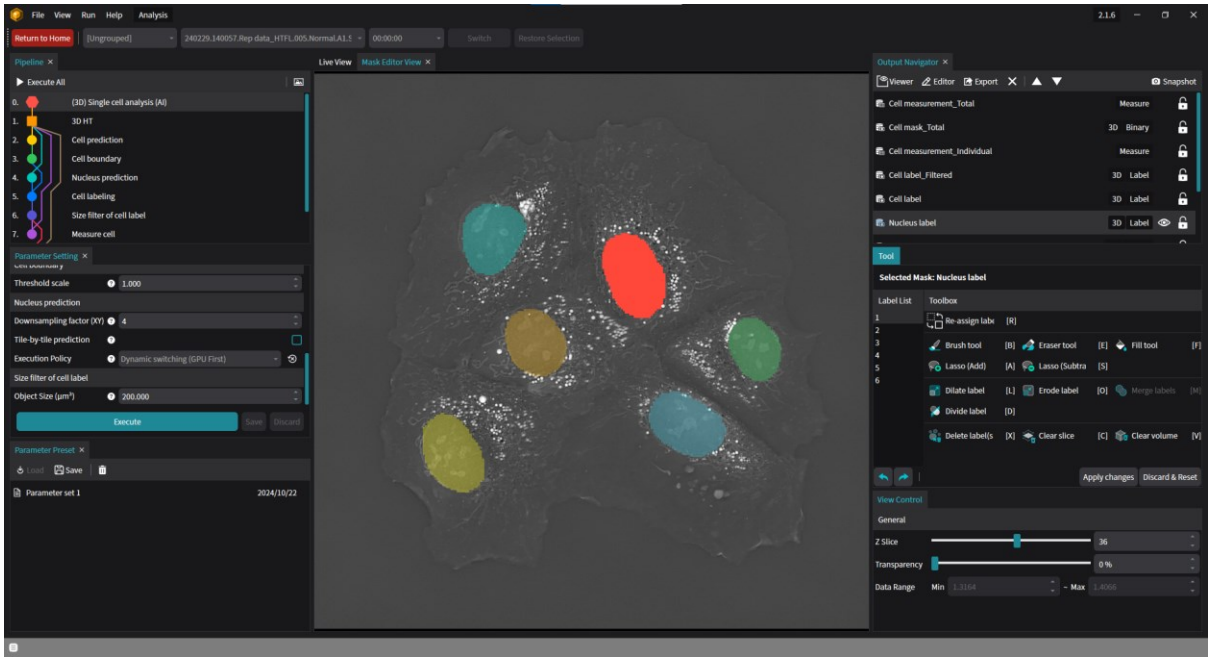
2.3 Pipeline Editor



The **Pipeline Editor** is used to create or modify an analysis pipeline. Most of the panels are shared with other modes, while the **Process** panel and the **Data Preview** panel are unique to this screen. Refer to the **UI section** of this chapter for detailed descriptions of each panel.

#	Panels	Description
1	Process	Lists available processes that can be added to the pipeline.
2	Pipeline	Shows the structure of the pipeline with input/output connections among processes.
3	Parameter Setting	Displays and controls the parameters for the selected process in the Pipeline panel.
4	Details	Shows detailed information about the pipeline.
5	Live View	Displays the image output data with various image contrast options.
6	Data Preview	Loads a TCF file for testing the selected process or the entire pipeline.
7	Output Navigator	Lists the output data produced by the process execution.
8	Visualization Control	Controls the image contrast shown in the Live View.

2.4 Mask Editor



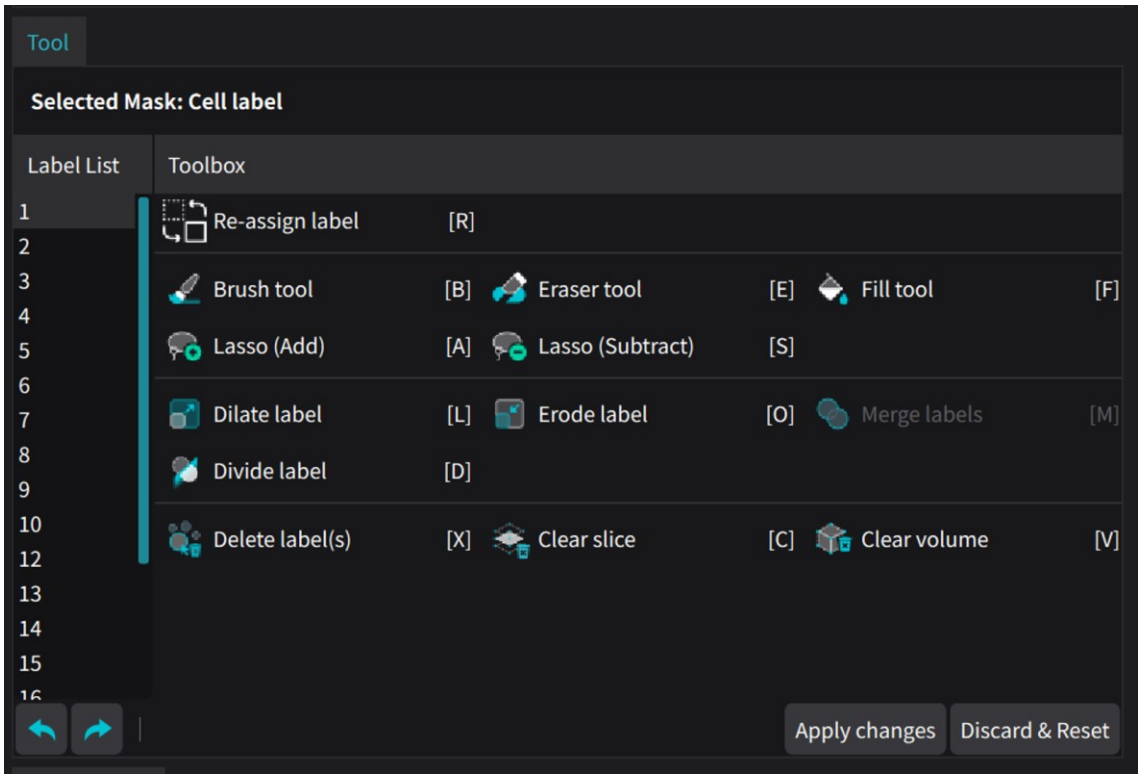
The **Mask Editor** is an add-on window that allows users to edit labels or binary masks registered in the **Output Navigator**. The **Mask Editor** can be loaded from both the **Pipeline Editor** and **Analysis Perspective** modes by pressing the Edit button in the **Output Navigator** panel.

When the **Mask Editor** is opened, it brings up three panels: the **Mask Editor View**, the **Tool** panel, and the **View Control** panel.

The **Mask Editor View** panel works similarly to the **Live View** panel, except that only one Label or Binary Mask output can be displayed along with the image data registered as the sources, such as HT and fluorescence (FL) data. The displayed Mask Output data can be selected from the **Output Navigator**.

The image contrast shown in the **Mask Editor View** can be controlled through the **View Control** panel. The **View Control** panel works similarly to the **Visualization Control** panel for the **Live View** panel, but with fewer options required for mask editing.

Tool Panel



The **Tool** panel gathers all the functions required to edit a mask in the **Mask Editor.** The buttons for these functions are grouped based on features that allow editing by pixels, objects, or regions.

Label List

The **Label List** on the left side shows the indices of the object labels in the selected label/binary mask output in the **Output Navigator.** Users can select a label or multiple labels from the **Label List** to apply the functions in the **Toolbox.**

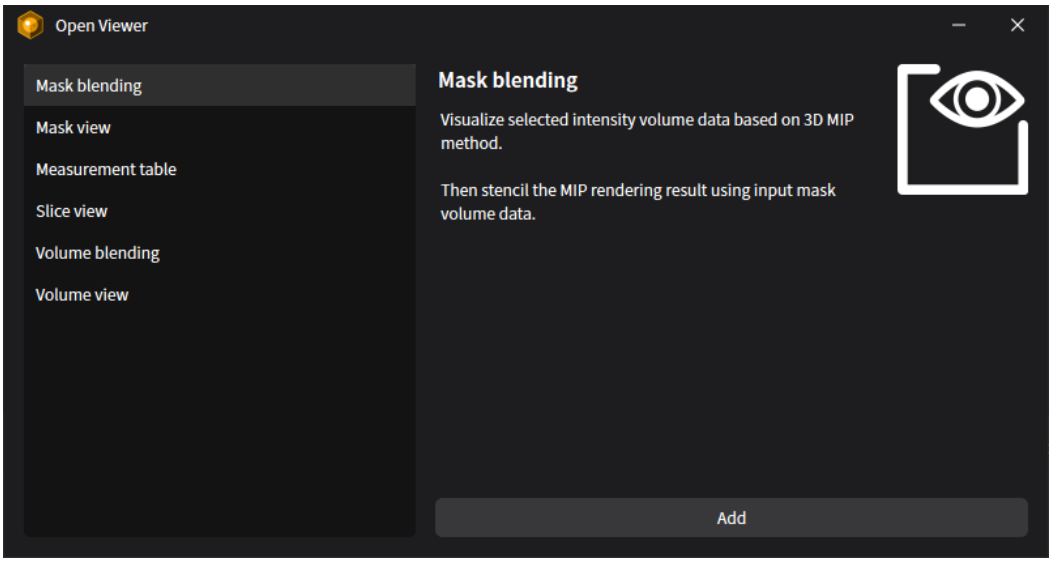
Toolbox

The **Toolbox** on the right side displays the functions that can be applied after selecting a label or labels from the **Label List.** When one of the functions is selected, the bottom side of the **Toolbox** changes according to the chosen function.

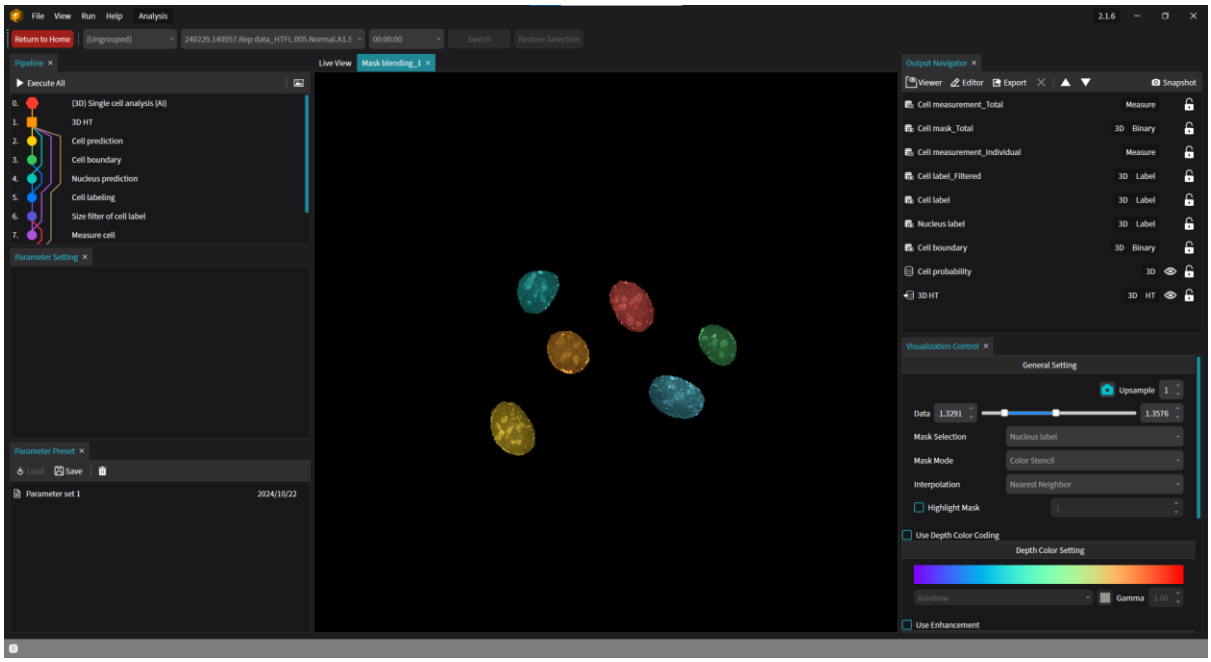
Icon	Tool	Shortcut	Description
	Re-assign label	[R]	Change the index for a selected label object (for Label Mask only)
	Brush	[B]	Draw spots to add pixels/voxels to a selected object
	Erase	[E]	Erase spots to remove pixels/voxels from a selected object
	Fill	[F]	Fill regions for a selected object
	Lasso (Add)	[A]	Draw a closed line to add regions to a selected object
	Lasso (Subtract)	[S]	Erase a closed line to remove regions from a selected object
	Dilate label	[L]	Dilate a selected object in the XY plane
	Erode label	[O]	Erose a selected object in the XY plane
	Merge labels	[M]	Combine two selected label objects into a single label object
	Divide label	[D]	Draw a free-drawn line to divide a selected label object into multiple separated label objects
	Delete label(s)	[X]	Remove selected label object(s)
	Clear slice	[C]	Remove all the label objects in the current XY plane
	Clear volume	[V]	Remove all the label objects in the whole volume (3D Mask only)

2.5 Viewers

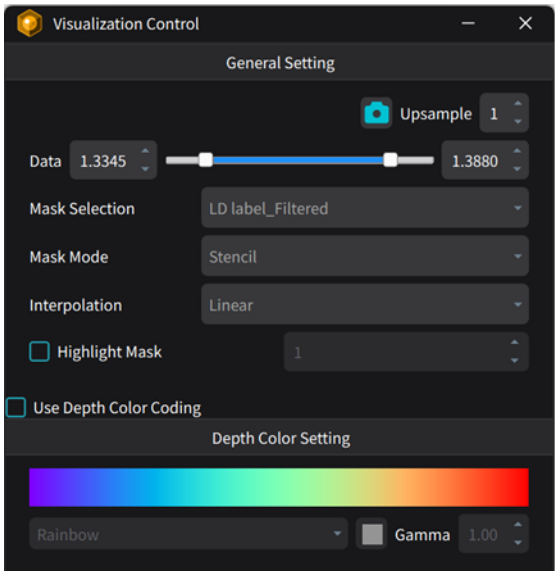
While the **Live View** panel is the default image display for data analysis in **TomoAnalysis**, users can add extra viewers to investigate the data using different rendering options by pressing the **Viewer** button in the **Output Navigator** panel.

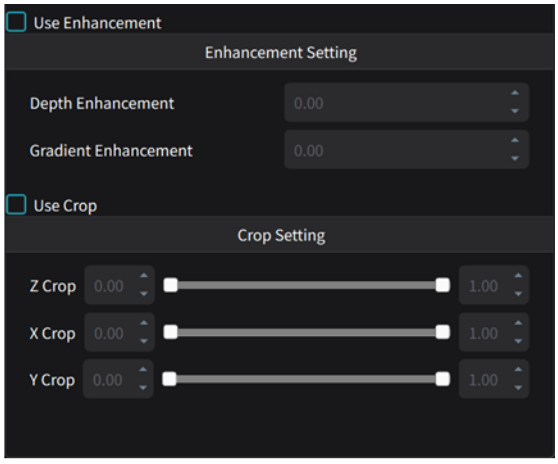


2.5.1. Mask Blending



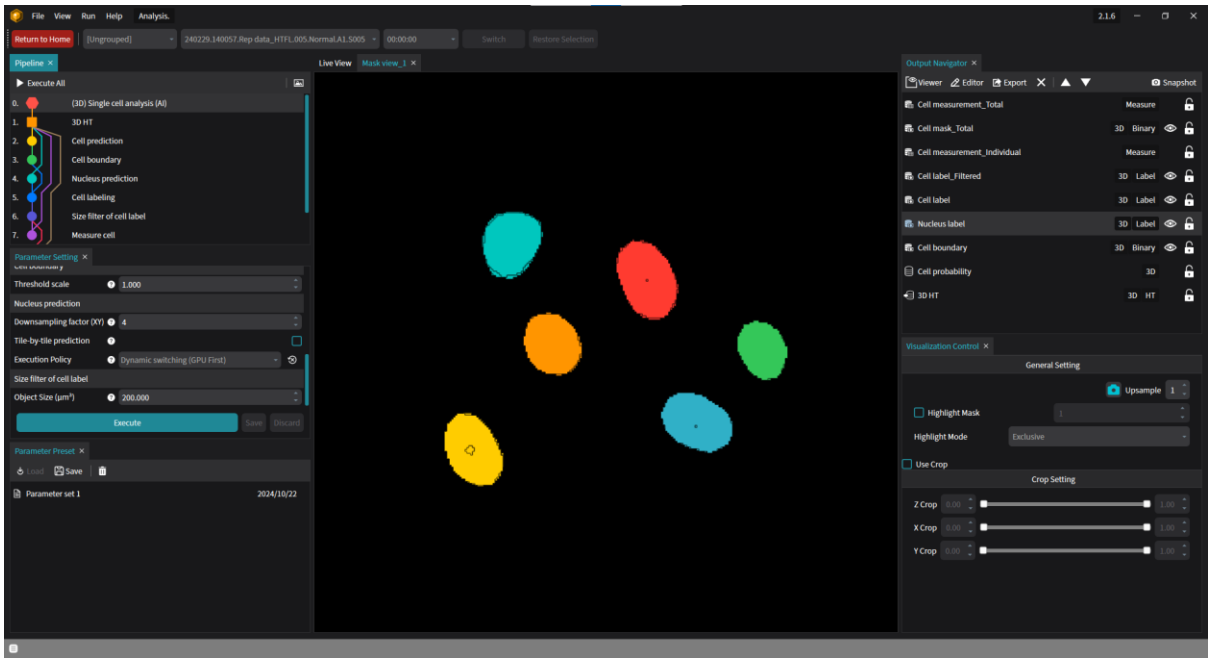
The **Mask Blending** viewer shows a blended image by selecting one image output data, including the source, and one mask output data. When a **Mask Blending** viewer is opened, the **Visualization Control** panel adjusts accordingly with options to control the blending.





Options	Description
Data	The range of the data selected in the Output Navigator
Mask Selection	The Mask Outputs in the Output Navigator which can be selected for mask blending
Mask Mode	The method of masking by the selected Mask
Interpolation	The rendering method for visualization
Highlight Mask	Enable/disable highlighting a chosen object index of the Mask Output
Use Depth Color-coding	Enable/disable the Depth Color-coding for the MIP rendering
Use Enhancement Setting	Enable/disable reflecting the depth effect and/or the gradient effect of the chosen Image data
Depth Enhancement	Ratio to apply the depth enhancement effect
Gradient Enhancement	Ratio to apply the gradient enhancement effect
Use Crop	Enable/disable cropping the X, Y, and Z range
Z Crop	A set Z range of the selected image data to be displayed in the viewer
X Crop	A set X range of the selected image data to be displayed in the viewer
Y Crop	A set Y range of the selected image data to be displayed in the viewer

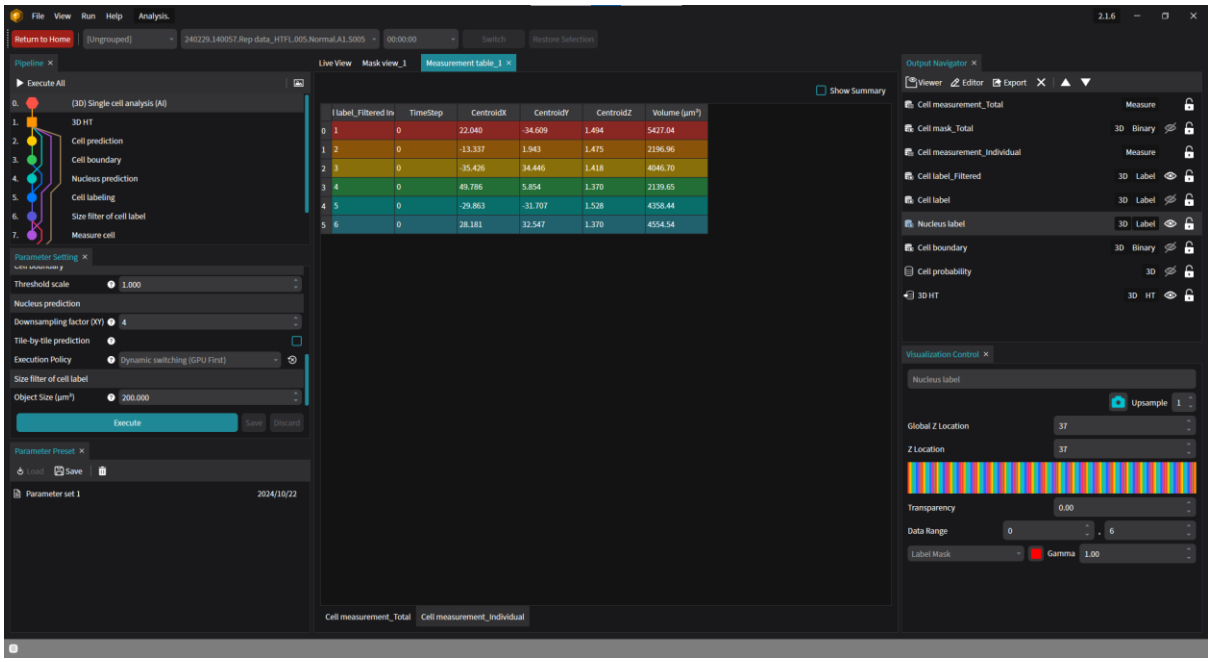
2.5.2. Mask View



The **Mask View** shows a rendered 3D image of the selected Mask output data from the **Output Navigator**. When a **Mask View** is opened, the **Visualization Control** panel adjusts accordingly with options to control the mask rendering.

Options	**Description**
Highlight Mask	Enables or disables highlighting of a chosen object index in the Mask Output.
Highlight Mode	The method used to highlight the selected object. In Exclusive mode, only the chosen masks are shown. In Use Transparency mode, the selected mask appears opaque, while other masks are shown as transparent.
Use Crop	Enables or disables cropping of the X, Y, and Z range.
Z Crop	A set Z range of the selected image data displayed in the viewer.
X Crop	A set X range of the selected image data displayed in the viewer.
Y Crop	A set Y range of the selected image data displayed in the viewer.

2.5.3. Measurement Table

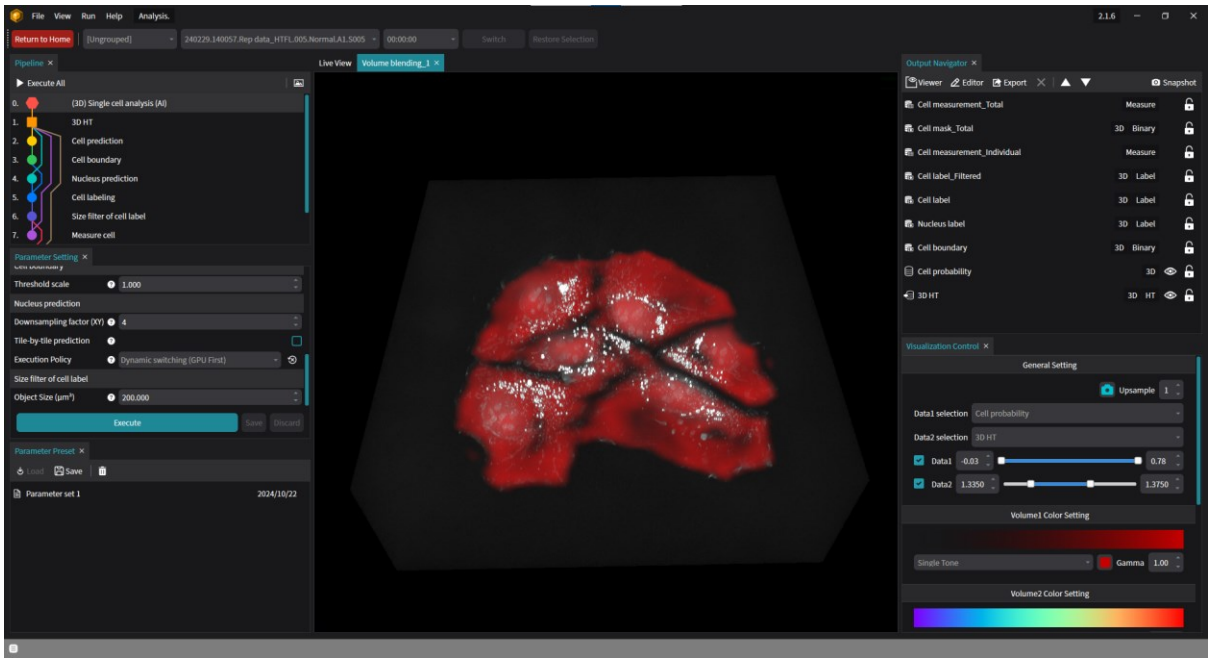


The **Measurement Table** viewer displays a duplicate of the measurement table panel shown in the **Live View** panel. The **Measurement Table** can be used to compare multiple measurement results on the same screen.

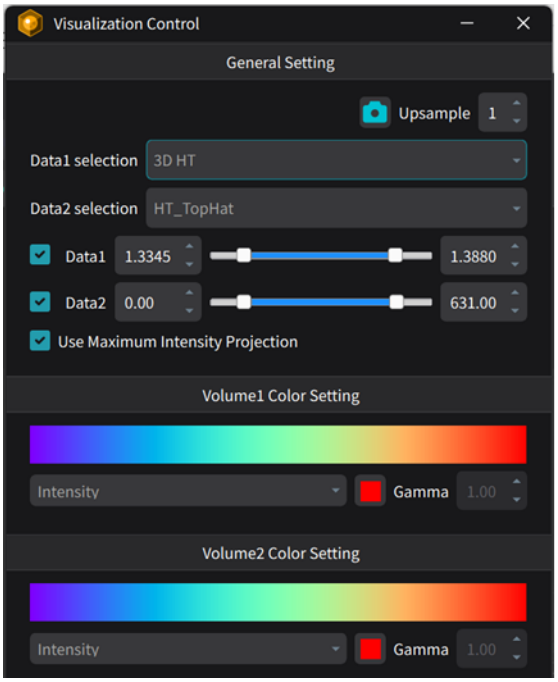
2.5.4. Slice View

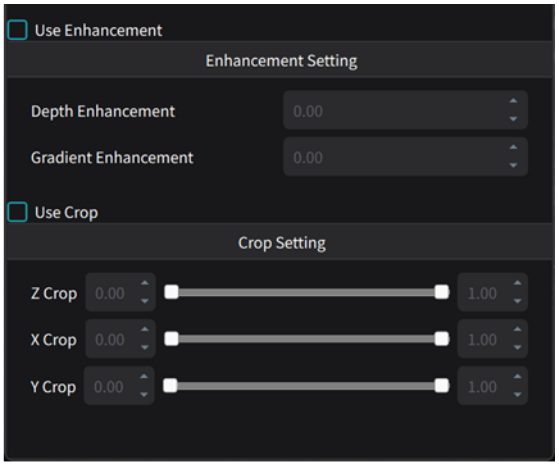
The **Slice View** duplicates the image layers shown in the **Live View**. The user interface for the **Slice View** is the same as the **Live View**, except for the **Snapshot** button on the **Output Manager**. When pressed, the **Snapshot** button fixes the data output in the **Output Navigator**, even if the processes in the pipeline are re-executed. This feature is intended to compare changes in the output made by process execution.

2.5.5. Volume Blending



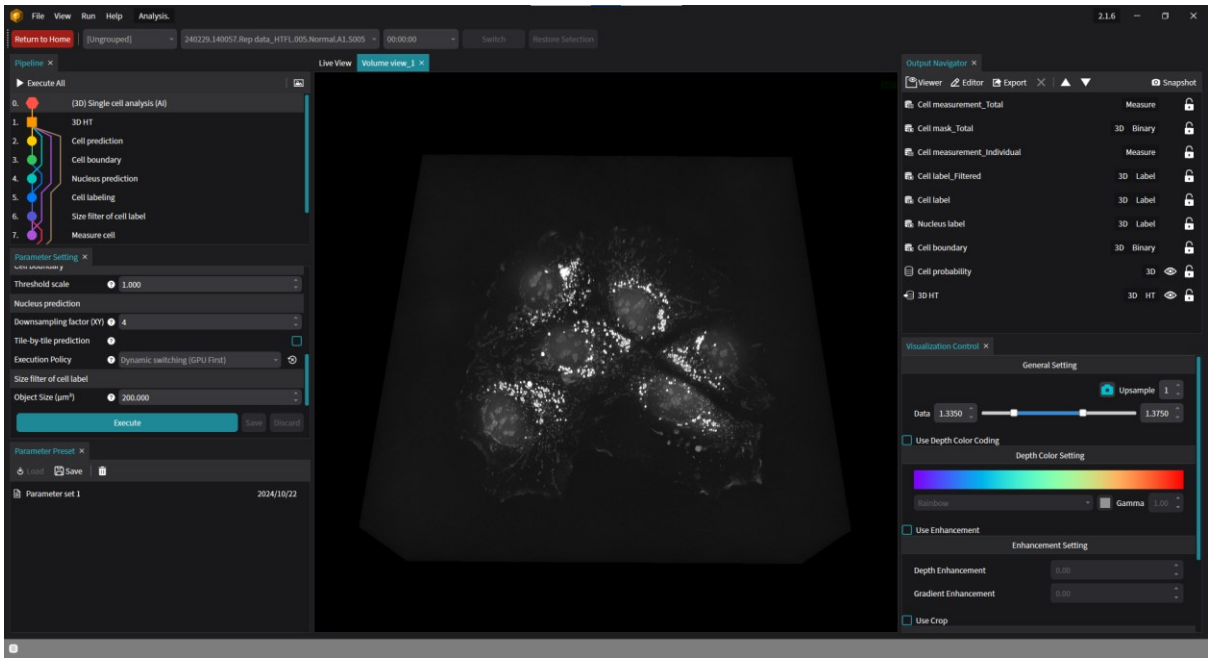
The **Volume Blending** panel visualizes two different 3D Image outputs from the **Output Navigator**. When a **Volume Blending** panel is opened, the **Visualization Control** panel adjusts with options to control the rendering. The two images for rendering are chosen from the **Visualization Control** panel, along with their respective image contrast settings.



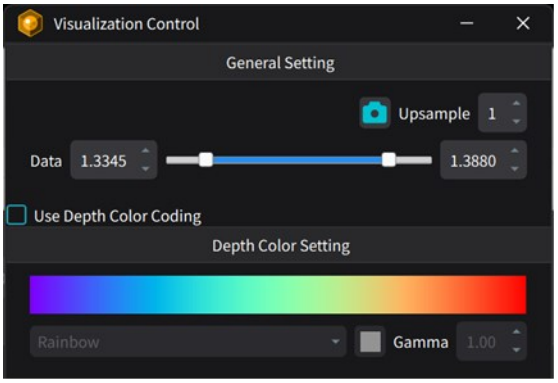


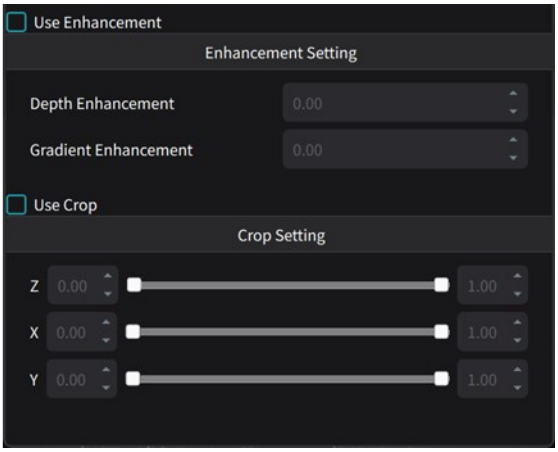
Options	Description
Data1 Selection	The first Image to be displayed
Data2 Selection	The second Image to be displayed
Data1	The range of Data1 for image contrast
Data2	The range of Data2 for image contrast
Use Maximum Intensity Projection	Option to render the images in maximum intensity projection
Volume1 Color Setting	Color selection for rendering Data1
Volume2 Color Setting	Color selection for rendering Data2
Use Enhancement Setting	Enable/disable reflecting the depth effect and/or the gradient effect of the chosen Image data
Depth Enhancement	Ratio to apply the depth enhancement effect
Gradient Enhancement	Ratio to apply the gradient enhancement effect
Use Crop	Enable/disable cropping the X, Y, and Z range
Z Crop	A set Z range of the selected image data to be displayed in the viewer
X Crop	A set X range of the selected image data to be displayed in the viewer
Y Crop	A set Y range of the selected image data to be displayed in the viewer

2.5.6. Volume View



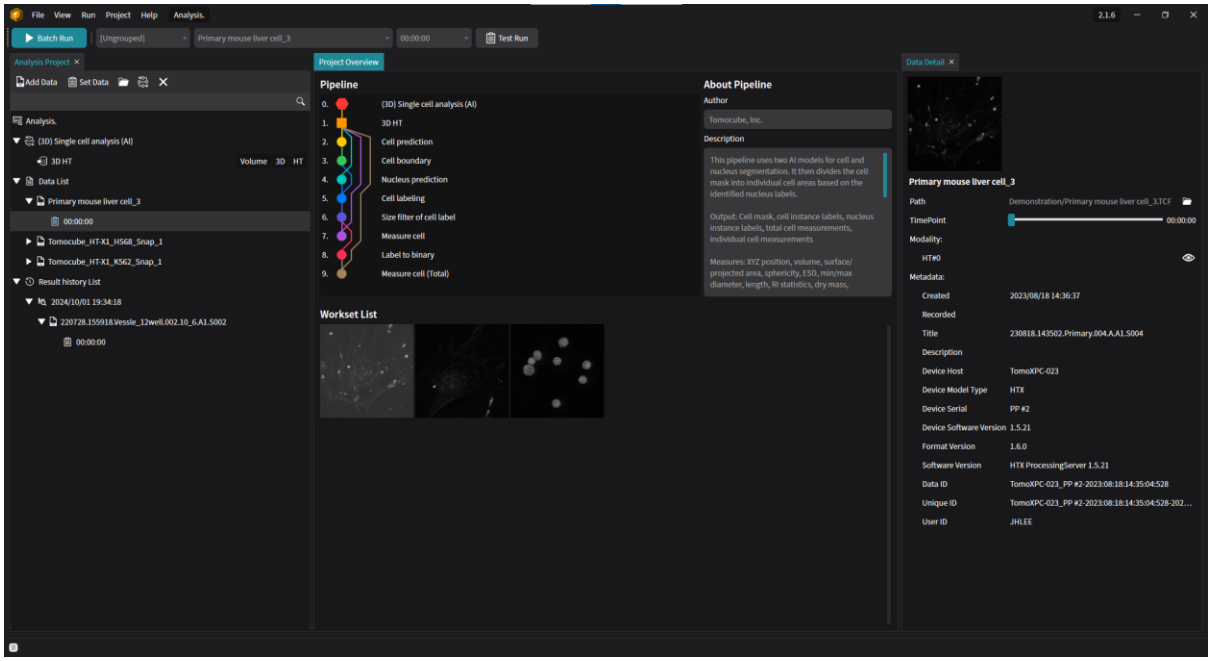
The **Volume View** shows a rendered 3D image of a selected image output from the **Output Navigator**. When a **Volume View** panel is opened, the **Visualization Control** panel adjusts accordingly with options to control the rendering.





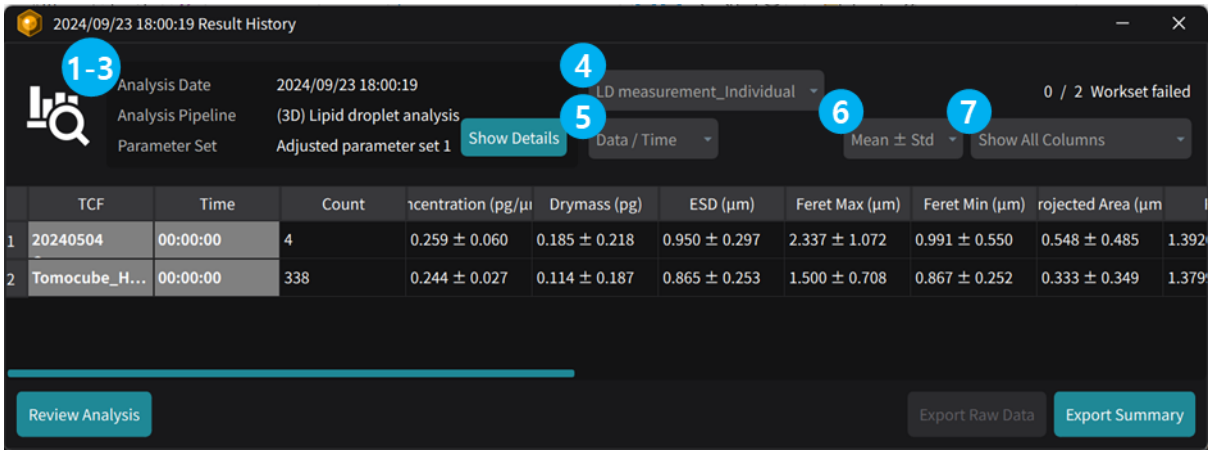
Options	Description
Data	The range of the data selected in the Output Navigator
Use Depth Color coding	Enable/disable the Depth Color-coding for the MIP rendering
Use Enhancement Setting	Enable/disable reflecting the depth effect and/or the gradient effect of the chosen image data
Depth Enhancement	Ratio to apply the depth enhancement effect
Gradient Enhancement	Ratio to apply the gradient enhancement effect
Use Crop	Enable/disable cropping the X, Y, and Z range
Z Crop	A set Z range of the selected image data to be displayed in the viewer
X Crop	A set X range of the selected image data to be displayed in the viewer
Y Crop	A set Y range of the selected image data to be displayed in the viewer

2.6 Analysis Perspective



In the **Analysis Perspective** mode, users can analyze multiple data files in a batch by running a selected analysis pipeline.

2.6.1. Dashboard

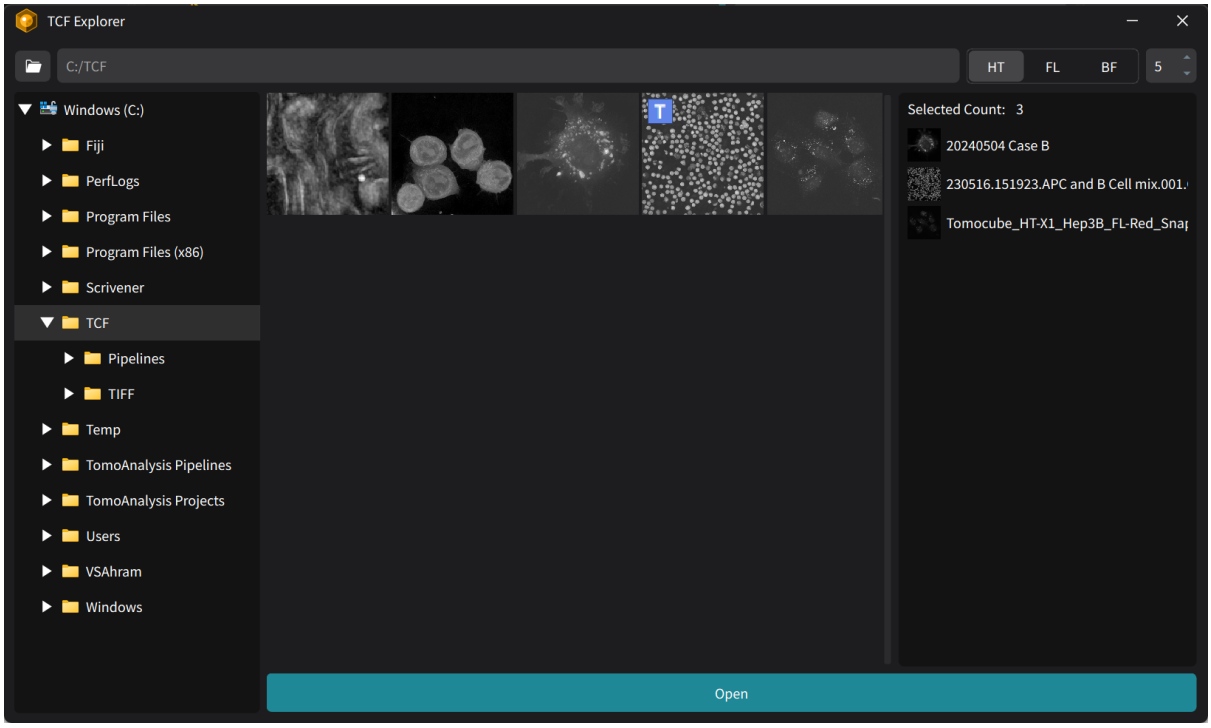


The dashboard displays the summarized results of a batch analysis record. It provides general information about the batch run as well as statistics for the worksets in the batch analysis.

#	Features	Description
1	Analysis Date	The time when the batch run was executed.
2	Analysis Pipeline	The pipeline used for the batch run analysis.
3	Parameter Set	The parameter set of the pipeline used in the batch run.
4	Measurement Option	The measurement data shown in the summary dashboard.
5	Grouping Option	The method used for grouping to calculate the statistics of the results.
6	Statistics Display Option	The type of statistics to display.
7	Empty Cell Option	Option to show empty slots.

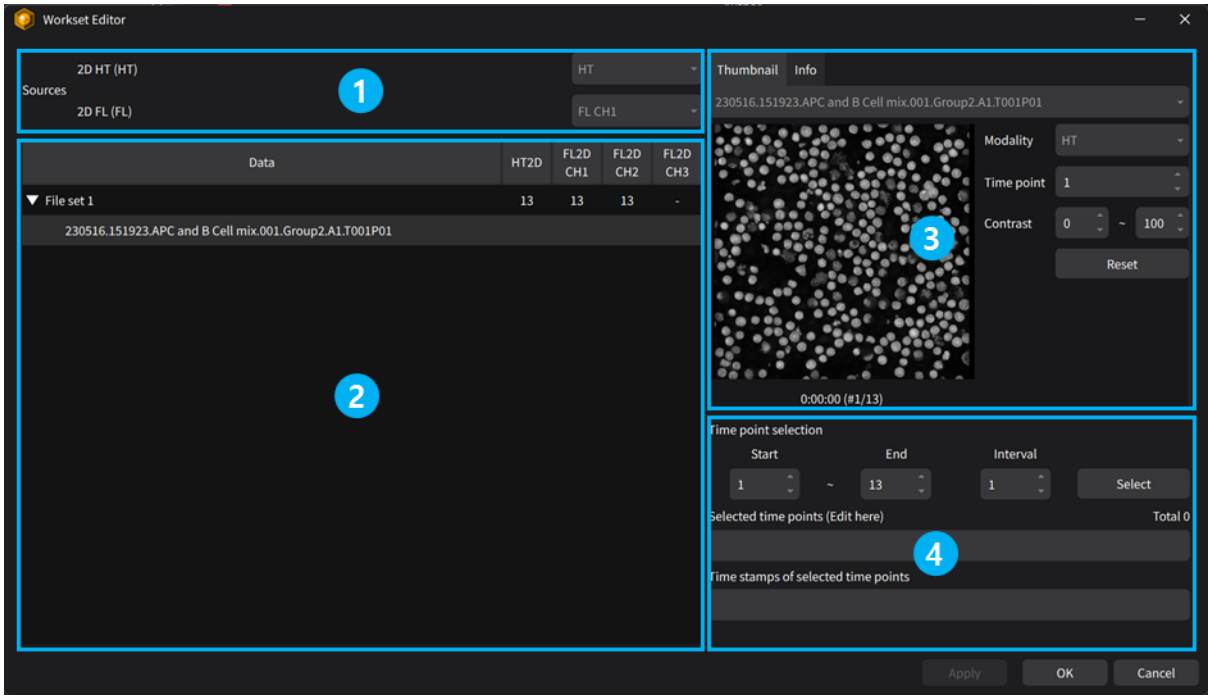
Depending on the UI option, the summary table will be updated accordingly.

2.6.2. TCF Explorer



The **TCF Explorer** is used to select TCF files from storage so that they can be loaded into the **Data List**. To select a folder, use the Windows tree on the left side. When the folder is selected, the TCF files in that folder will be displayed in the middle panel. The right panel lists the selected TCF files.

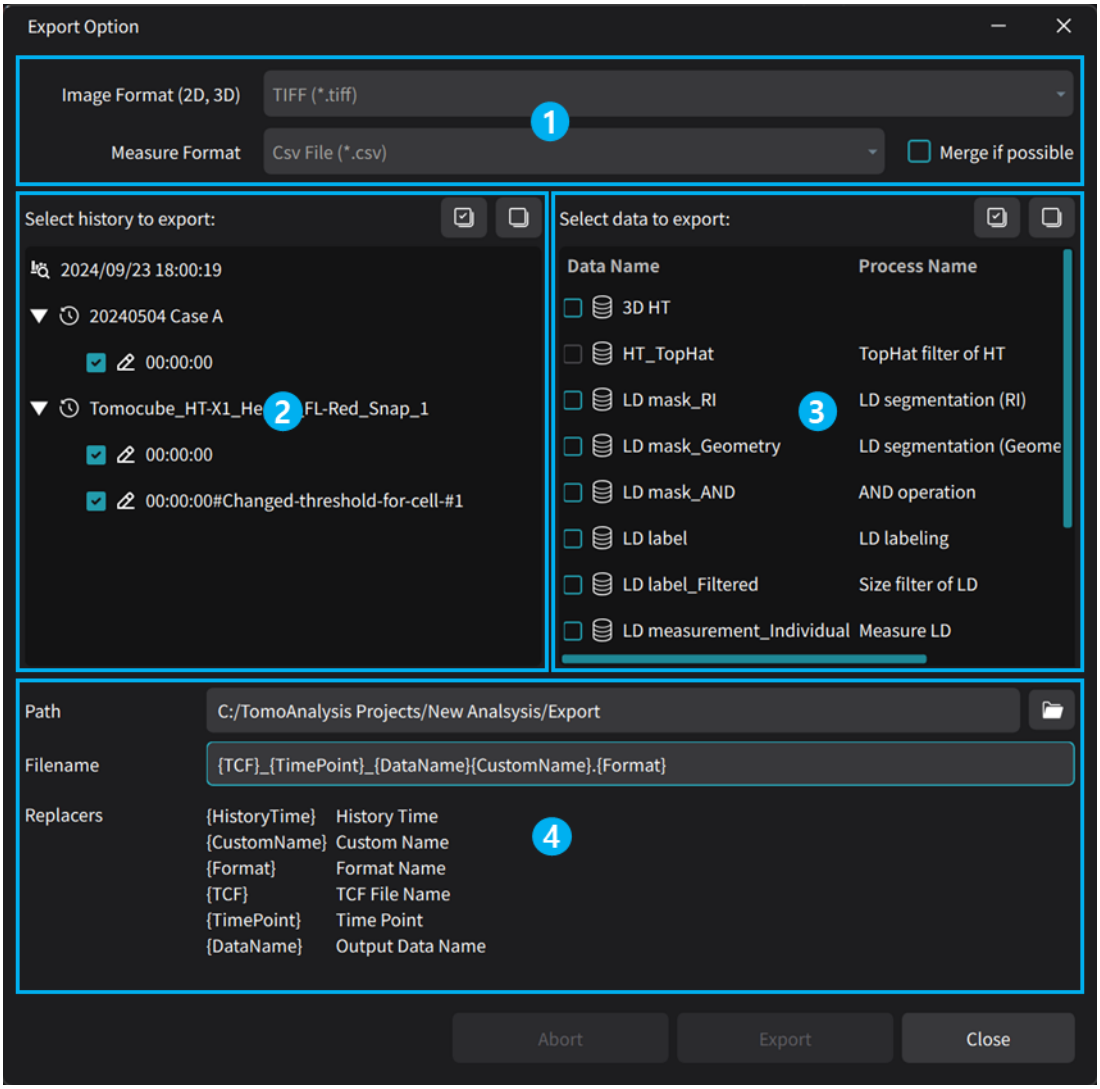
2.6.3. Workset Editor



The **Workset Editor** is used to assign time points and data channels of TCF files in the **Data List** to the pipeline.

#	Panels	Description
1	Source selection	Assign the channels of TCF files to the source of the pipeline.
2	File table	Select the files for time point selection.
3	Thumbnail	Thumbnail view of the selected file for time point selection.
4	Time point selection	Assign the time point for the selected TCF files.

2.6.4. Export Manager



The **Export Option** window is for saving analysis results to storage so they can be used by other software for further analysis.

#	Panels	Description
1	Format setting	Set the file format for exporting image type data and measurement data.
2	Data selection	Select the data for export.
3	Output selection	Select the outputs for export.
4	Path & Name setting	Select the folder path for the export, and set the filename rules to be used for the exported data.

2.7 Test Run Mode and Review Mode

The **Test Run** mode and **Review** mode share the same user interface and are similar to the Pipeline Editor mode.

The **Test Run** mode is used to test the pipeline for analysis and determine the appropriate parameter set for the analysis pipeline, while the **Review** mode is used to evaluate the analysis results from the pipeline and re-analyze the results if necessary. Most of the panels are shared with other modes; however, the **Parameter Preset** panel is unique to this screen. Please refer to **Chapter 2.2 Panels**, for detailed descriptions for each panel.

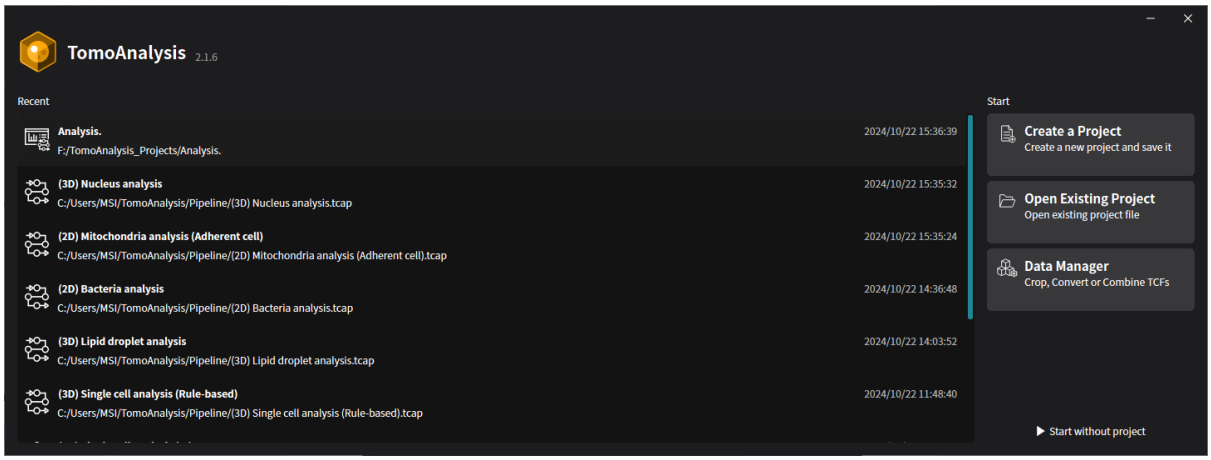
#	Panels	Description
1	Pipeline	Shows the structure of the pipeline, including input/output connections among the included processes.
2	Parameter Setting	Displays and controls the parameters for the selected process in the Pipeline panel.
3	Parameter Preset	Saves and loads parameter settings for the pipeline.
4	Live View	Displays the image output data with various image contrast options.
5	Output Navigator	Lists the output data produced by the process execution.
6	Visualization Control	Controls the image contrast shown in the Live View.

Chapter 3. Beginning the First Analysis

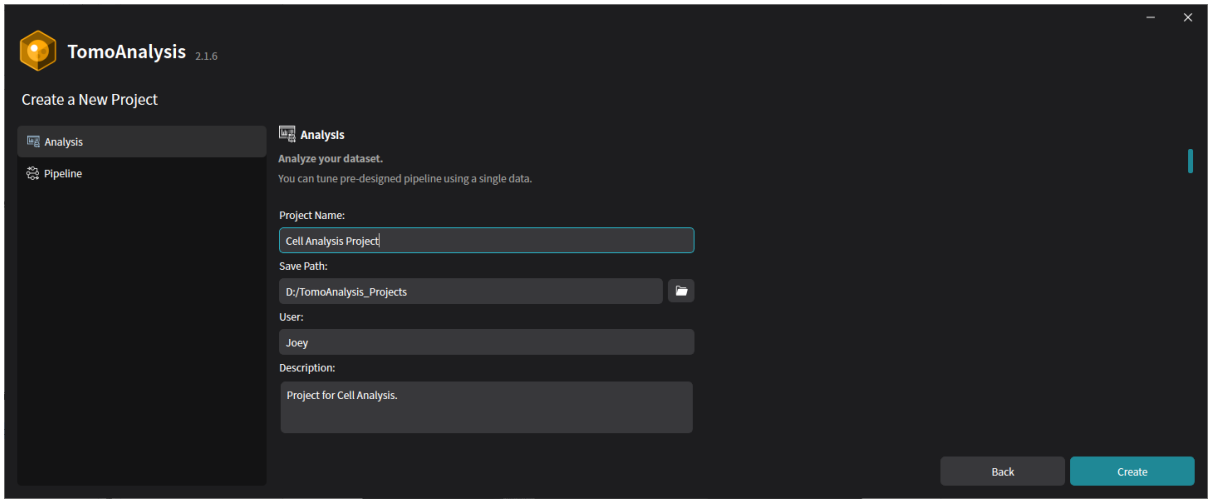
3.1 Introduction

This chapter provides a step-by-step guide for data analysis in **TomoAnalysis** using an example analysis pipeline that detects lipid droplets in cells.

3.2 Creating New Analysis Project

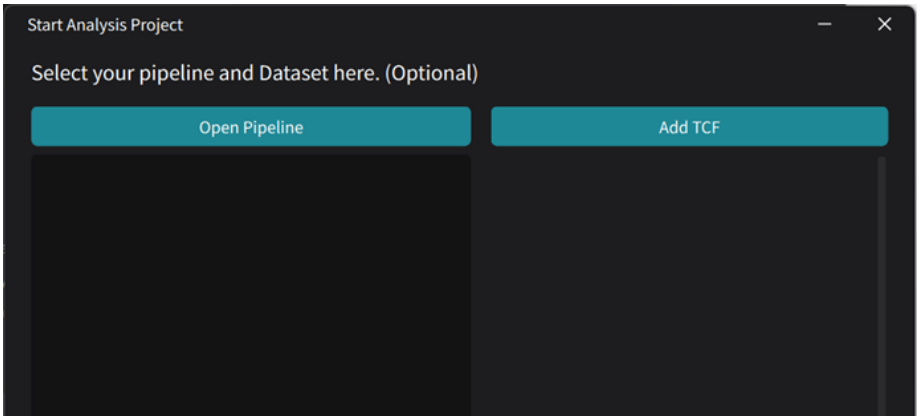


After launching the program, the initial step is to create a new analysis project. In the initial window, press the **Create a Project** button.



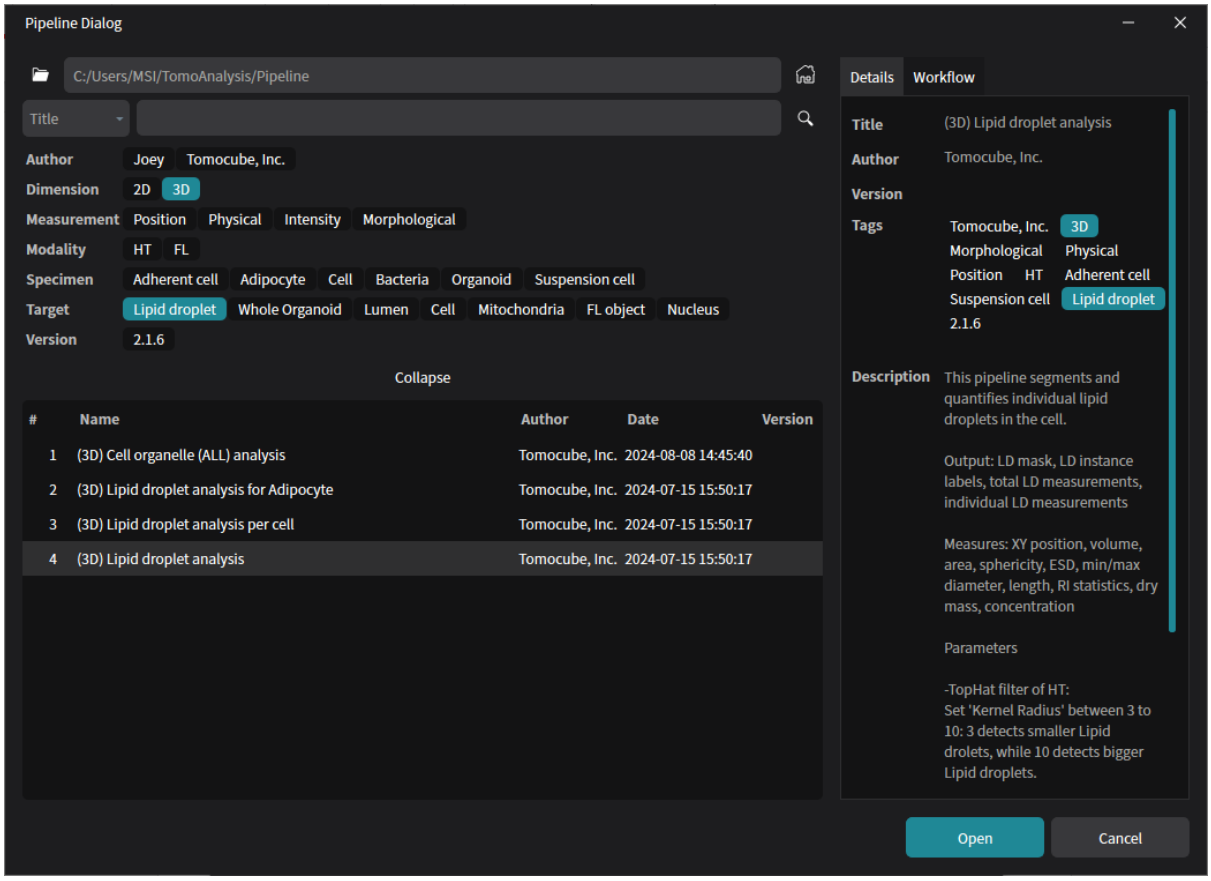
In the next window, ensure that **Analysis** is selected. If it is not selected, click the **Analysis** option. Then, enter the Project Name, Save Path, User, and Description fields. The User and Description fields are optional and can be left empty. The Description can also be edited later if needed.

After filling in the necessary information, click the **Create** button at the bottom right.



Next, select an analysis pipeline and the data files to be analyzed for the project. Click the Open Pipeline button at the top left to select a pipeline, and click the Add TCF button at the top right to add data files.

NOTE: This sequence can be skipped by selecting a pipeline and data files directly from the main window. For more details, refer to **Chapter 5. Analysis Perspective.**

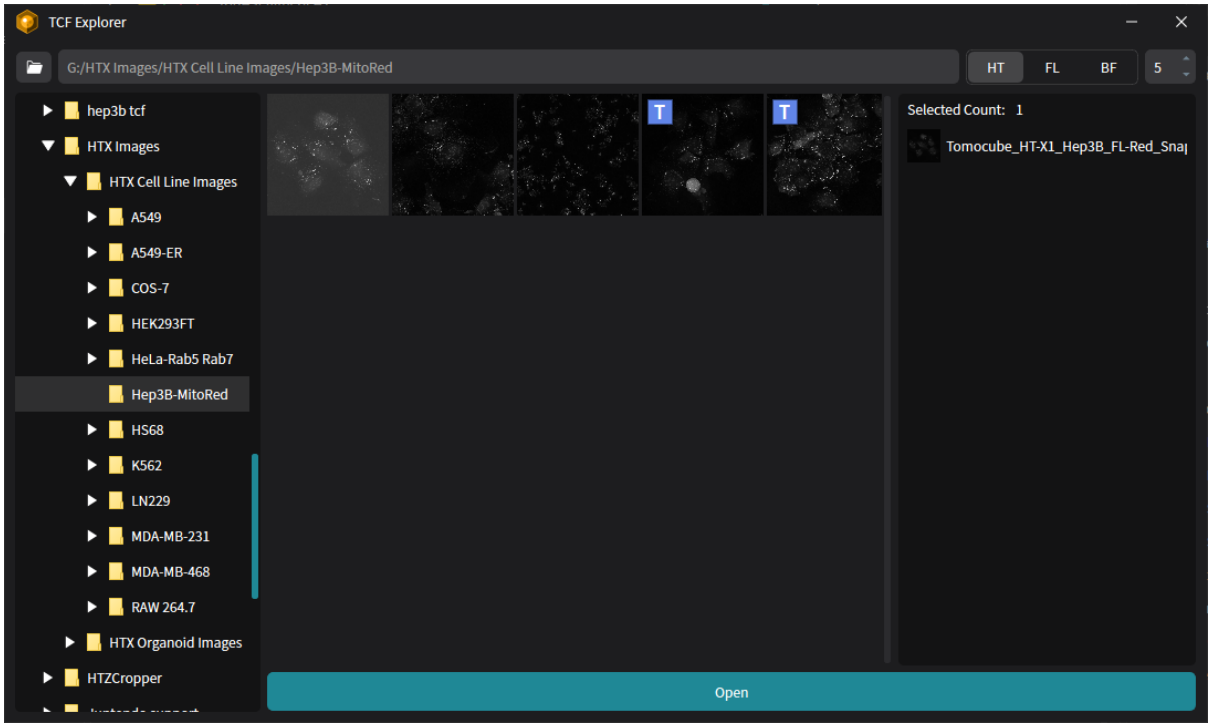


In the **Pipeline Dialog** window, the folder path where the pipelines are stored is displayed at the top. The available pipelines are listed on the left side of the window. To use a pipeline from a different folder, select the appropriate folder location. If numerous pipelines are listed, filtering by tags on the panel can help narrow the selection.

For example, to list pipelines related to lipid droplets and 3D analysis, click the *"3D"* and *"Lipid droplet"* tags in the keyword list, as shown in the figure.

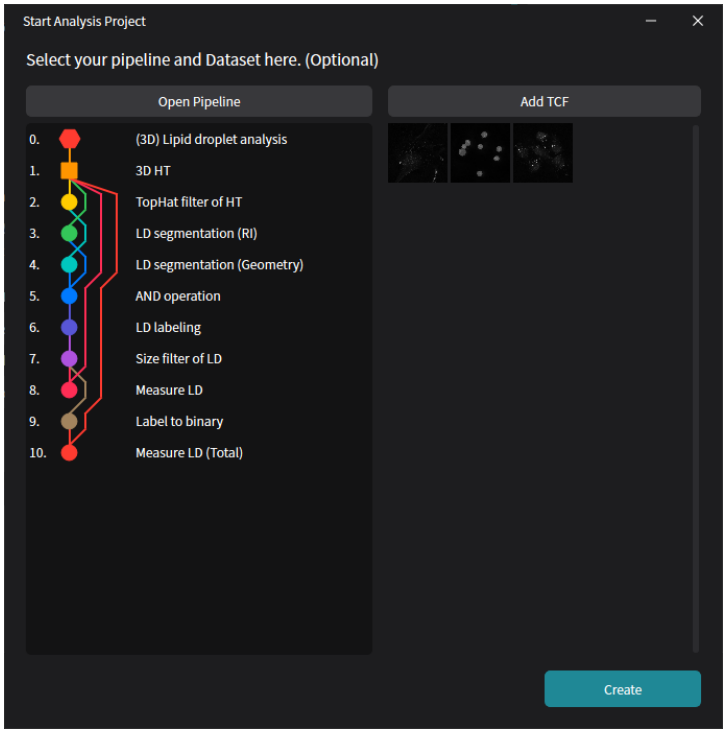
In this example, four pipelines related to the selected tags are displayed. Select *"(3D) Lipid droplet analysis"* pipeline for this project. Information about the selected pipeline is displayed on the right side of the window to ensure it meets the analysis requirements.

After selecting the pipeline, click the **Open** button at the bottom.

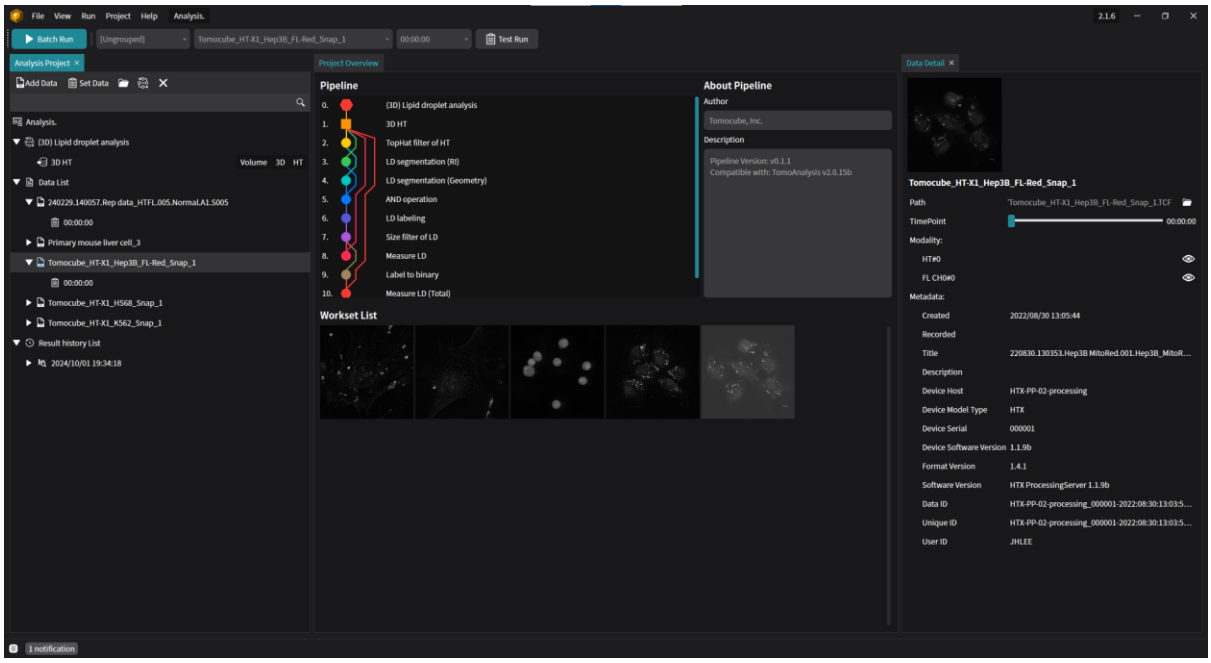


Next, add data files for the analysis project. From the left side of the **TCF Explorer** window, navigate to the folder where the data is stored. When a folder is selected, thumbnail images of the data files in that folder will appear on the right side. Select the desired files by clicking them, and they will be listed on the right side of the window. After selecting the data files, click the Open button to return to the **Start Analysis Project** window.

NOTE: Multiple files can be selected in the same way as in Windows Explorer, using the **Ctrl** and **Shift** keys for multi-file selection.



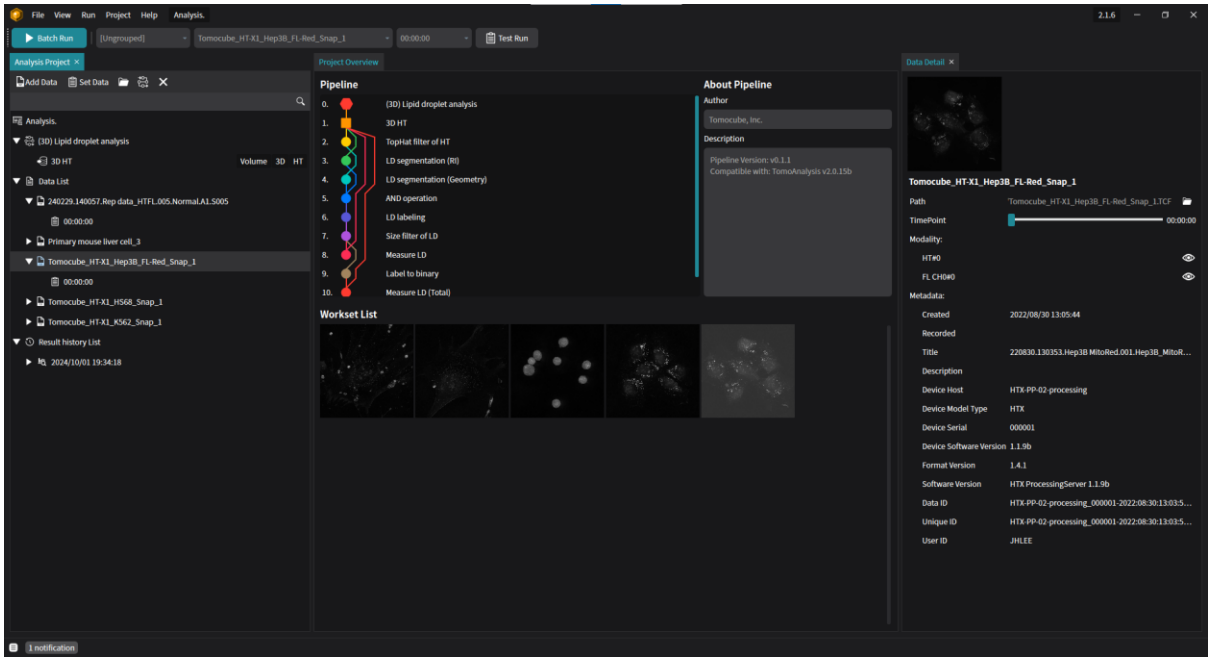
Back in the **Start Analysis Project** window, the selected pipeline and data files will be listed. Review the displayed items to ensure they match the selection. If everything is correct, click the **Create** button to proceed with the analysis.



In the main window, the analysis pipeline and data files are registered in the **Project Overview** panel in the center of the window.

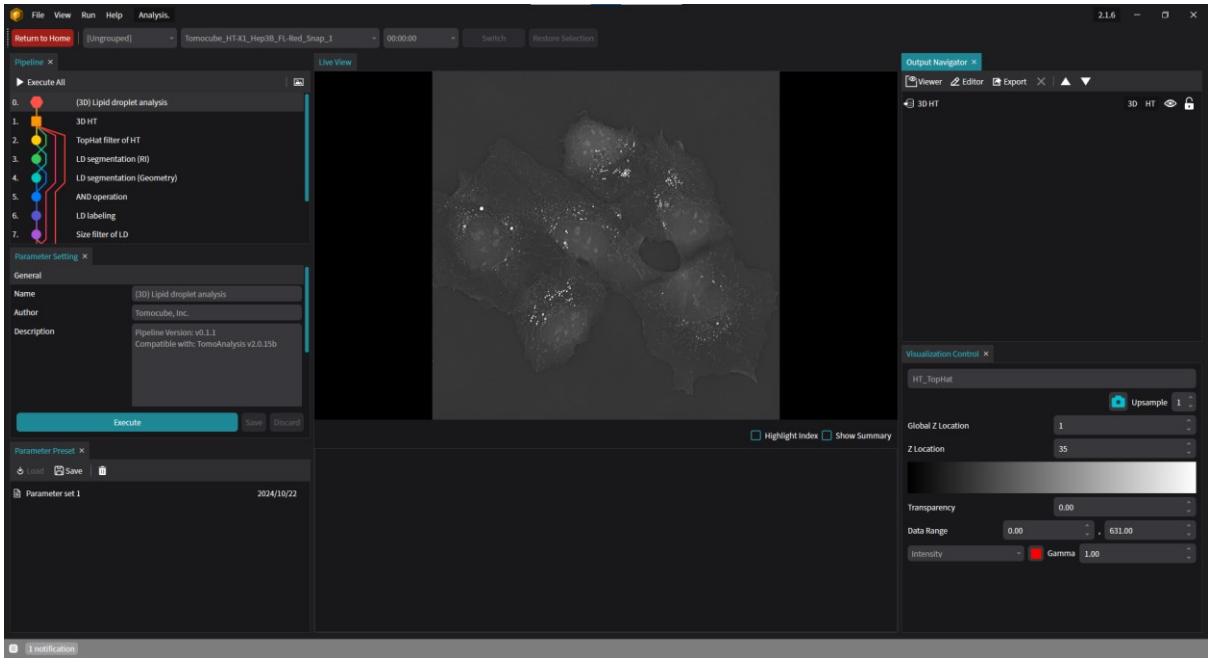
3.3 Testing Pipeline

Once the pipeline and data files are selected, it is recommended to use the **Test Run** mode in **TomoAnalysis** to find an appropriate parameter set for the analysis pipeline to achieve accurate results.



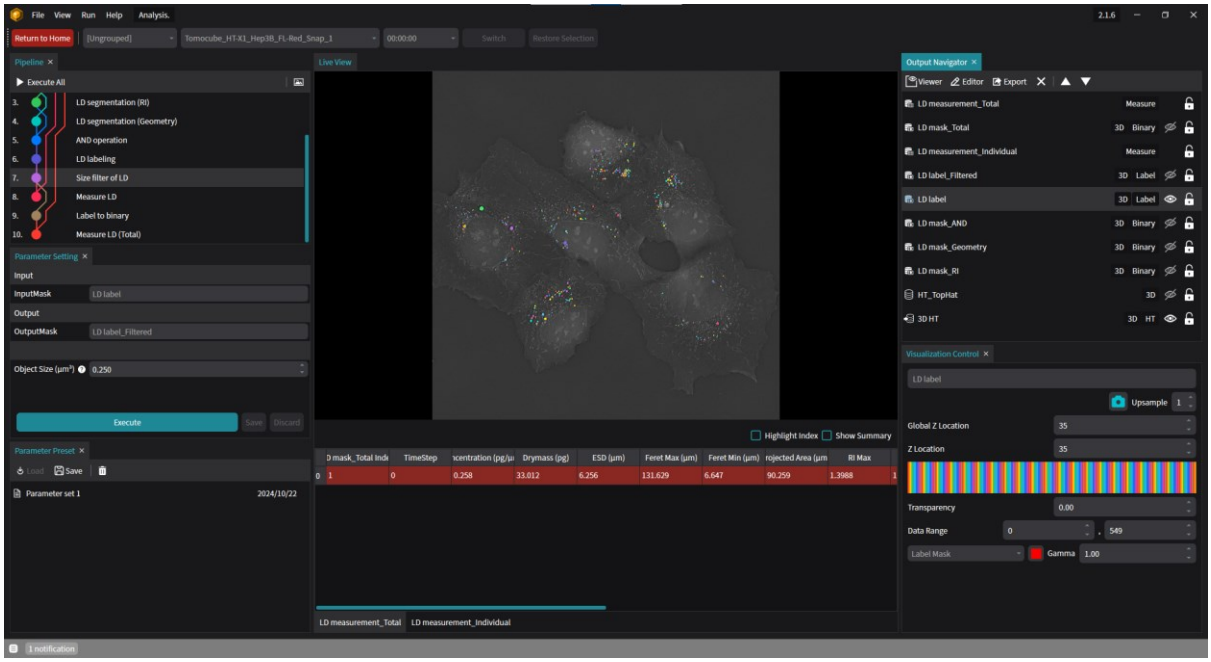
To access the **Test Run** mode, select one of the data in the **Data List** on the **Analysis Project** panel on the left side of the main window.

When data is selected, the **Test Run** button at the top of the panel will be activated. Click the **Test Run** button to enter the **Test Run** mode.

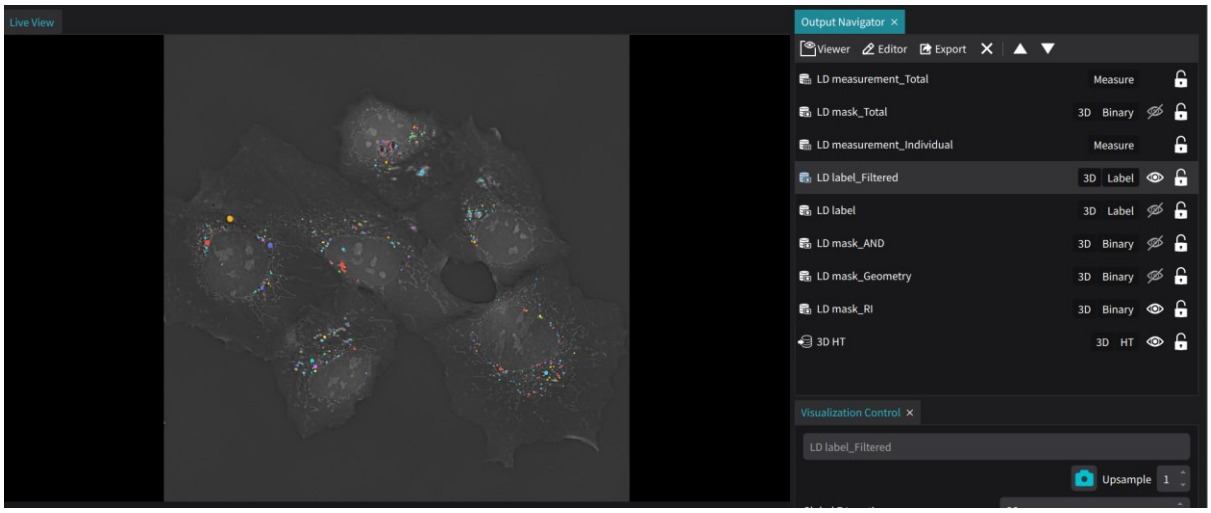


In **Test Run** mode, the structure of the selected pipeline is displayed in the **Pipeline** panel on the left. Selecting the top-most row, which is the pipeline name, will display the primary parameters in the **Parameter Setting** panel below. These parameters are key settings that need to be adjusted for the specific analysis, having been pre-selected when the pipeline was designed.

If there is uncertainty about how to adjust the parameter values, the **Execute** button can be clicked without making any changes. The default values will be used for the initial test.

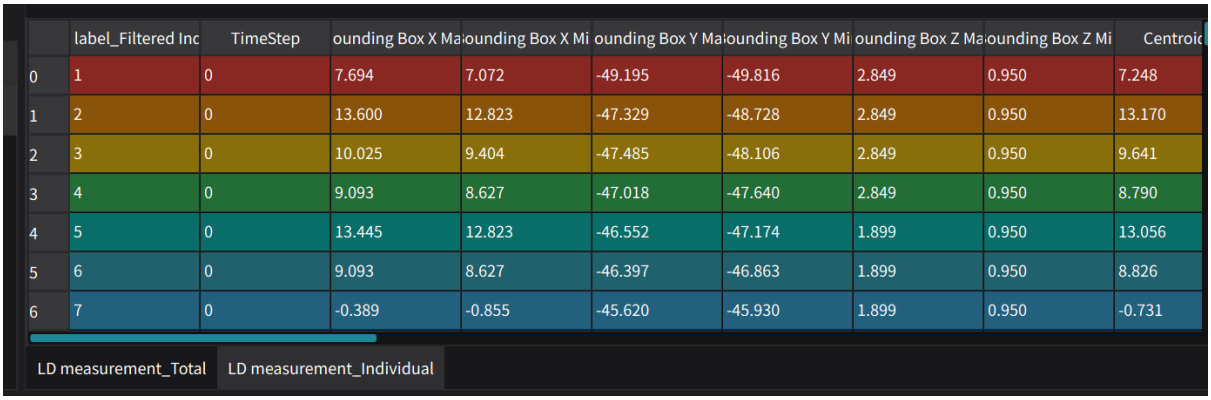


Once the initial run is completed without errors, the results will appear as shown in the figure above. Since this pipeline is designed to detect lipid droplets, the results can be reviewed in the **Live View** panel and the **Measurement** panel.



To view the lipid droplets detected by the pipeline, select the *"LD label_Filtered"* layer in the **Output Navigator**. If the selected layer is not displayed in the **Live View** panel, ensure that the **Show/Hide** option is enabled.

Other layers can also be displayed by enabling their **Show/Hide** options. Each output layer is linked to a specific process in the pipeline. The output layers can be verified to ensure they are properly processed by their linked process, and if necessary, the parameters can be adjusted from the **Pipeline** panel. For more details on parameter adjustment, refer to **Chapter 5. Analysis Perspective.**

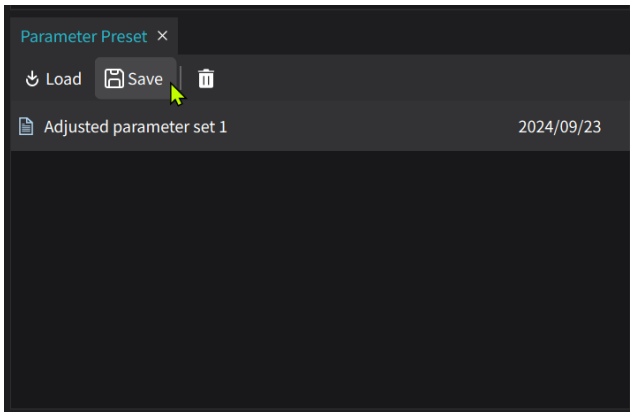


	label_Filtered Inc	TimeStep	ounding Box X Ma	ounding Box X Mi	ounding Box Y Ma	ounding Box Y Mi	ounding Box Z Ma	ounding Box Z Mi	Centroid
0	1	0	7.694	7.072	-49.195	-49.816	2.849	0.950	7.248
1	2	0	13.600	12.823	-47.329	-48.728	2.849	0.950	13.170
2	3	0	10.025	9.404	-47.485	-48.106	2.849	0.950	9.641
3	4	0	9.093	8.627	-47.018	-47.640	2.849	0.950	8.790
4	5	0	13.445	12.823	-46.552	-47.174	1.899	0.950	13.056
5	6	0	9.093	8.627	-46.397	-46.863	1.899	0.950	8.826
6	7	0	-0.389	-0.855	-45.620	-45.930	1.899	0.950	-0.731

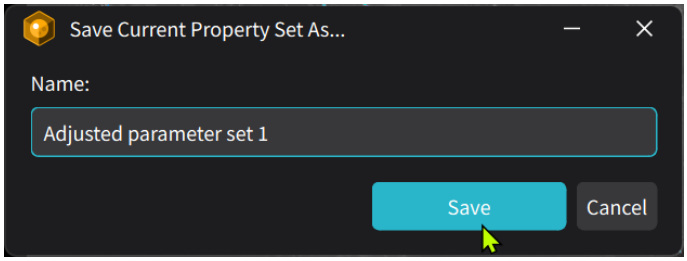
LD measurement_Total | LD measurement_Individual

For measurement results, check the **Measurement** panel below the **Live View** panel. The columns display metrics for the detected lipid droplets, and can be scrolled up and down to view all values. Click a column header to sort by the selected column's values. Repeatedly clicking the column header toggles between ascending and descending order.

To add more measurement metrics, modify the selection in the measurement processes within the pipeline. For this example, the processes *"Measure LD"* and *"Measure LD (Total)"* can be configured to change the metric selection.



Once satisfied with the test run results, save the parameter settings from the **Parameter Preset** panel at the bottom left of the **Test Run** window.

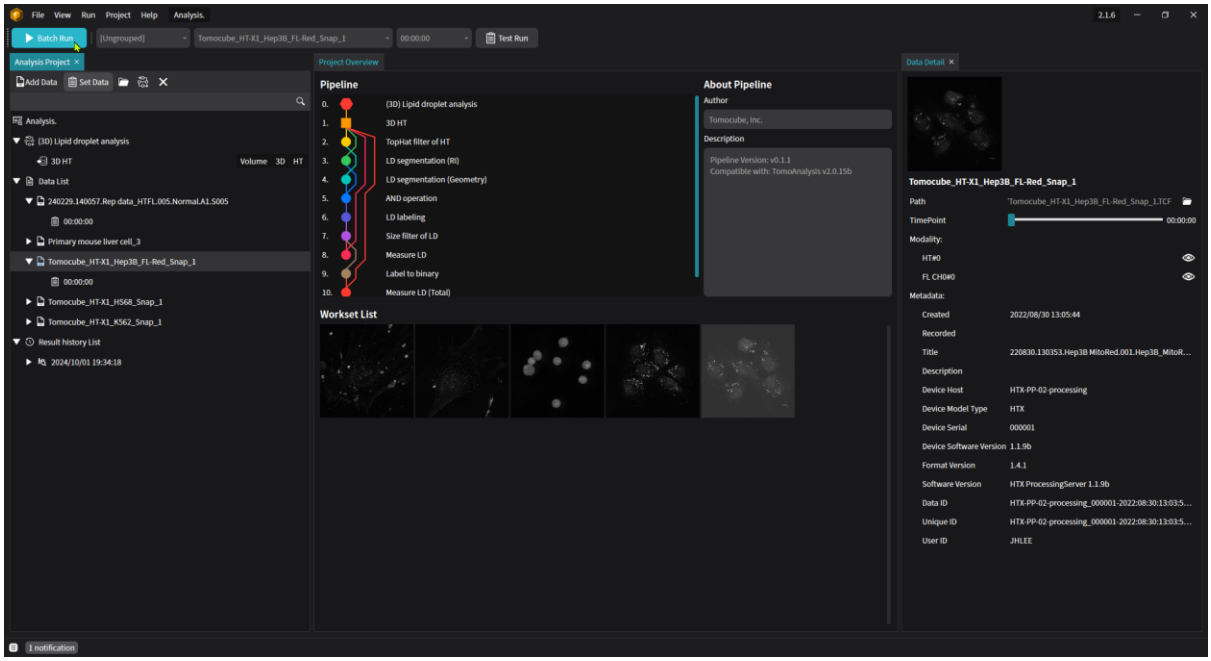


Press the **Save** button, and a window will appear to name the parameter set, which can be used during batch analysis.

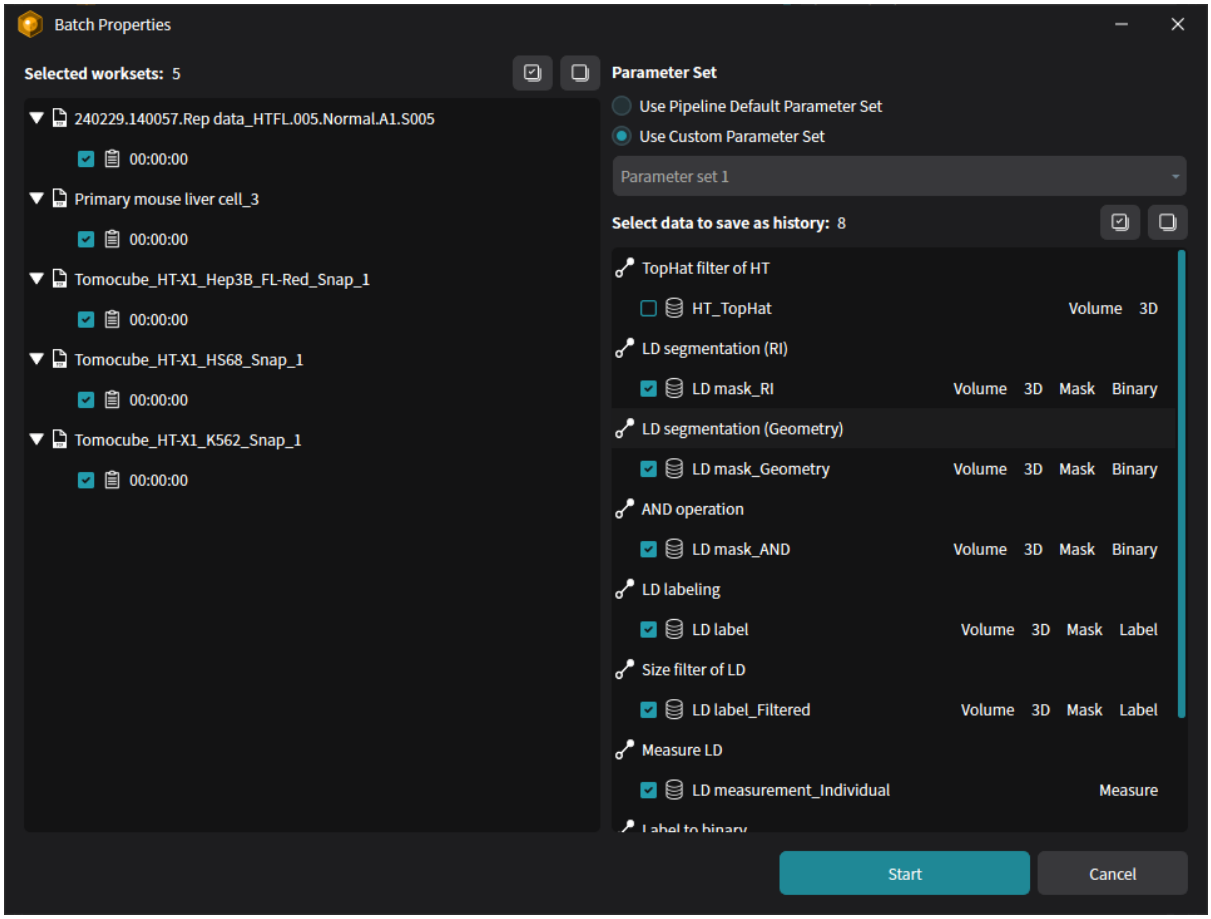
NOTE: If a parameter set is not saved, only the default parameter settings will be used for the pipeline during batch analysis.

After saving the parameter set, return to the main window by clicking the **Return to Home** button at the top left of the window.

3.4 Running Batch Analysis



After completing the **Test Run** and saving a parameter set for the pipeline, Progress to **Batch Run,** where analyze the registered TCF data files in a batch. Clicking the **Batch Run** button at the top left of the window will automatically open the **Batch Properties** window.



In the **Batch Properties** window, select the data files to be analyzed and determine which results to save after the analysis:

Workset Selection for Batch Run:

On the left side is the list of data files registered in the main window. Select or deselect the data files (worksets) to be analyzed.

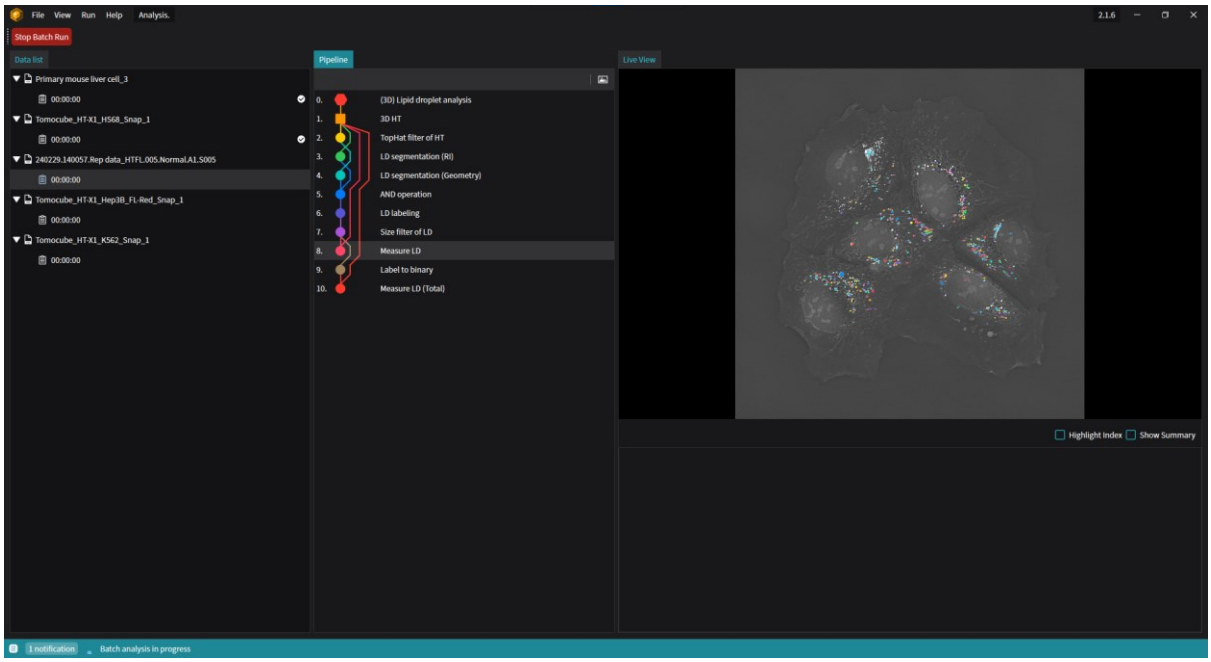
Output Selection to be Saved for Batch Run:

On the right side, select the outputs produced during the analysis. The default is to select all segmentation and measurement results except image data. For example, *"LD label"* and *"LD label_Filtered"* (two label data), four binary mask data, and *"LD measurement_Individual"* and *"LD measurement_Total"* (two measurement data) are selected by default.

Parameter Selection for Batch Run:

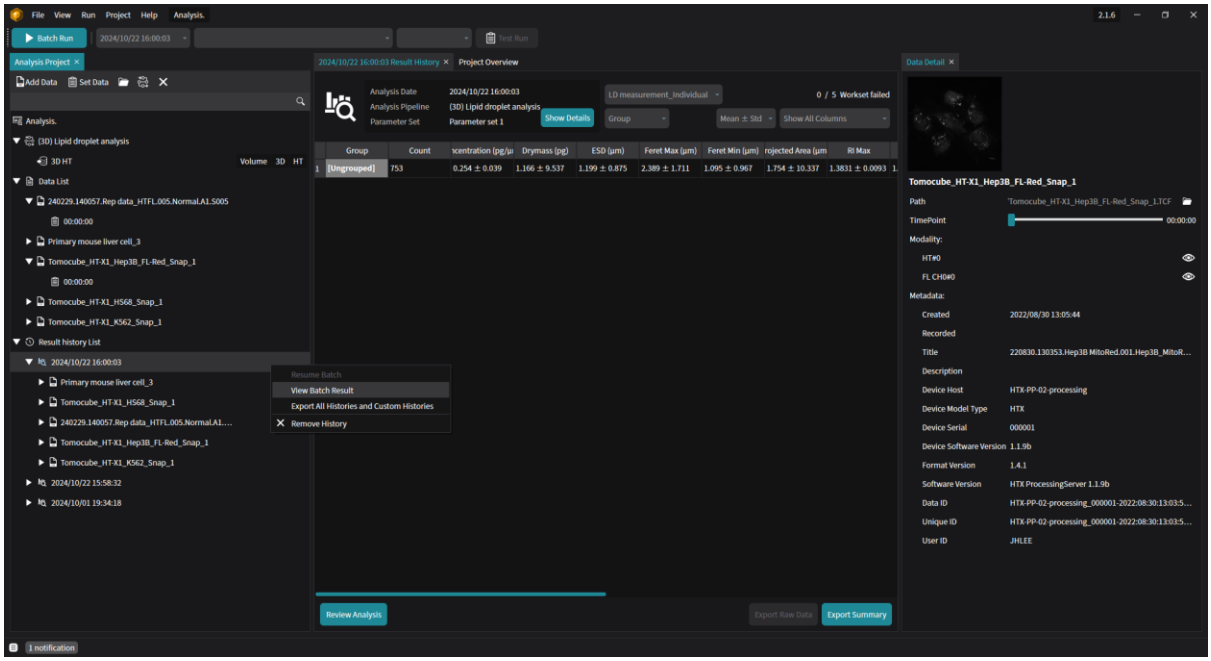
After selecting the TCF files, time points, and outputs, choose a parameter set for running the pipeline. If a parameter set was saved in **Test Run**, its name can be found in the **Parameter Set** menu at the top right. Select **Use Custom Parmeter Set** and choose the parameter set from the drop-down menu. If no parameter set was saved, or if the default settings are preferred, select **Use Pipeline Default Parameter Set**.

After selecting the TCF files, output data, and parameter set, click the **Start** button to begin the batch analysis.



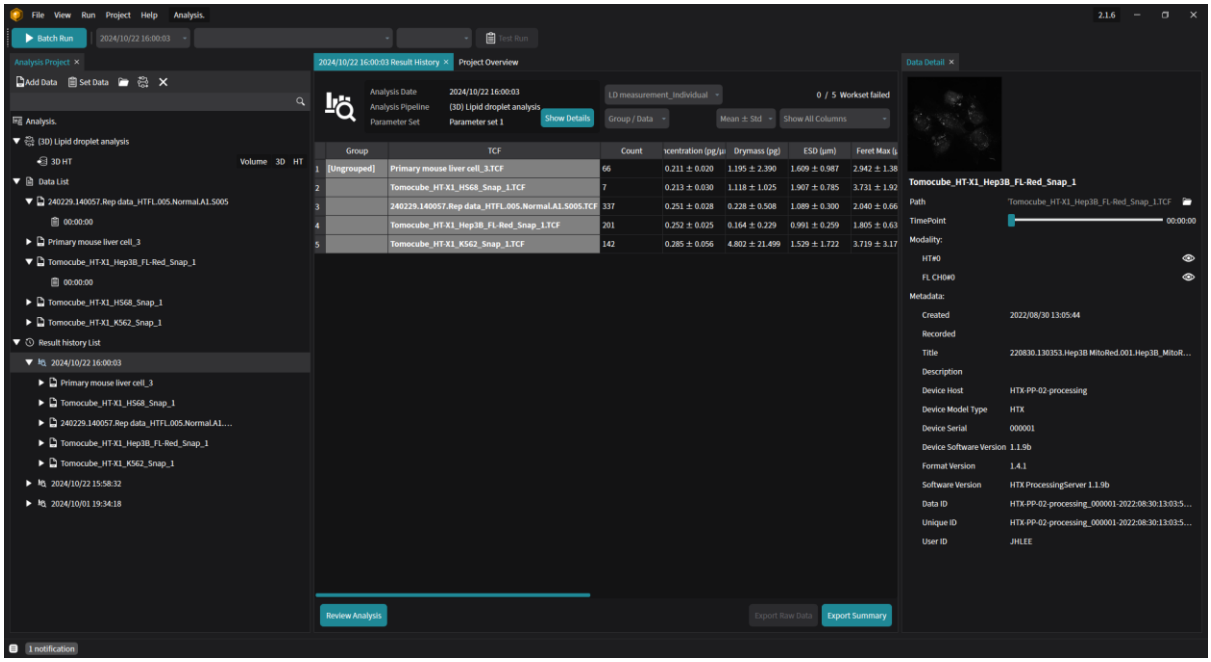
When the batch analysis starts, the window changes to display the progress in real time. After all the worksets are analyzed, the window returns to the main window.

3.5 Review Analysis Results



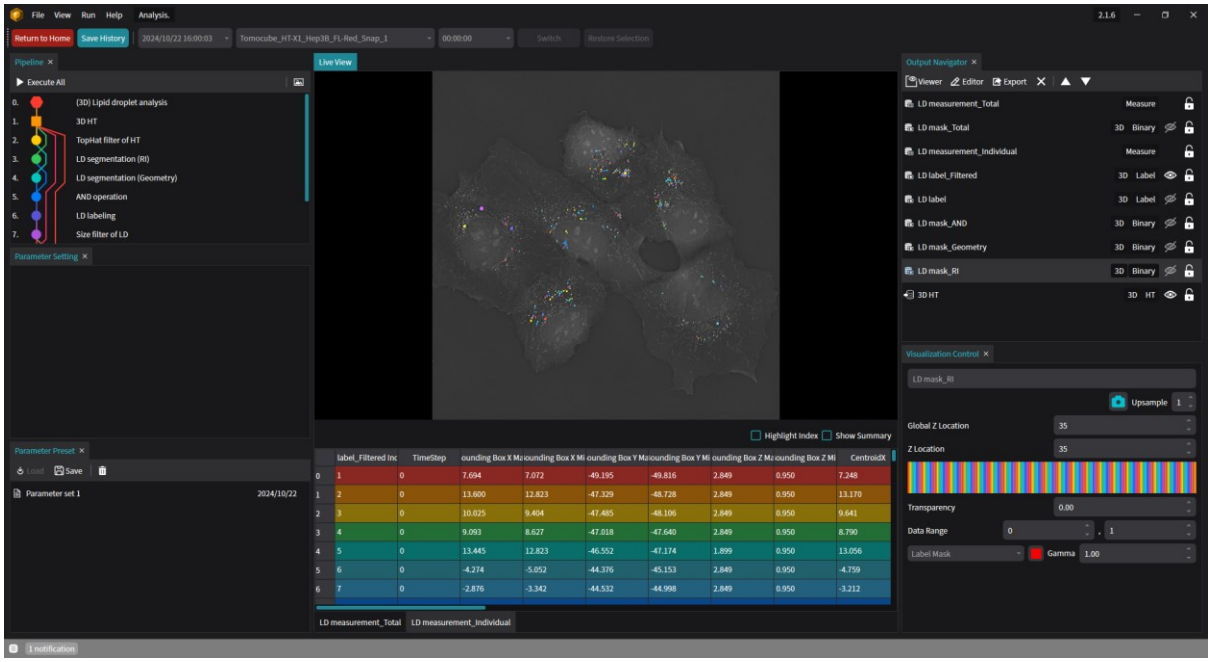
Once the batch analysis is complete, the results are added to the **Result History List** in the **Analysis Project** panel on the left side of the main window.

To review the overall results, select a record from the **Result History List**. For example, a record might be named "*2024/10/22 14:00:03*", depending on when the analysis was run. Right-click on the history record and select **View Batch Result** to open a dashboard summarizing the analysis results.



In the **Review Dashboard**, the analysis results are summarized in a table. The table can be displayed according to user settings as Group, Group/Data, Group/Time, Data, Data/Time, Time, Time/Group, or Time/Data.

To review individual data in detail, select a row and press the **Review Analysis** button at the bottom of the dashboard panel.



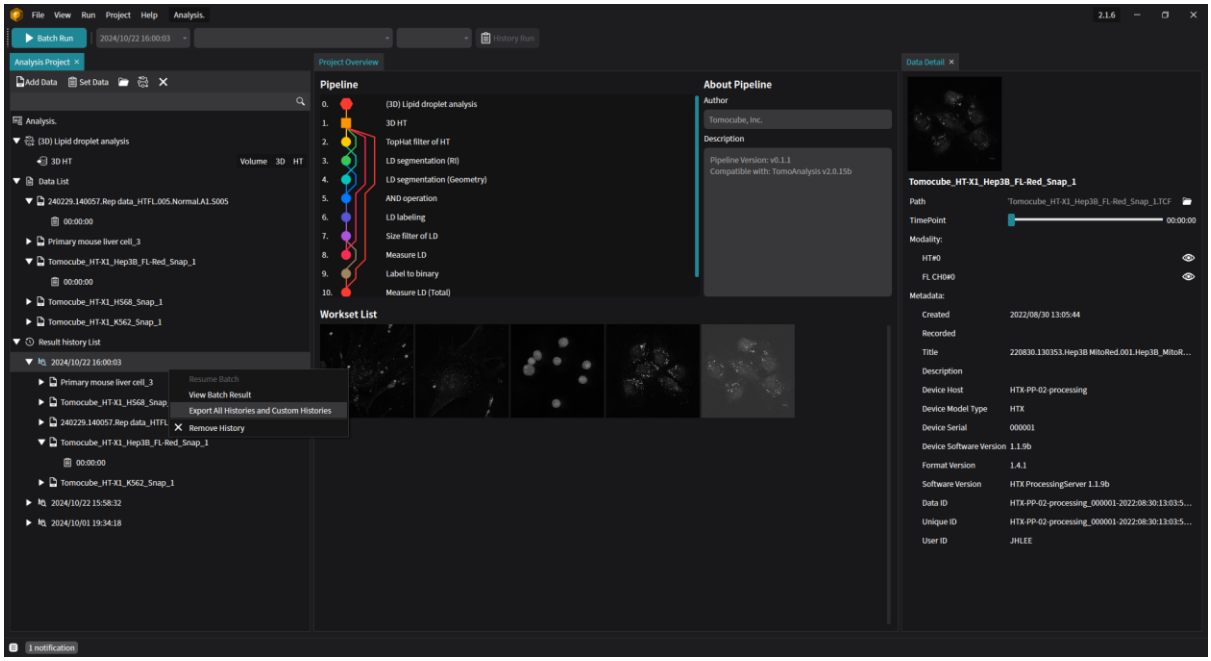
In **Review Mode**, the window layout is similar to **Test Run** mode, except for the **Parameter Setting** panel.

As in **Test Run** mode, output layers registered in the **Output Navigator** can be shown or hidden to review the accuracy of the analysis results. The measurement table can also be used to assess the analysis metrics.

To navigate through different TCF file records, use the navigation bar at the top of the window to select the desired data file and time point. For more information on loading specific TCF file records, refer to **Chapter 5. Analysis Perspective.**

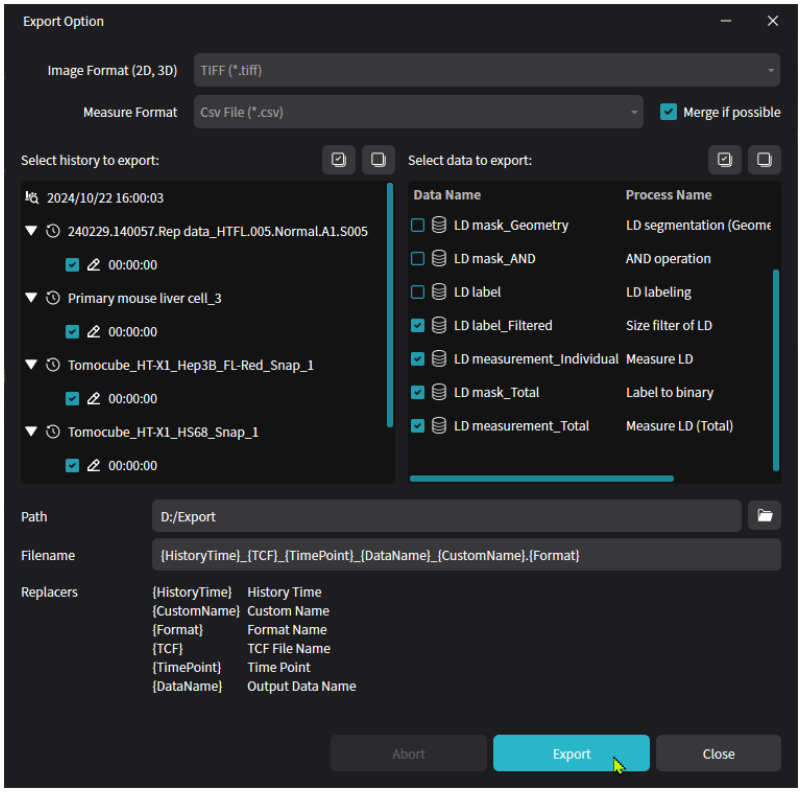
When the review is complete, click the **Return to Home** button to go back to the main window of the **Analysis Perspective**.

3.6 Export Results



After completing the batch analysis and reviewing the results, the data can be exported for further analysis with other software, either in TIFF format for image outputs or in CSV format for measurement table outputs.

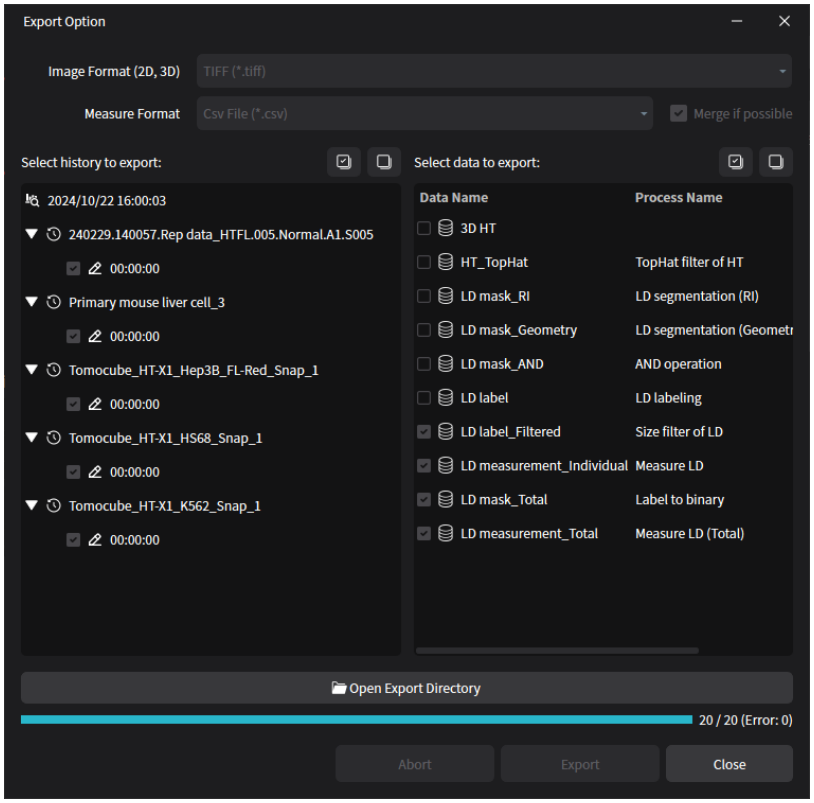
To export the results in batch, select the history record as in **Review Mode** and choose **Export All Histories** or **Custom Histories** from the pop-up menu to open the **Export Option** window.



In the **Export Option** window, select work and data outputs for export, similar to the **Batch Properties** window. The image data can be saved in TIFF, RAW, or NPY formats, while the measurement table data can only be saved in CSV format.

After selecting the TCF files and output formats, specify the save path and file naming format at the bottom of the window. For more details on file naming, refer to **Chapter 5. Analysis Perspective.**

When all selections are complete, click the **Export** button to begin the export process.



During the export, a progress bar will appear at the bottom of the window. When the export is complete, click the **Open Export Directory** button to open the Windows Explorer at the export location.

Press the **Close** button to return to the main window after exporting the results.

This is the general workflow for analysis in **TomoAnalysis**. For more detailed information about analysis and adjustments, refer to **Chapter 5. Analysis Perspective.**

Chapter 4. Pipeline Editor

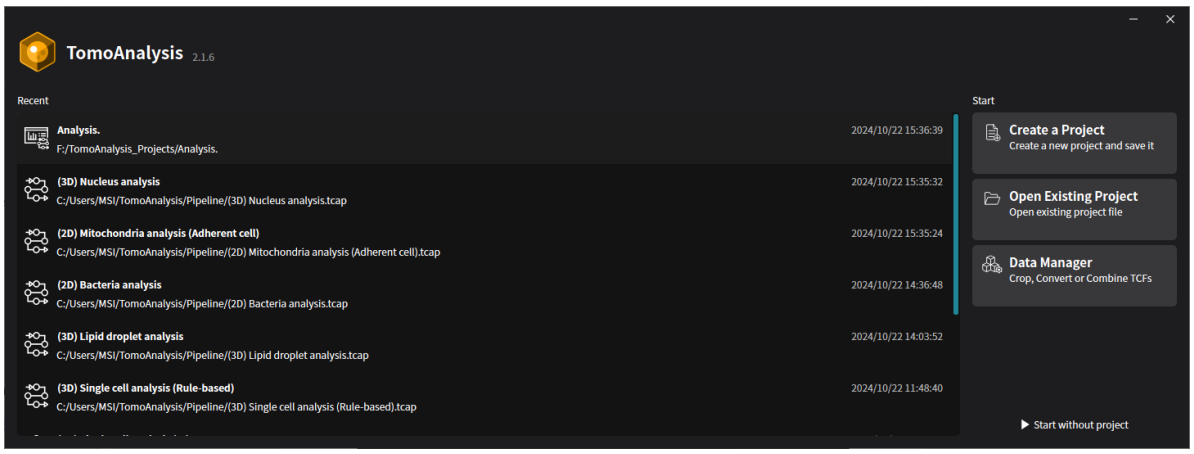
4.1 Introduction

Analyzing TCF data in **TomoAnalysis** involves applying a series of computational steps, each referred to as a process, which is managed as part of the analysis pipeline. The pipeline can be created or modified using the **Pipeline Editor** and is used in the **Analysis Perspective** to process data files.

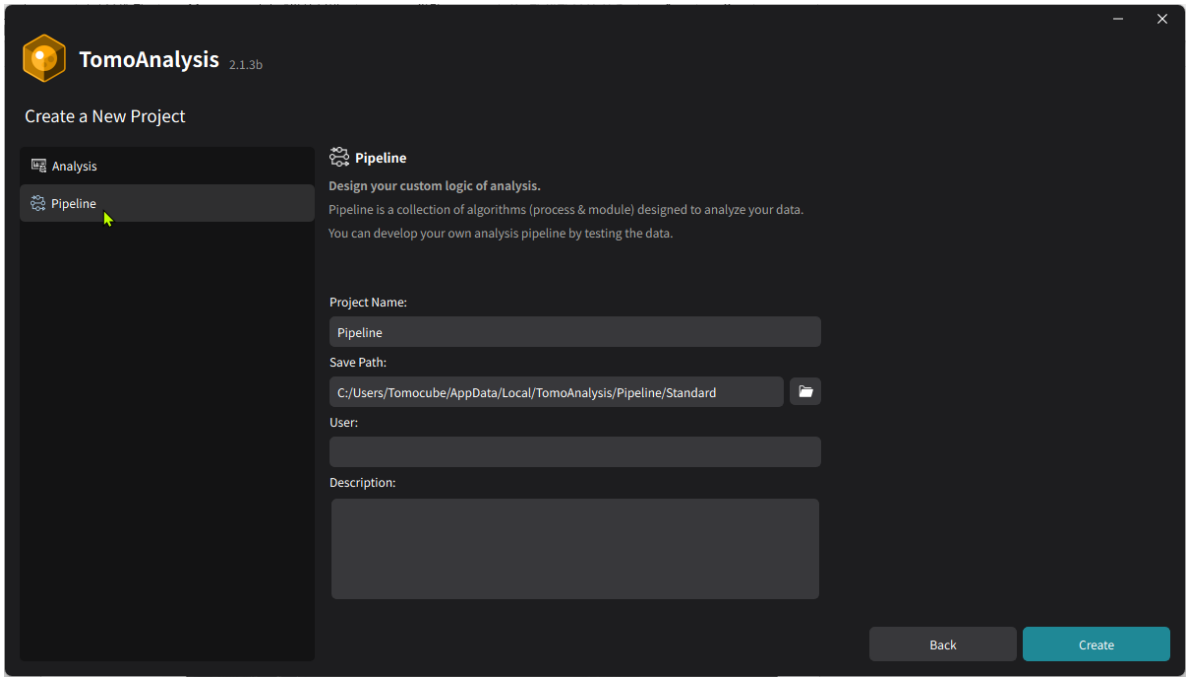
4.2 Creating New Pipeline

To create a new analysis pipeline in **TomoAnalysis**, follow these steps:

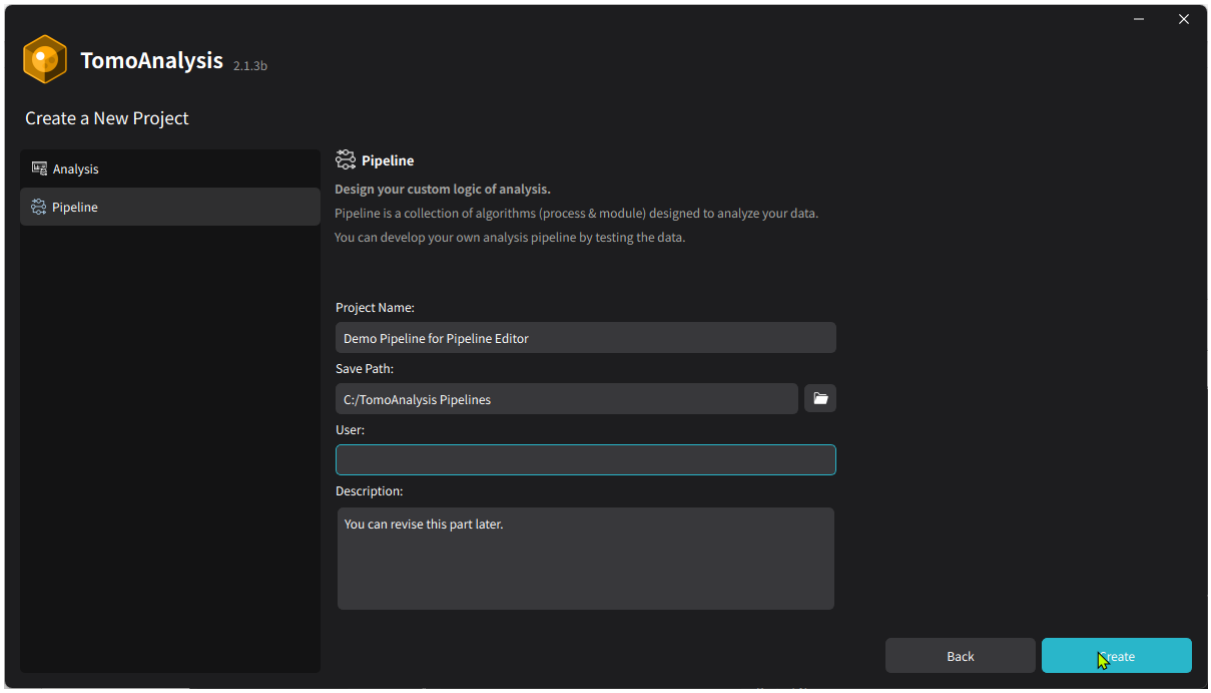
1. Start the software.



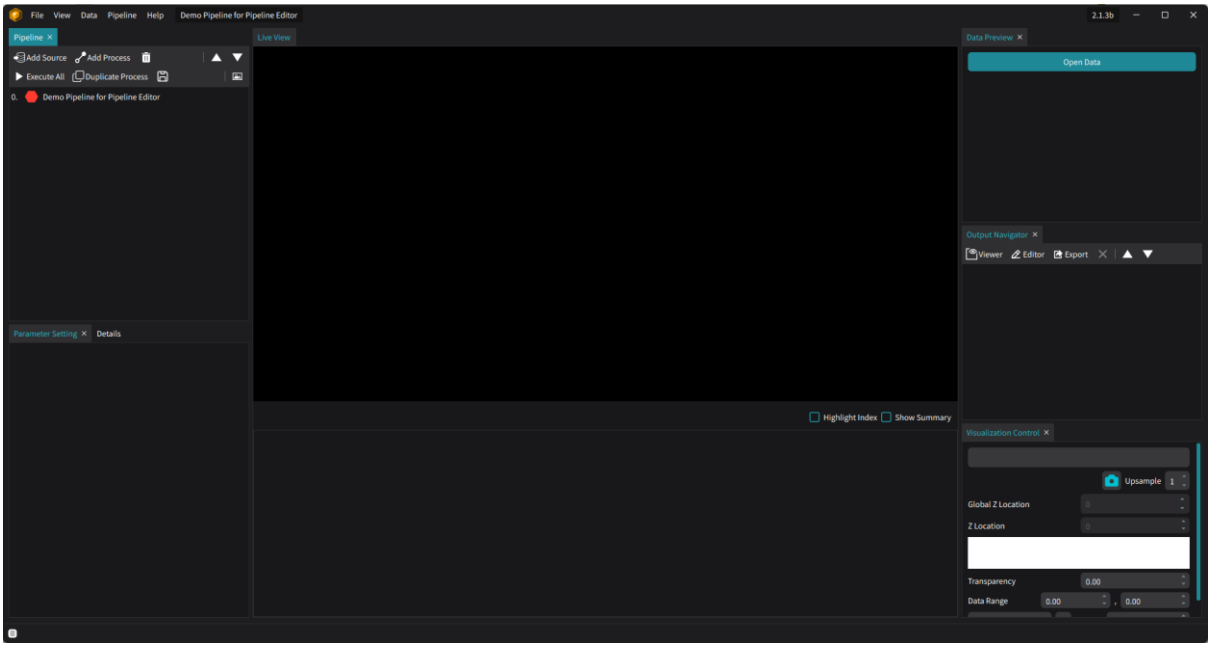
2. Click the **Create a Project** button.



3. In the **Create a Project** window, select the **Pipeline** menu from the left tab.



4. Enter a project name for the new pipeline and specify the folder path where it will be saved. Optionally, the pipeline author's name and a description can be entered, both of which can be revised later in the **Pipeline Editor**. After filling in the input text boxes, click the **Create** button to proceed.



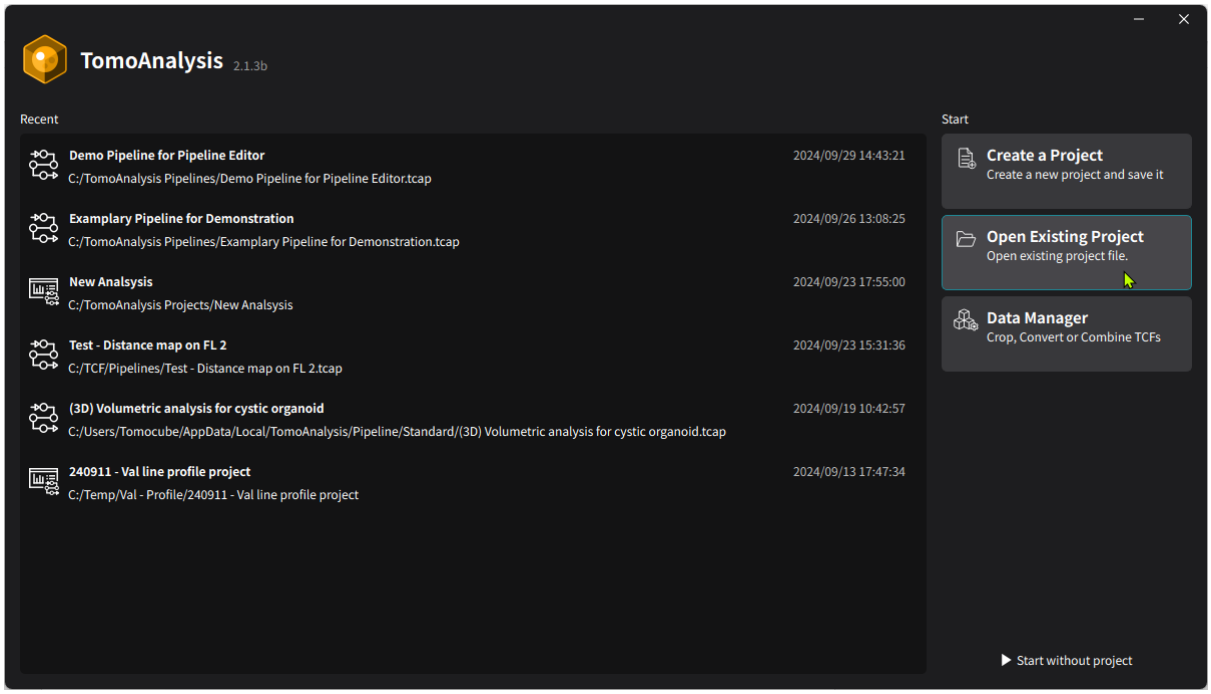
5. The main screen for the **Pipeline Editor** will appear, and the system is now ready for the creation of a custom analysis pipeline in **TomoAnalysis**.

6. Once editing is complete, click the **Save Pipeline** button on the toolbar.

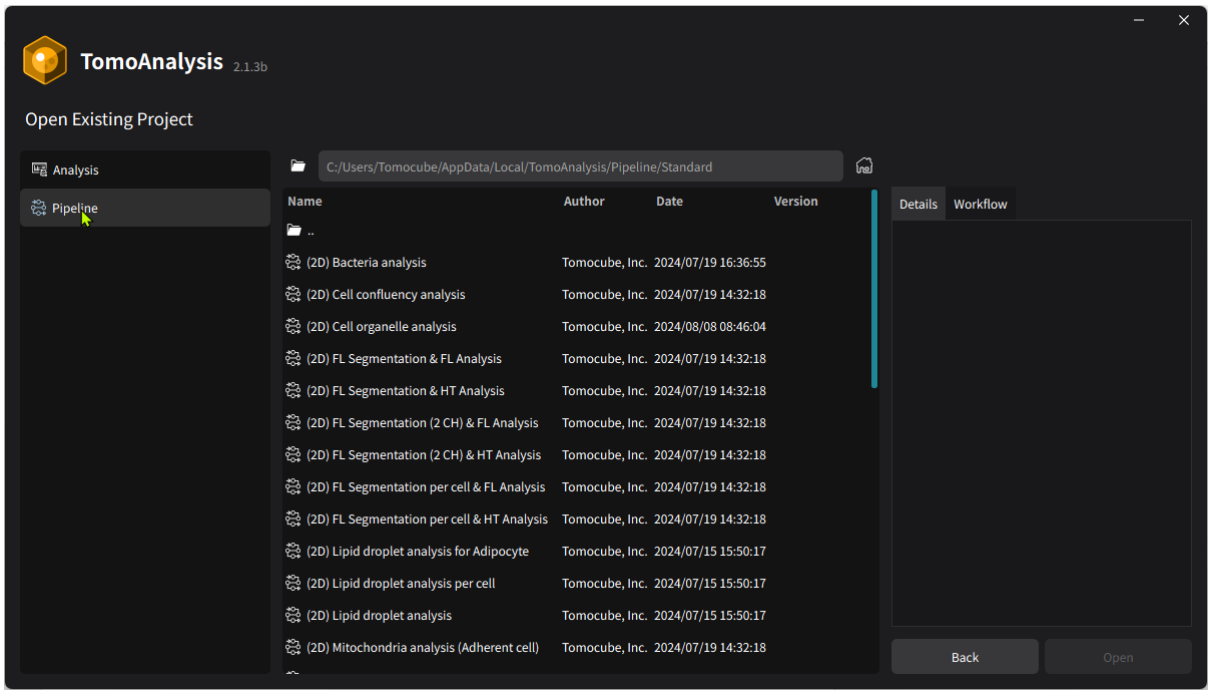
4.3 Editing Existing Pipeline

Existing analysis pipelines can be edited to update them or to create a new one based on an existing pipeline. To edit a pipeline in **TomoAnalysis**, follow these steps:

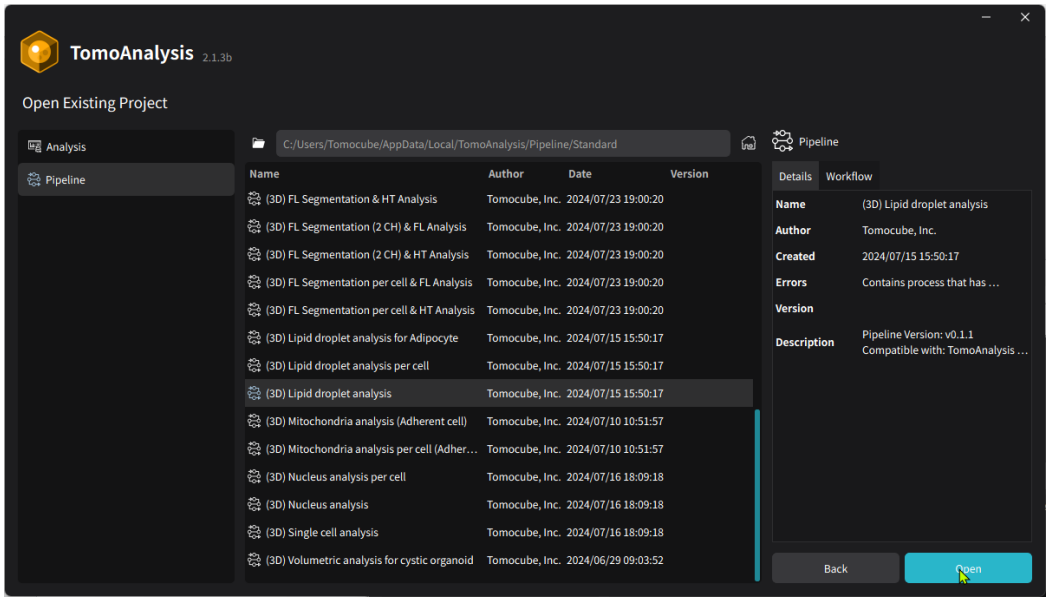
1. Start the software.



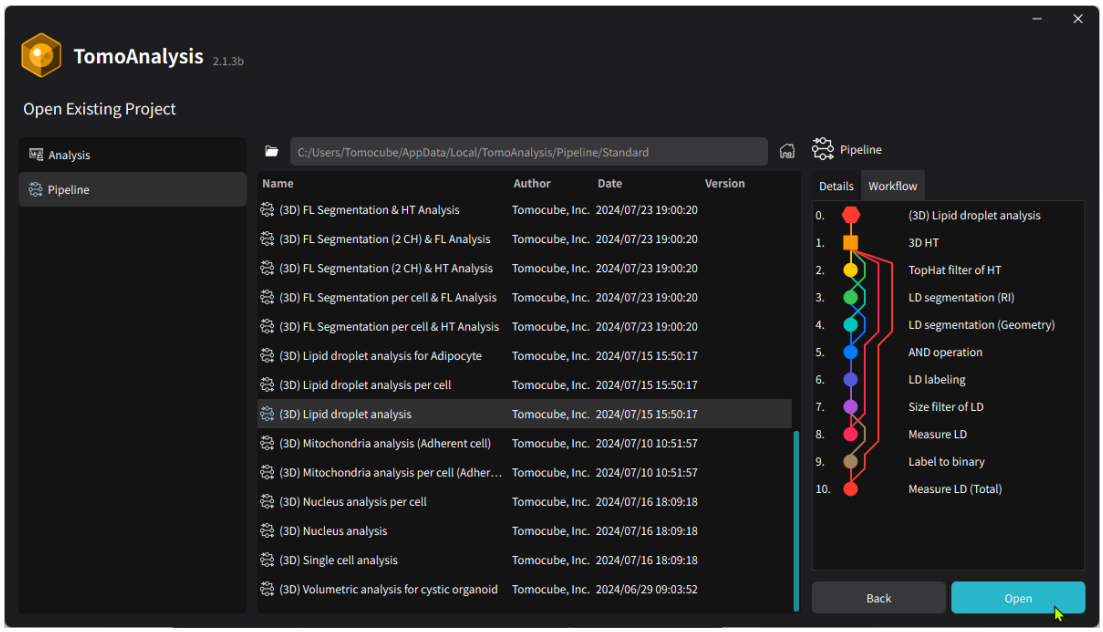
2. Click the **Open Existing Project** button.



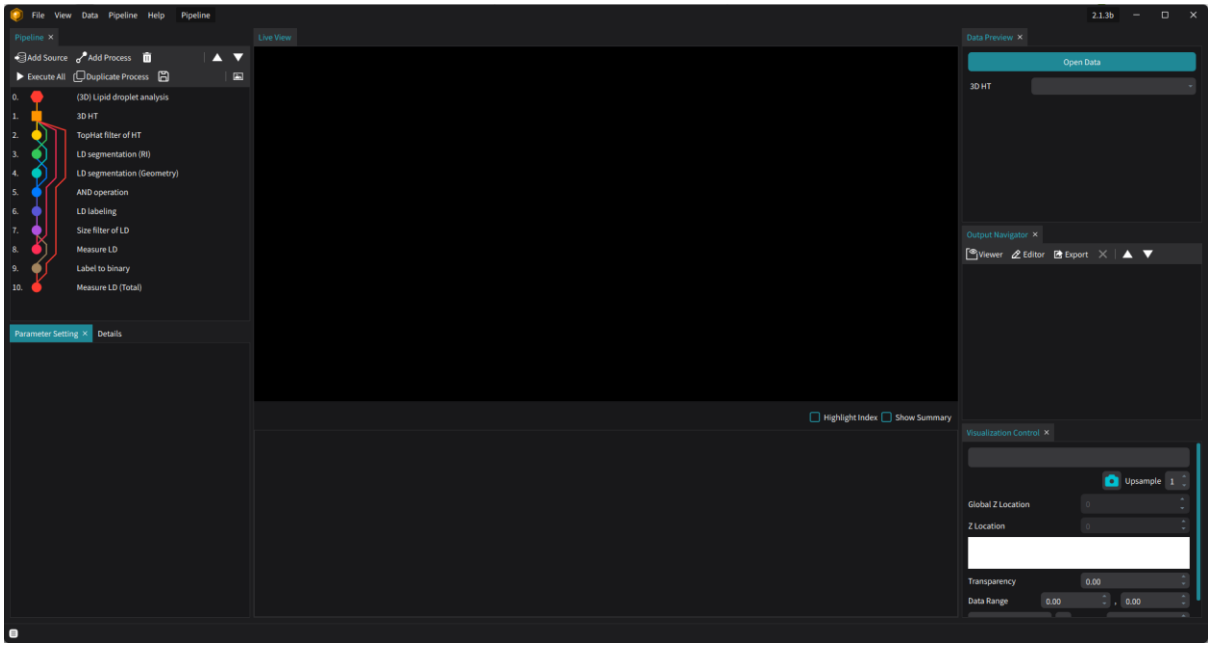
3. In the **Open Existing Project** window, select the **Pipeline** menu from the left tab.



4. When the **Pipeline** menu is clicked, the right panel shows the pipelines located in the folder displayed at the top of the panel. The folder can be changed by clicking the folder icon to the left of the folder path. Upon selecting a pipeline from the list, its description will appear on the right side of the panel. The **Details** tab displays basic information, including the pipeline's name and author. The **Workflow** tab shows the pipeline's structure, including processes and connections.



5. After selecting a pipeline to edit, click the **Open** button at the bottom right.



6. The main screen will display the structure of the opened pipeline in the **Pipeline** panel on the left.

7. Once editing is completed, click the **Save Pipeline** button on the toolbar.

4.4 Pipeline Components

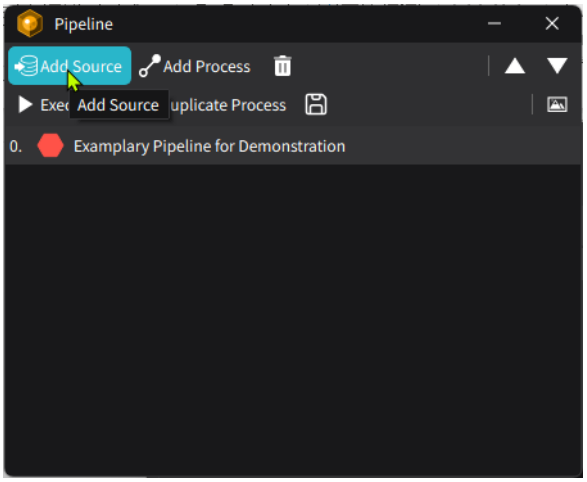
There are two types of components in a pipeline: **Source** and **Process**.

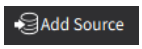
Source: Defines the type of data in the TCF file that the pipeline will analyze.

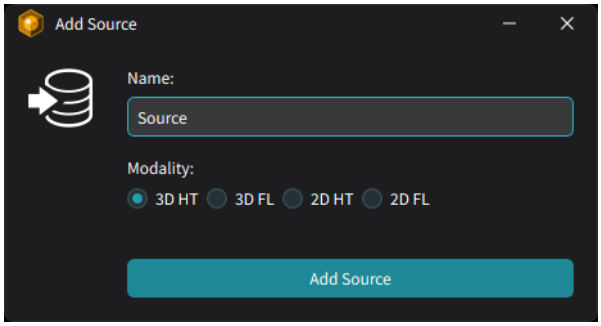
Process: Defines how a source or its processed output is used to produce results or intermediate outputs for further processing.

4.4.1. Data source

In **TomoAnalysis**, the analysis pipeline needs to know the type of data from the TCF files that will be processed. Defining the data source for the pipeline is required.



1. Press the **Add Source**  button in the **Pipeline** panel to open the **Add Source** dialog box.

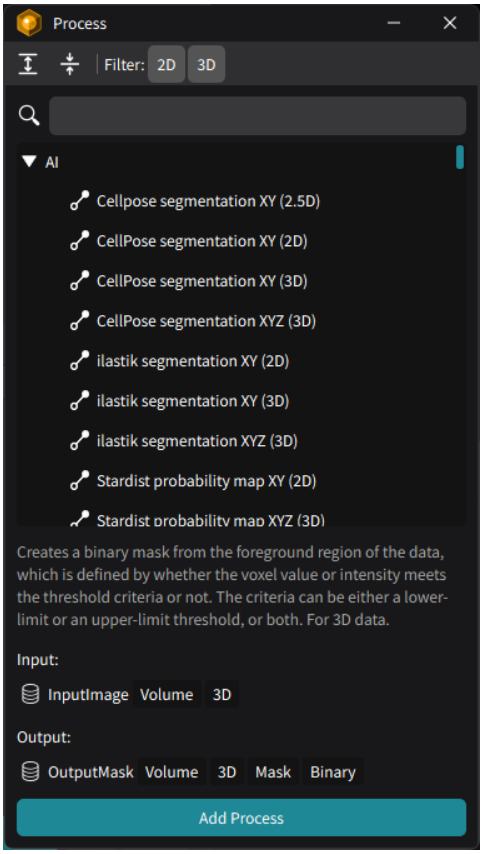


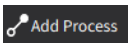
2. In the **Add Source** dialog box, select the modality of the data to be analyzed and assign a name to the source that will be used in the pipeline's processes.

   - The source name can be anything, except wildcard characters such as asterisks (*) and question marks (?).
   - For the modality type, four options are possible for TCF files: 3D HT, 3D FL, 2D HT, and 2D FL.

4.4.2. Process

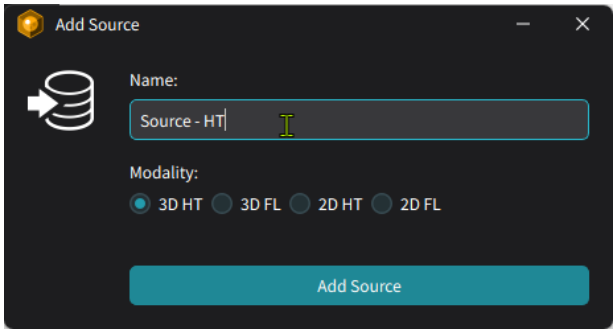
The second component type is the **Process**, which performs calculations on its input to generate its output.



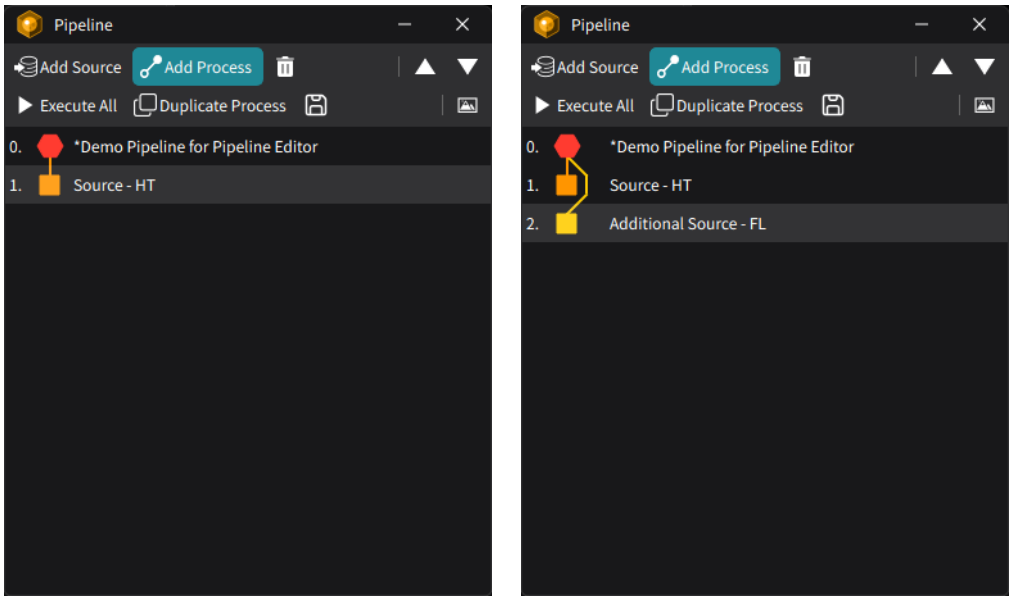
1. To add a process to the pipeline, press the Add Process 

button to open the **Process** panel.
2. The **Process** panel lists available processes by category. The list can be scrolled through or searched using the search box to find a specific process.
3. After selecting the desired process, click the **Add Process** button at the bottom of the panel. Multiple processes can be added in one session. Once finished, close the **Process** panel.
4. The **Process** panel can be accessed at any point during the editing to additional processes.

4.5 Adding Data Sources



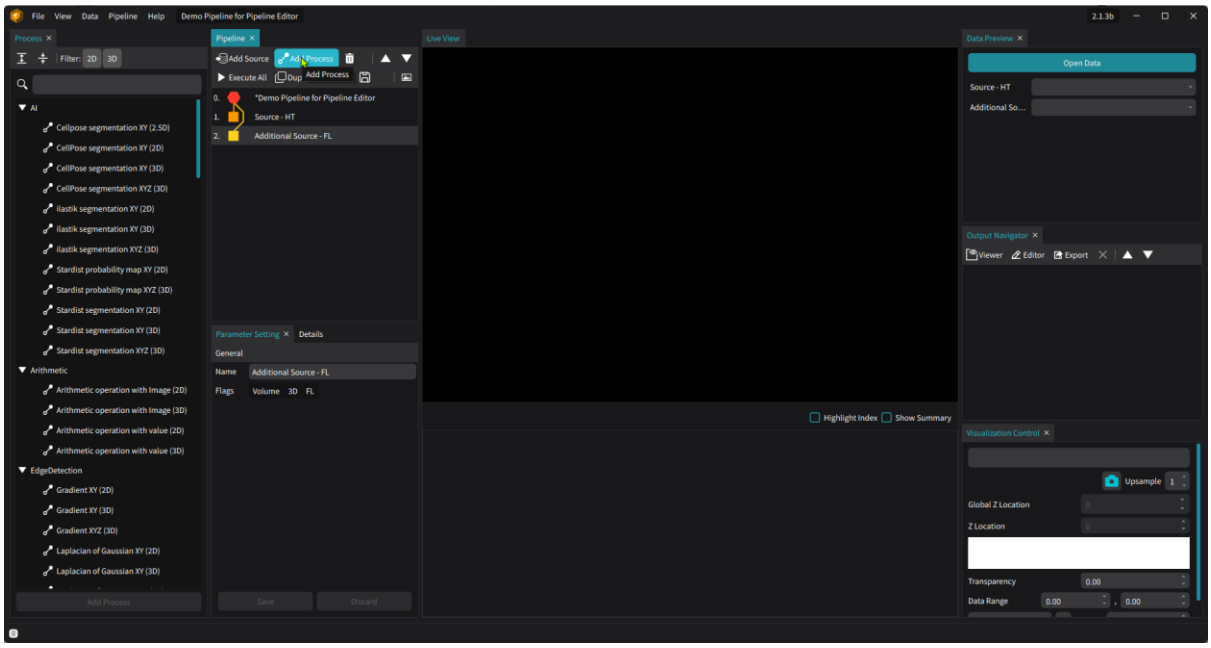
1. For each pipeline, it is necessary to specify the type of data in the TCF file that will be used for analysis. Click the **Add Source** button at the top of the **Pipeline** panel.
2. This enables the selection of input data, such as 3D HT, 2D HT, 3D FL, 2D FL.

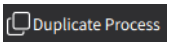


3. After selecting a data type, assign a name that will be referenced by the processes in the pipeline. Once named, click the **Add Source** button to register the source. This task can be repeated to add more data sources.

4.6 Adding Processes

After selecting one or more data sources, processes can be added to transform the sources into results.



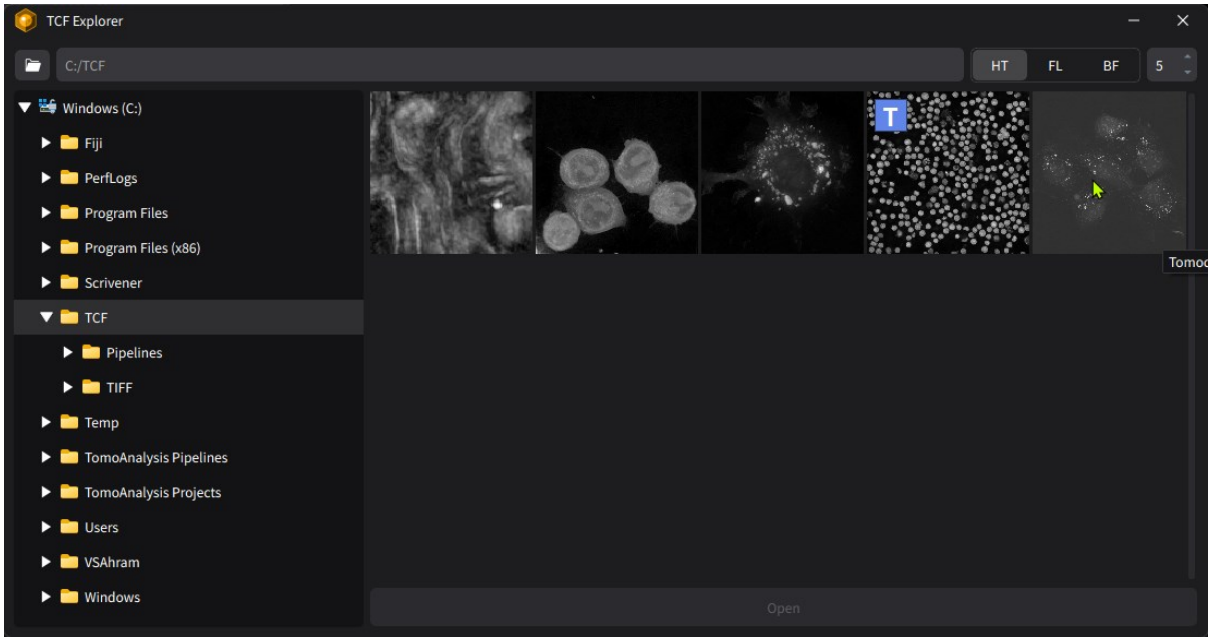
1. Click the **Add Process** button next to the **Add Source** button.
2. This opens a menu showing available processes, grouped into categories like AI, Arithmetic, and Edge Detection.
3. Choose a process by clicking on it (e.g., *"Stardist Segmentation XYZ (3D)"*), and it will be added to the pipeline.
4. To duplicate a process, click the **Duplicate** 

button after selecting the desired process. The duplicated process will appear below the original.
5. To remove a process, select it and click the **Remove**  button. Note that any processes dependent on the removed process will need to be updated.

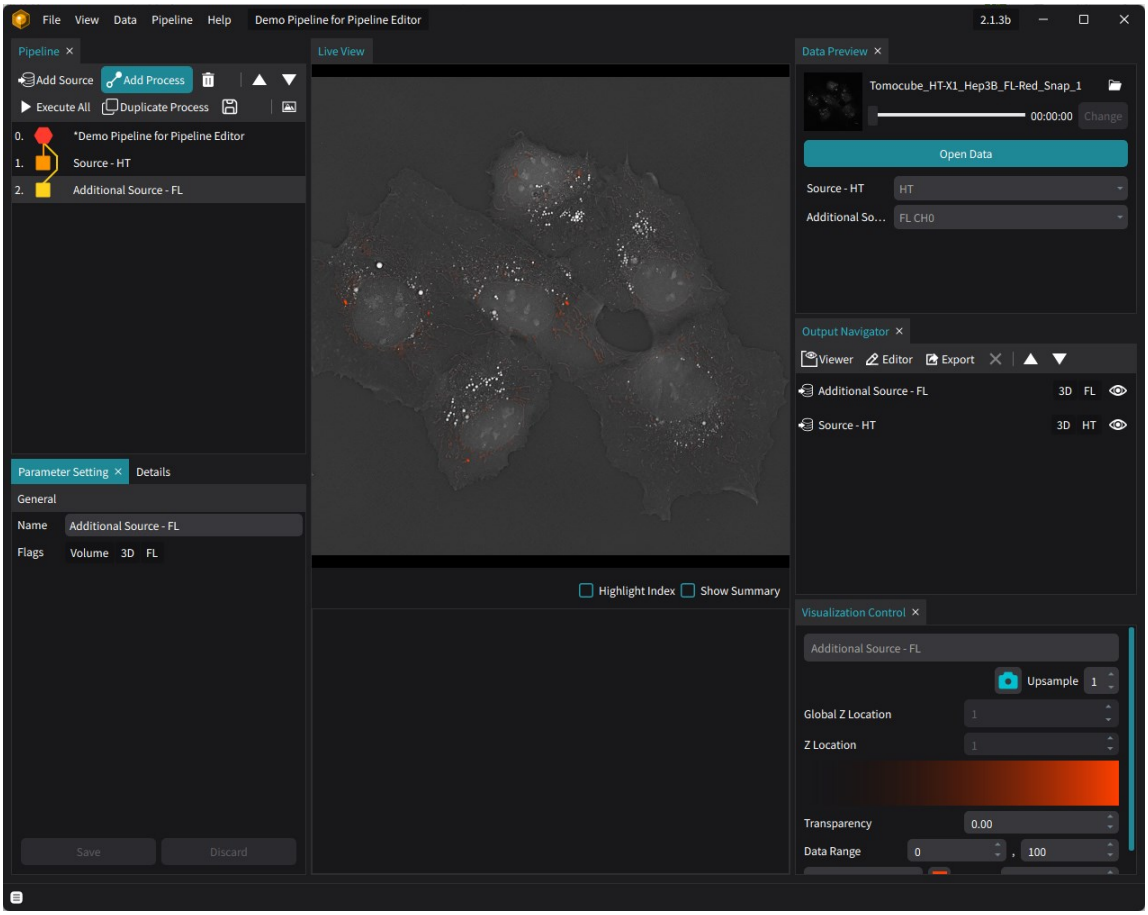
4.7 Loading Test Data

When creating a pipeline, it is important to test it with data to ensure it functions as expected.

1. Click the **Open Data** button on the **Data Preview** panel on the right side of the screen.

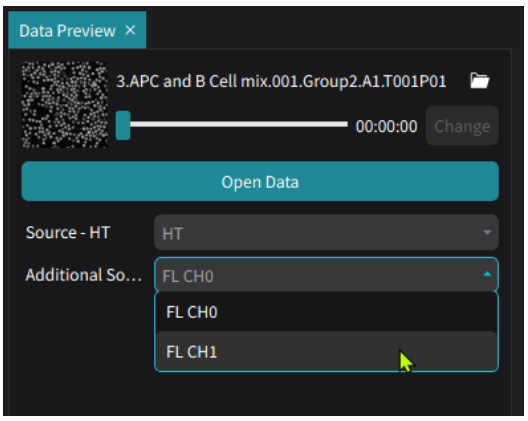


2. The **TCF Explorer** window will open, allowing the selection of a TCF file for testing.
3. After selecting a file, click the **Open** button to load the data into the pipeline test.



4. Once loaded, the relevant data channels registered as sources in the pipeline will automatically appear in the main window. The loaded data sources will also be listed in the **Output Navigator** panel on the right.

NOTE: If the TCF file contains multiple data channels corresponding to the source type registered in the pipeline, another channel can be selected using the dropdown menu.

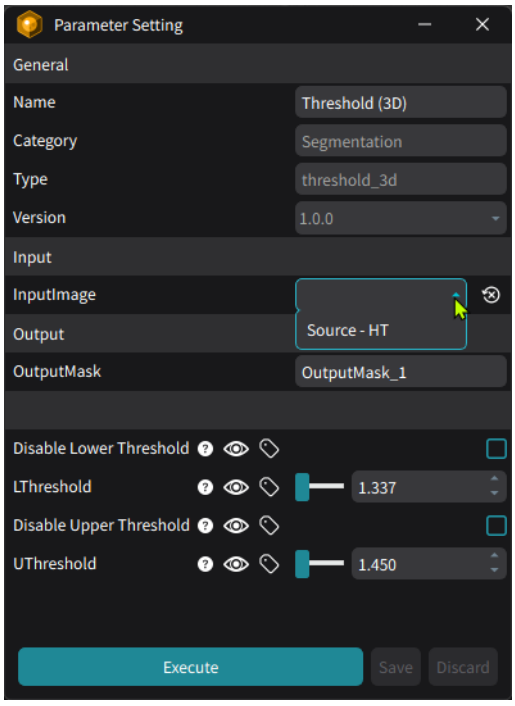


4.8 Adjusting Process Parameters

Each process has configurations for inputs, outputs, and parameters, which must be properly set for desired results.

Inputs

Each process requires one or more inputs to generate an output. Select a process in the pipeline to manage its inputs and outputs.



Since a process requires one or more inputs, the input must be assigned in the **Input** section of the **Parameter Setting** panel. By clicking the dropdown box in the Input section, the available data names will be listed. For example, the Threshold (3D) process requires one image type data as input, and the available data name, Source – HT, will be listed in the dropdown menu.

Output

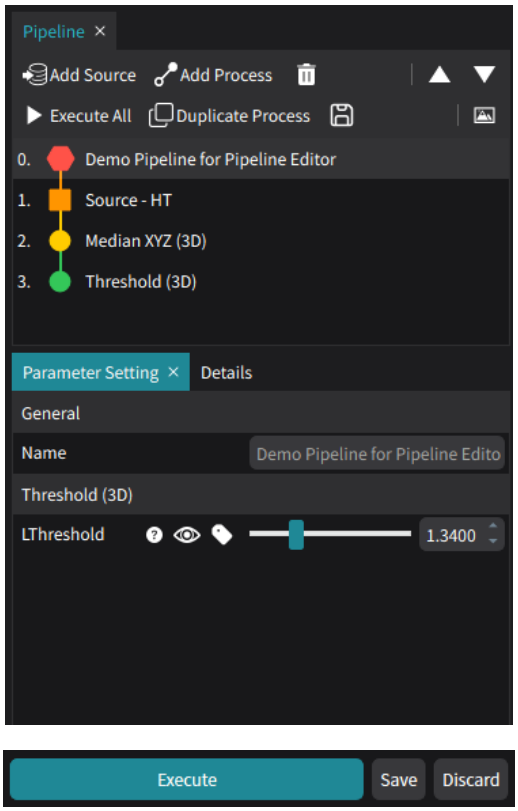
Each process produces one output upon execution, which can be renamed from the default. To rename the output, select the text box in the **Output** section and enter the desired name.

Parameters

Parameters vary by process and can be adjusted in the **Parameter Setting** panel. For details on specific parameters, refer to **Chapter 8. Processes.**

Show in Pipeline Summary

Among the parameter controls, there are the tagging icons for each parameter. This indicates if the process parameter is shown up in the summarized controls of the pipeline. The icon can be toggled to , where the former indicates that the process does not show up in the summary while the latter does.



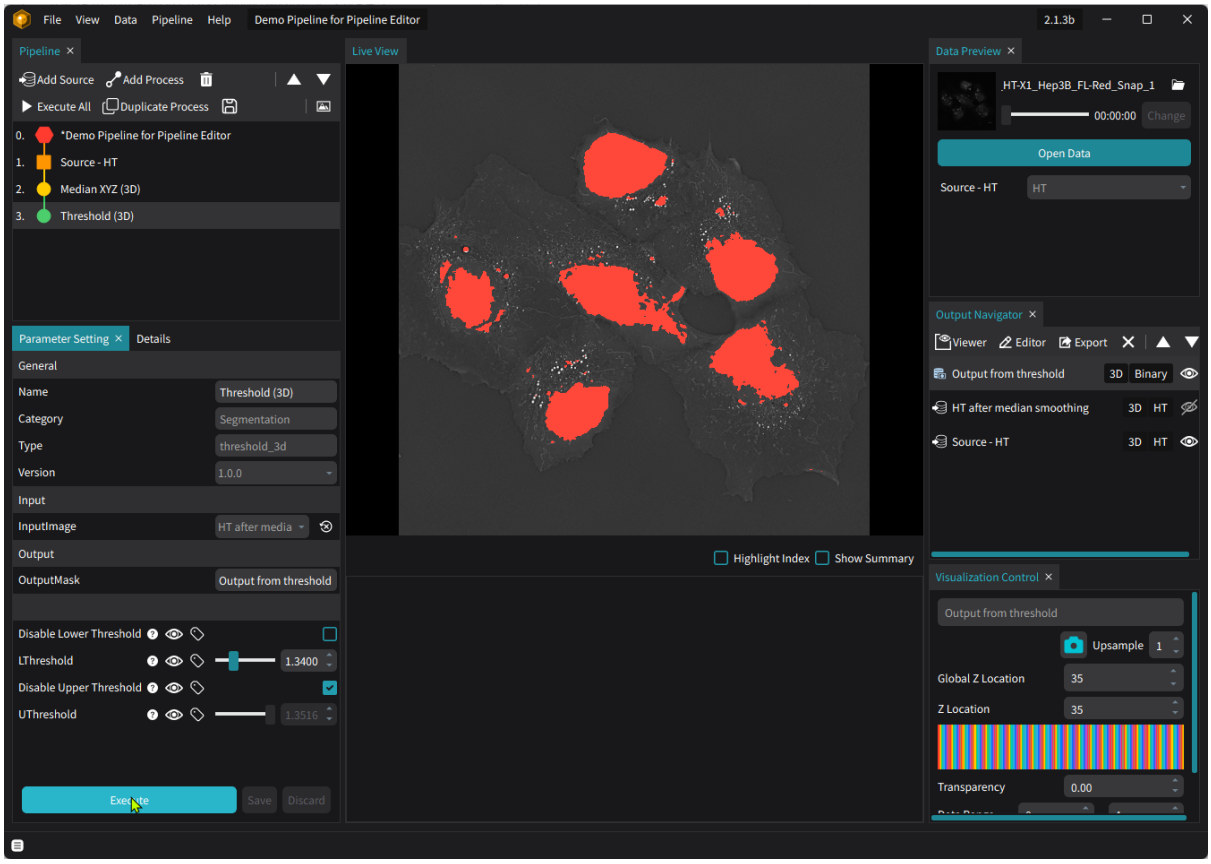
After configuring the inputs, outputs, and parameters, press the **Save** button to store changes. To test the process, press the **Execute** button.

4.9 Testing Processes

In the **Pipeline Editor** mode, any process added to the **Pipeline** panel can be executed individually.

After adjusting the process parameters, inputs, and outputs, click the Execute button in the **Parameter Setting** tab. If the output is image data (e.g., Image, Label, Mask), it will appear in the Live View panel. The output will be listed in the **Output Navigator** tab.

For example, when running the *"Threshold (3D)"* process with the output named *"Output from the threshold"*, the results will appear as follows.



NOTE: The process can be executed as many times as needed to find the optimal conditions. Parameters of other processes that affect or are affected by the current one can also be adjusted to achieve the best configuration for the entire pipeline.

To run all the processes in the pipeline sequentially, click the **Execute All** 

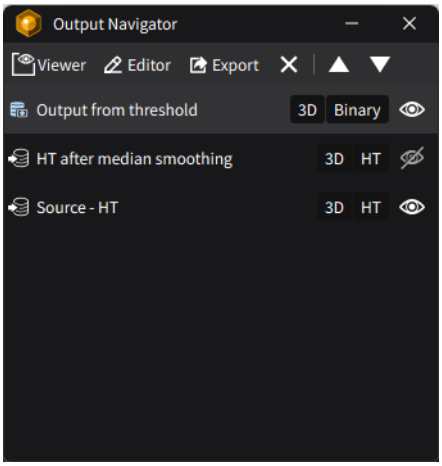
button on the pipeline toolbar. All the processes in the pipeline will be executed in descending order.

4.10 Adjusting Views

While editing the pipeline, results can be evaluated by comparing them with the source data or other outputs. All image-type outputs are displayed in the **Live View** panel, where users can adjust contrast and display order.

Stacking order

The most recently added image is stacked on top of others. Adjust the stacking order in the **Output Navigator** panel to make hidden layers visible.



The output at the top layer hides the other layers underneath. To move an output to the top and make it visible, click the **Up** button.

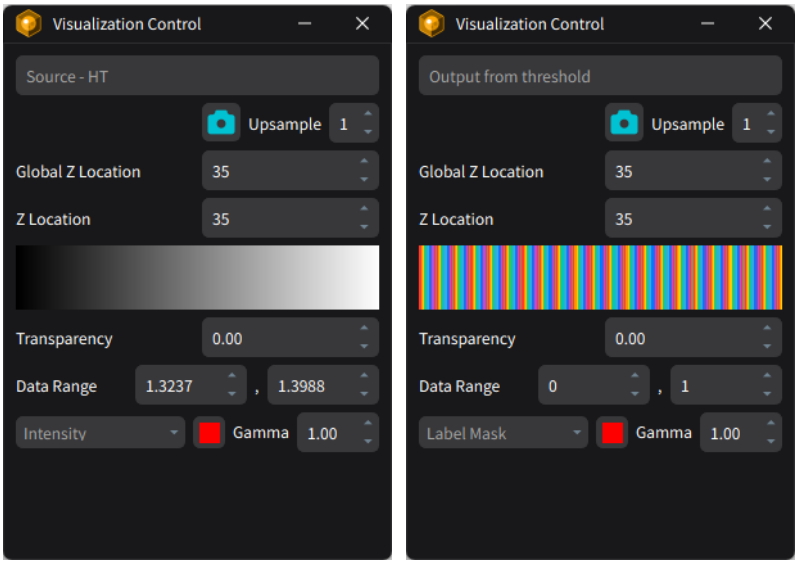
To hide the selected output beneath other layers, click the **Down** button.

To hide the selected output from the **Live View** panel, click the Show/Hide toggle button next to the output name.

- While the **Show/Hide** icon is set to Hide, the selected layer is ignored by the **Live View** panel.
- The layer will reappear when the icon is reverted to the Show state.

Image contrast

Select an output in the **Output Navigator** panel to adjust contrast and transparency in the **Visualization Control** panel.



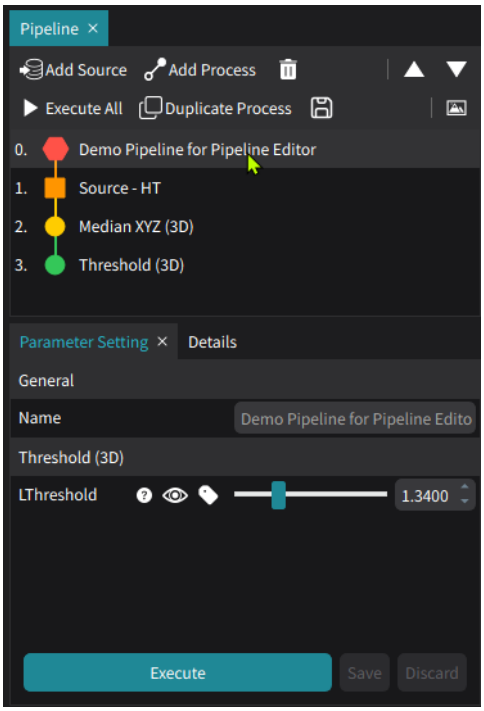
The transparency of the selected output can be controlled by the **Transparency** value, where 0 corresponds to a total opacity of the selected output and 1 to a total transparency.

The contrast of the image can be controlled by assigning the lowest and highest value of the image from the **Data Range** section that is mapped to the lowest and highest intensity value of the chosen look-up table, respectively.

The look-up table can be changed by selecting the color for the table, where the gamma value can be applied to fine-tune the intensity change of the look-up table.

4.11 Authoring a Pipeline

After completing pipeline edits, additional descriptions or other information may need to be added.

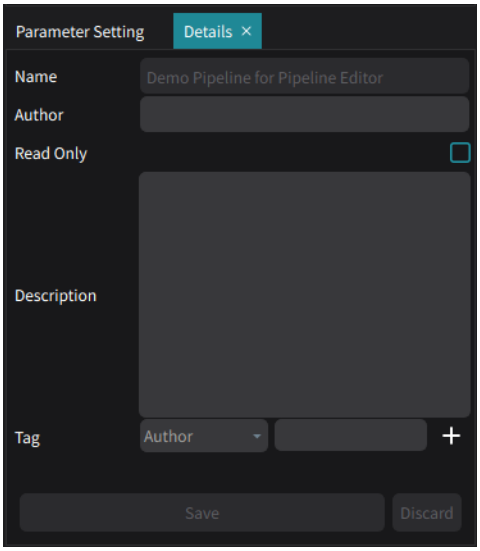


Summarized Parameters

Click the pipeline name at the top of the process list to show a summary of selected parameters in the **Parameter Setting** panel. To add or remove process parameters in the **Parameter Setting** panel, refer to **Chapter 4.8. Adjusting Process Parameters**.

Pipeline Information

To add details such as author information, description, and tags for easier searching, select the **Details** tab. The pipeline also be set to **Read Only** to prevent overwriting.



After adding the necessary information, press the **Save** button to store changes.

Chapter 5. Analysis Perspective

5.1 Introduction

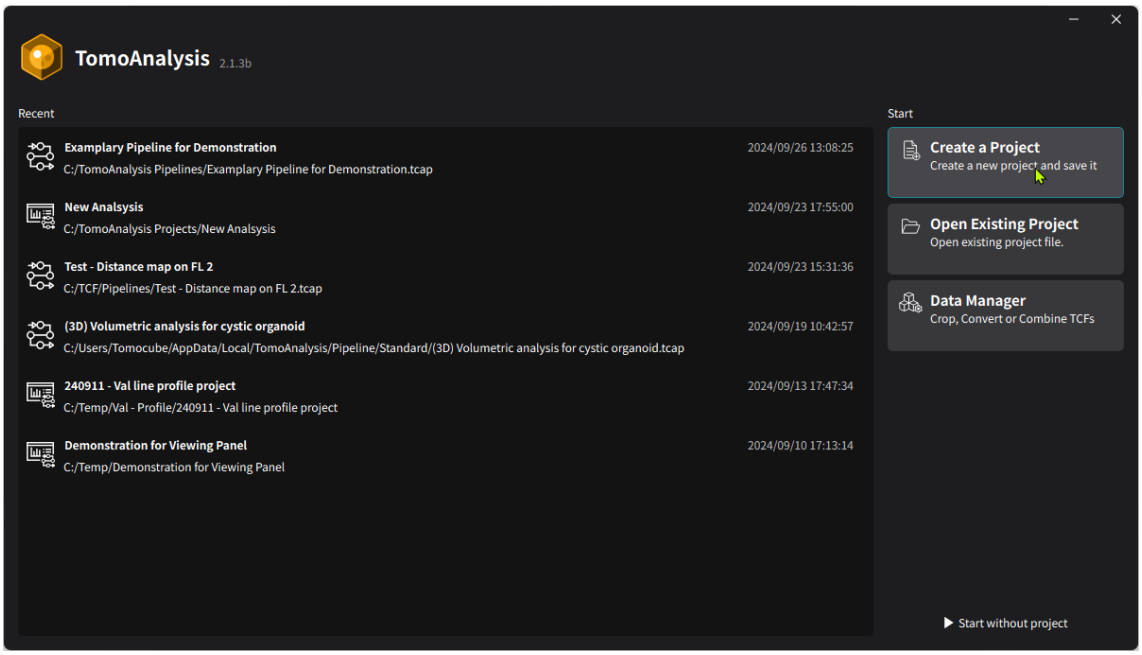
When analyzing TCF data, **TomoAnalysis** can produce results by applying analysis pipelines. Since the pipeline can be applied to multiple datasets, **TomoAnalysis** provides batch processing through the **Analysis Perspective** mode.

In the **Analysis Perspective**, it's possible to select datasets for analysis, choose an analysis pipeline, and adjust the parameter sets to produce optimal results from the batch processing. After generating the results, they can be reviewed and exported for further analysis.

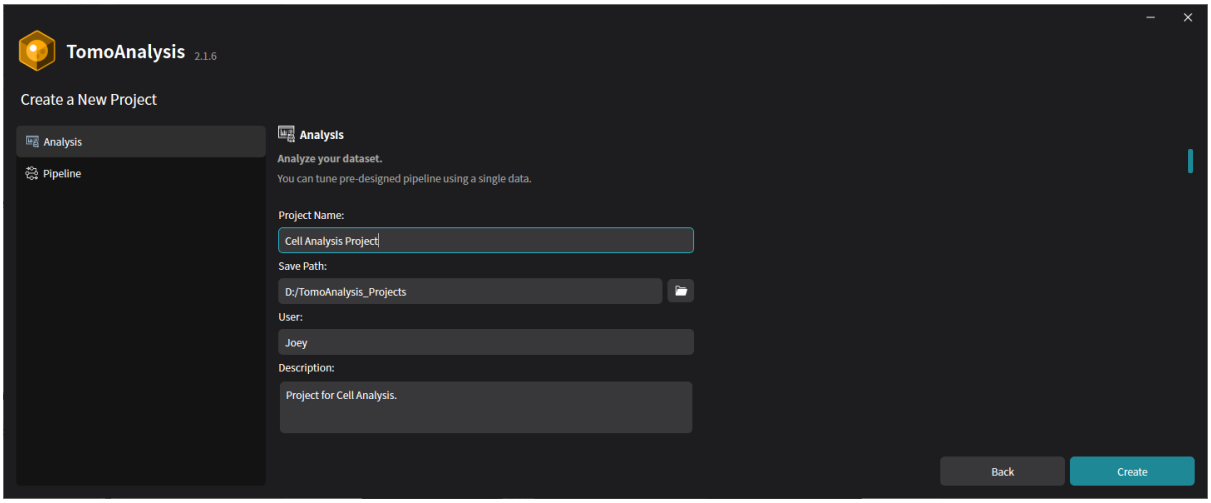
5.2 Creating a New Analysis Project

To create a new analysis project, follow these steps:

1. Start the software. The initial interface displays options for creating new projects, opening existing projects, and accessing **Data Manager** which can crop TCF files.

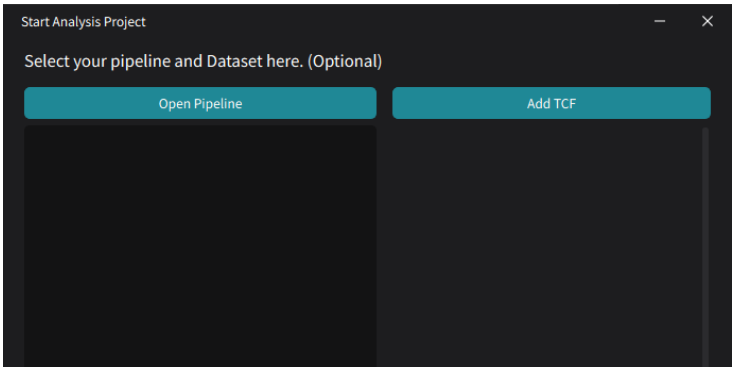


2. Click the **Create a Project** button in the right panel to make a new analysis project.

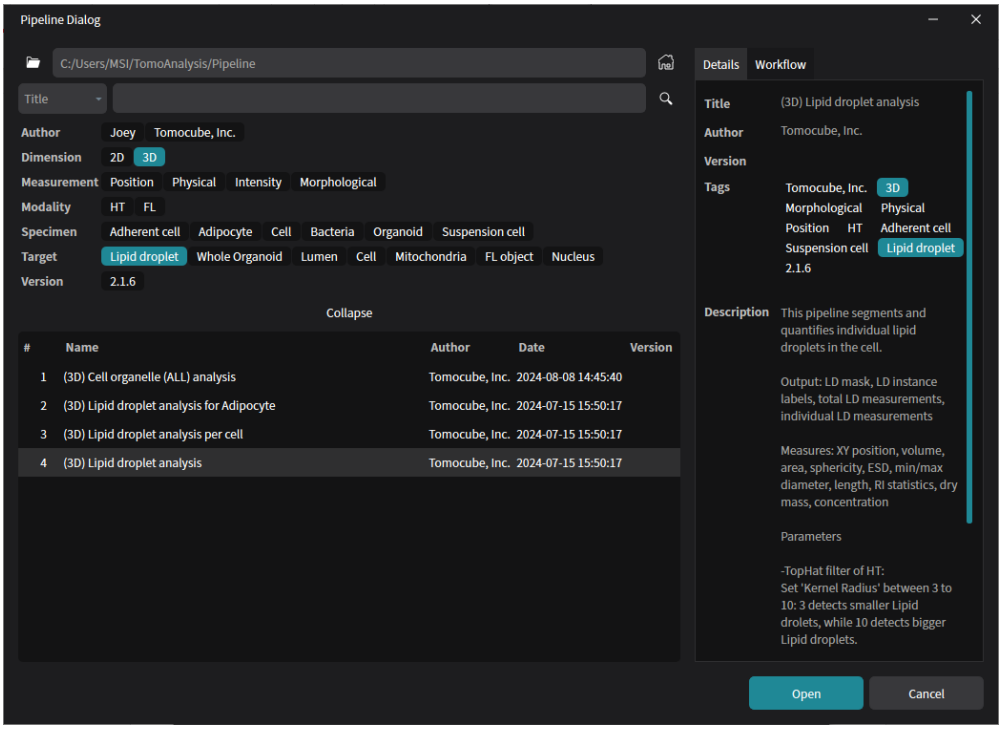


3. On the **Create a Project** window, select the **Analysis** menu from the left tab.
4. Enter a suitable project name and the folder path where it will be saved. Optionally, add user information and a description for this project, both of which can be edited later in the **Analysis Perspective.** After completing the required fields, click the Create button to proceed to the main screen of the **Analysis Perspective.**
5. (Optional) Before proceeding to the main screen of the **Analysis Perspective**, the user can select a pre-defined analysis pipeline and the TCF data files to begin the analysis. On the left side is the section for selecting an analysis pipeline and the right side is for registering TCF data files.

NOTE: It is optional to select an analysis pipeline and TCF data files at this stage. These selections can be made on the main screen. To skip this step, proceed directly to step 10.



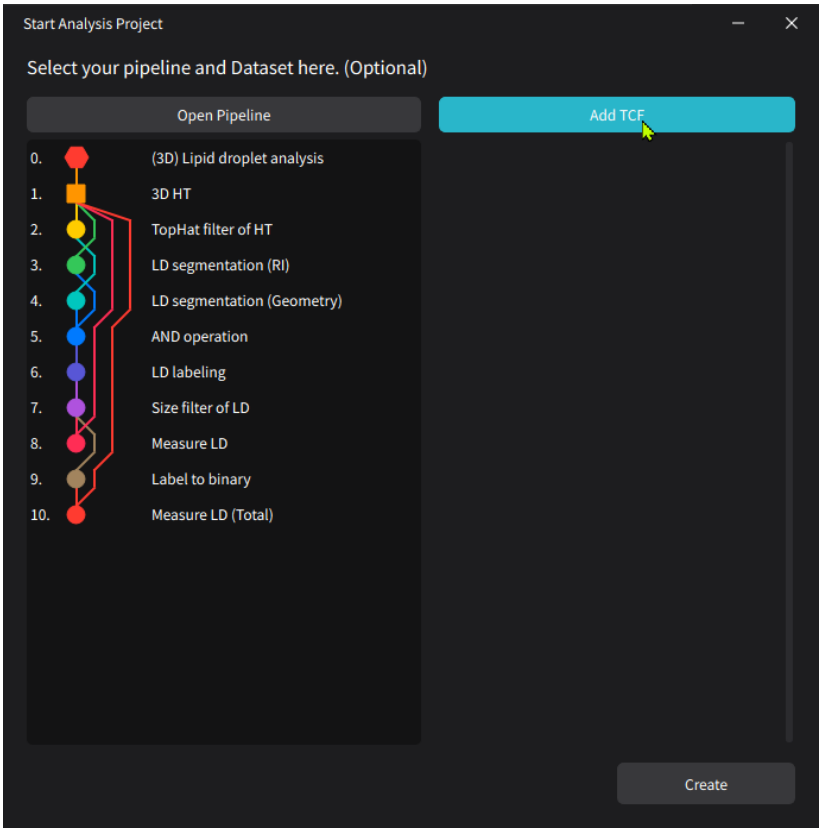
6. Click **Open Pipeline** to choose a pre-defined analysis pipeline from available pipelines.



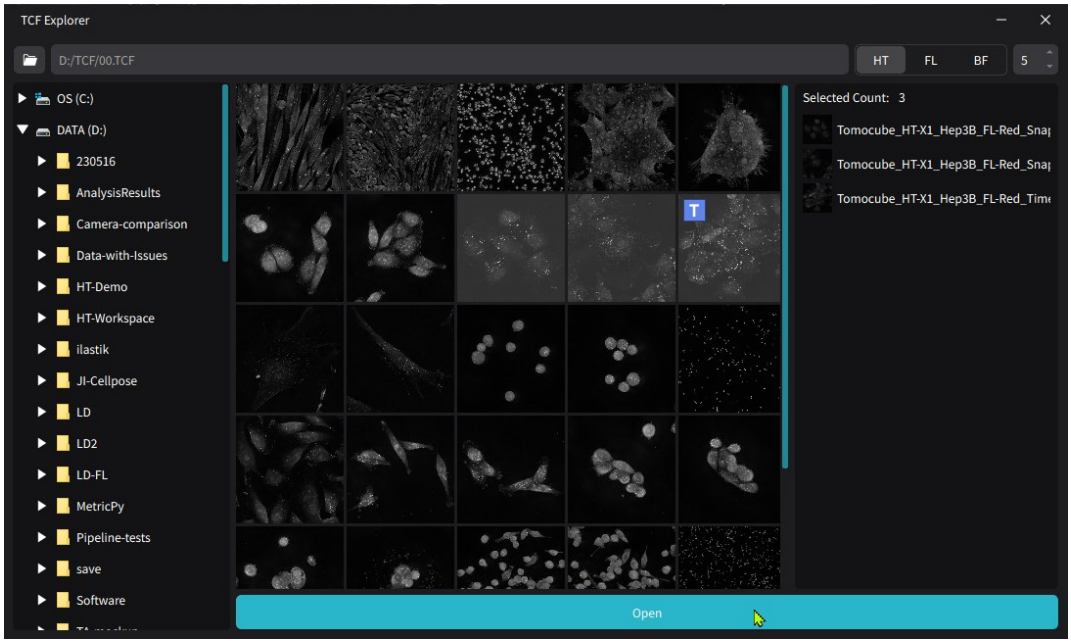
7. In the **Pipeline Dialog** window, select one of the listed pipelines. If the pipeline is in a different folder, the directory can be changed by clicking the **Folder** icon at the top. To return to the default directory, click the **Home** icon. When a pipeline is selected, information about it will be displayed in the **Detail** panel on the right, and the **Workflow** tab will show the pipeline's structure.

   If the chosen pipeline is suitable, click the **Open** button to proceed.

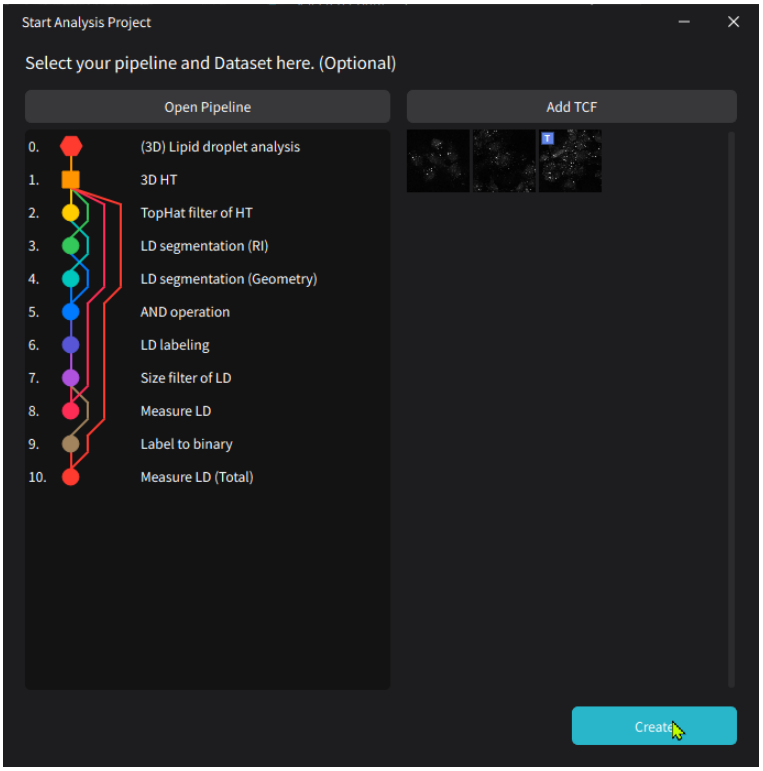
NOTE: Pipelines can be filtered using the search box or by selecting **Tags** at the top of the window.



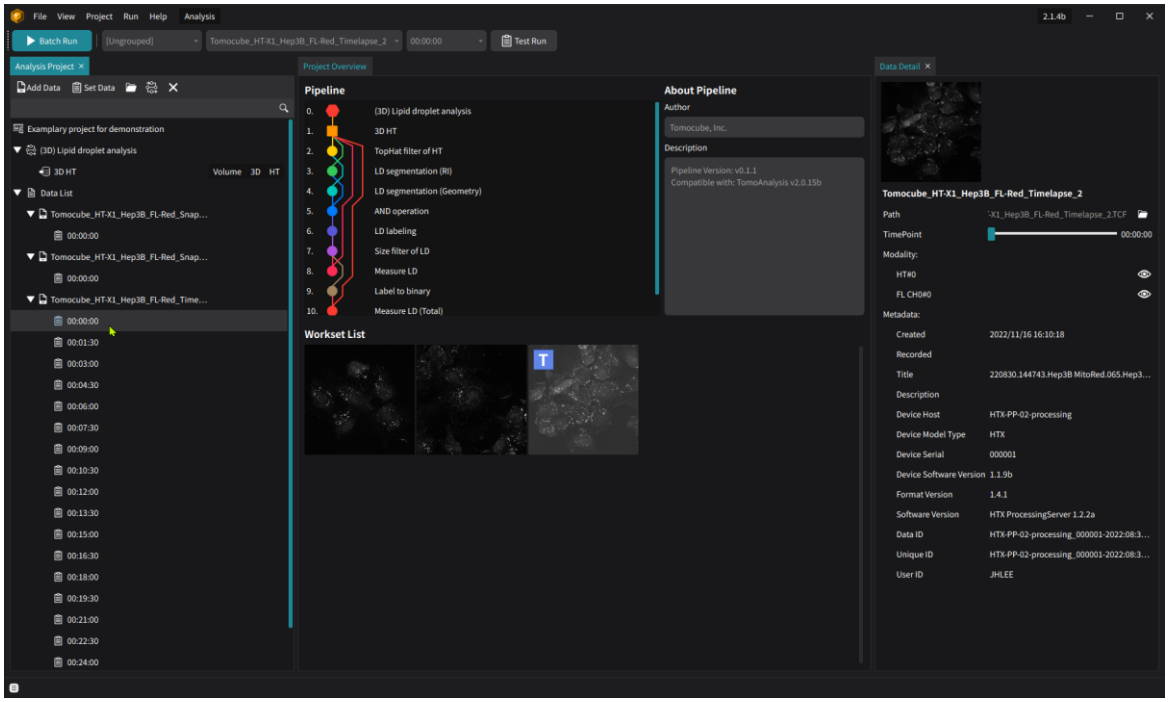
8. After selecting the pipeline, TCF data files can also be registered in this window. Click the **Add TCF** button to open the **TCF Explorer** window, and change the directory using the **Folder**  icon or **Explorer** tab on the left. Select one or multiple data files, using the **Ctrl** and **Shift** keys in the **TCF Explorer** panel.



9. Press the **Open** button to register the selected TCF data files in the analysis project.



10. Once the analysis pipeline and TCF data files are selected, click the **Create** button to finalize the initiation of the analysis project.

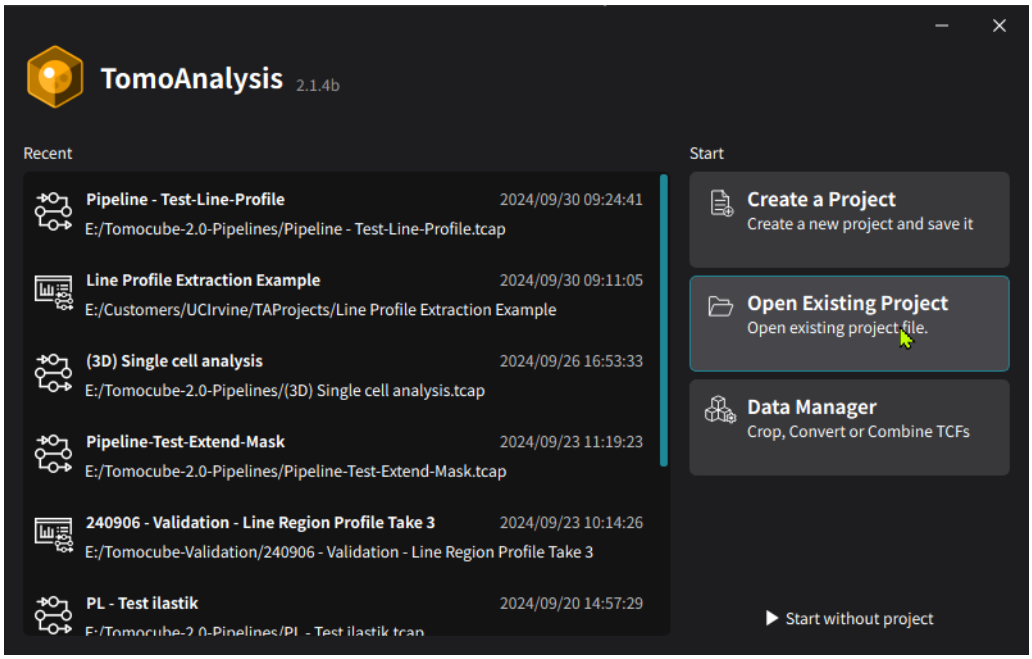


11. The main screen for the **Analysis Perspective** will now appear, and it's ready to begin the analysis.

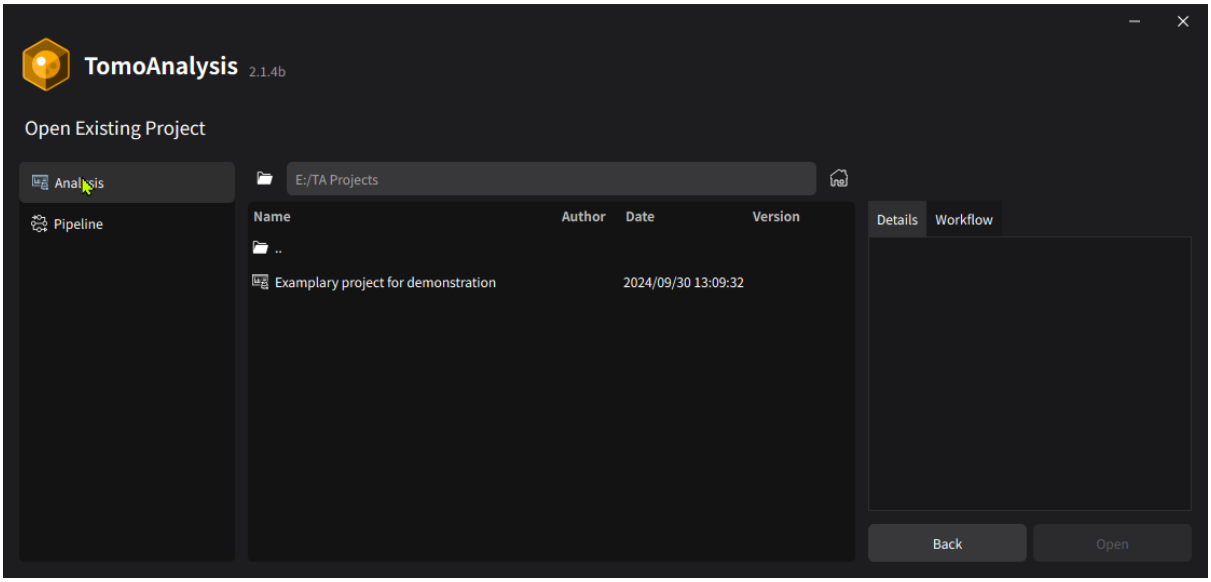
5.3 Opening an Existing Analysis Project

The user can open existing analysis projects to review or rerun them. To open an analysis project in **TomoAnalysis**, follow these steps:

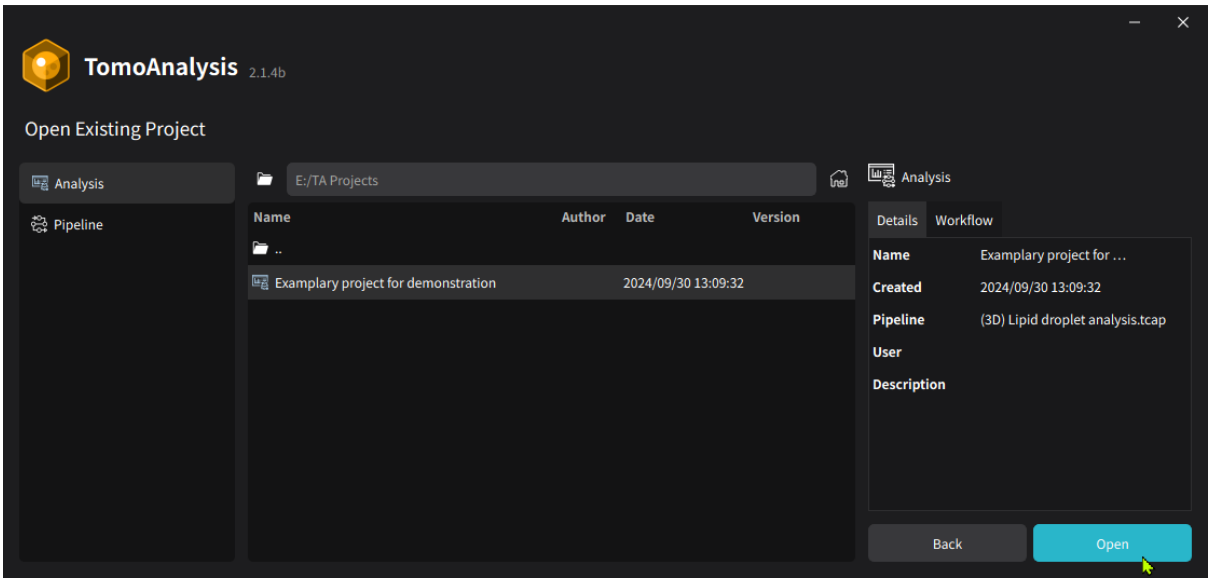
1. Start the software.



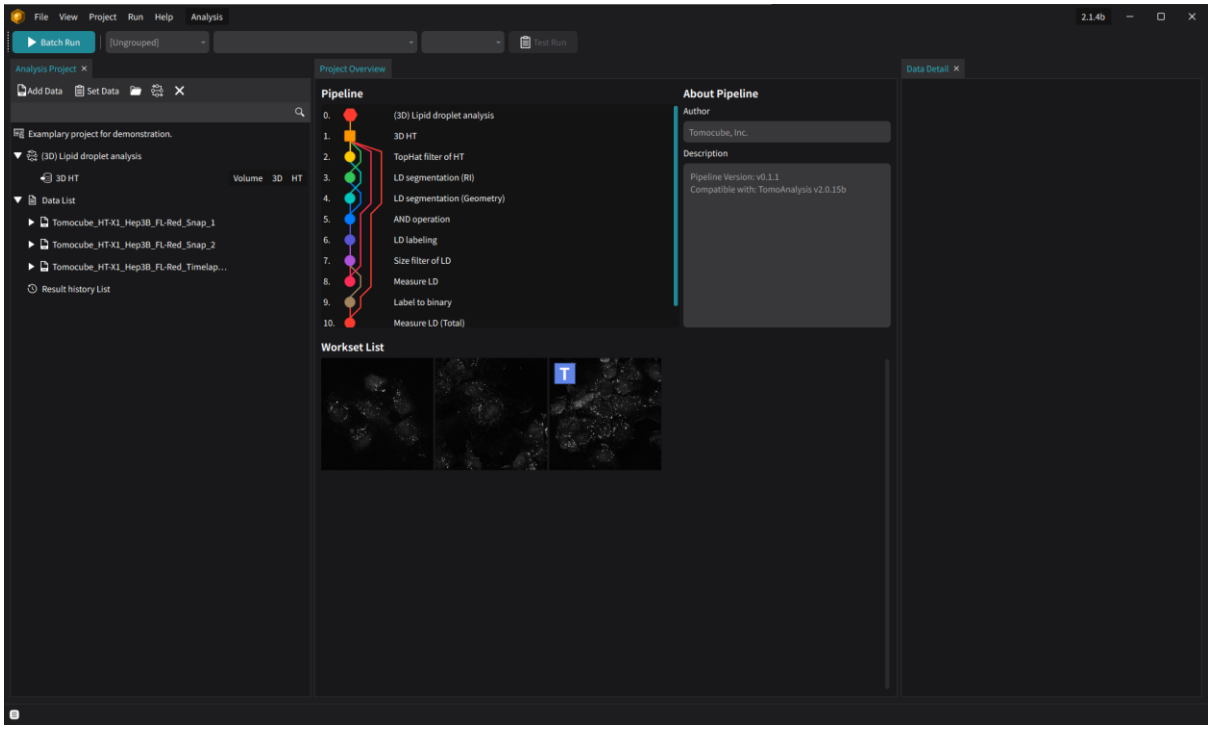
2. Click the **Open Existing Project** button.



3. In the **Open Existing Project** window, select the **Analysis** menu from the left tab. A list of available analysis projects will be displayed. The directory of the listed projects is shown at the top of the middle section. The user can change the directory by clicking the **Folder** icon. The project list will be updated according to the changed directory.



4. When a project is selected from the list, details of the selected project—including its name, creation date, assigned pipeline, username, and description—are displayed on the right side of the window.

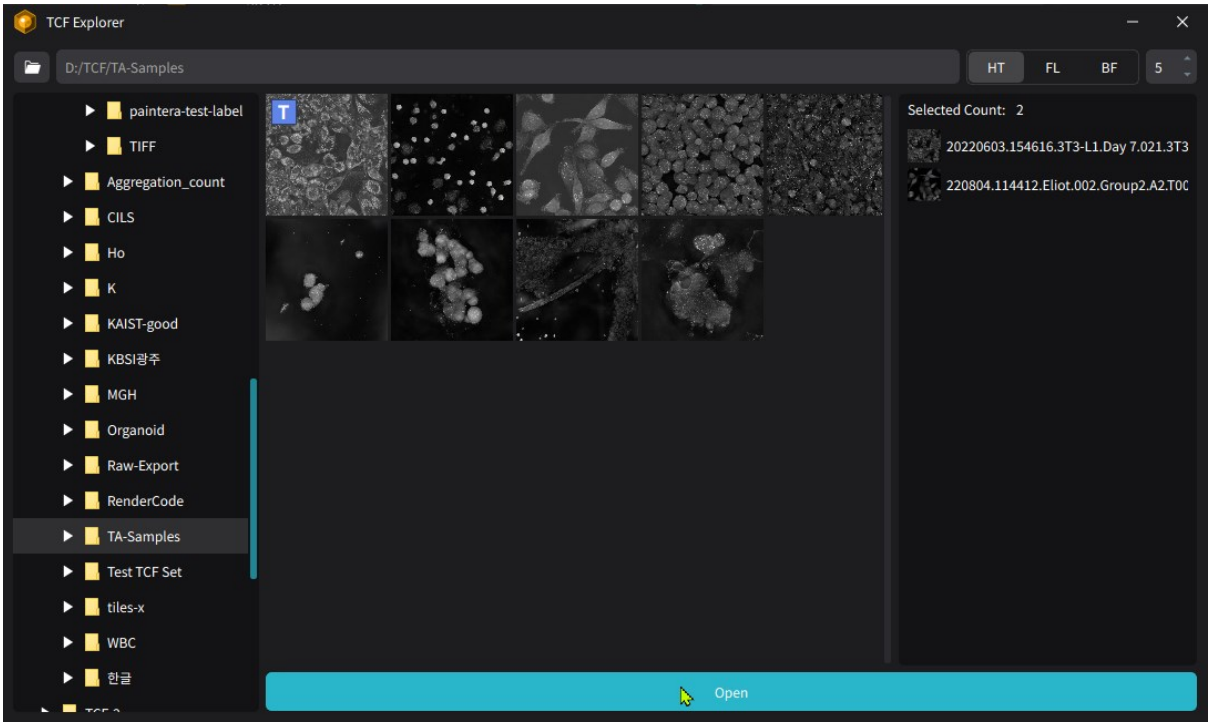


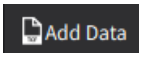
5. To open the selected project, press the **Open** button. This will open the main window for **Analysis Perspective**, and ready to continue the selected project.

5.4 Loading TCF Files into the Analysis Project

In the main window of the **Analysis Perspective**, additional TCF files can be added to an existing project, even if there are already TCF files registered in the **Data List**.

To register additional TCF files:

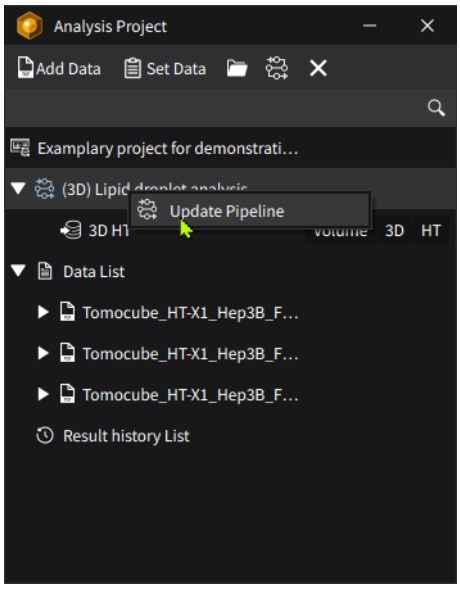


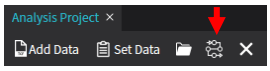
1. Click the **Add Data** 

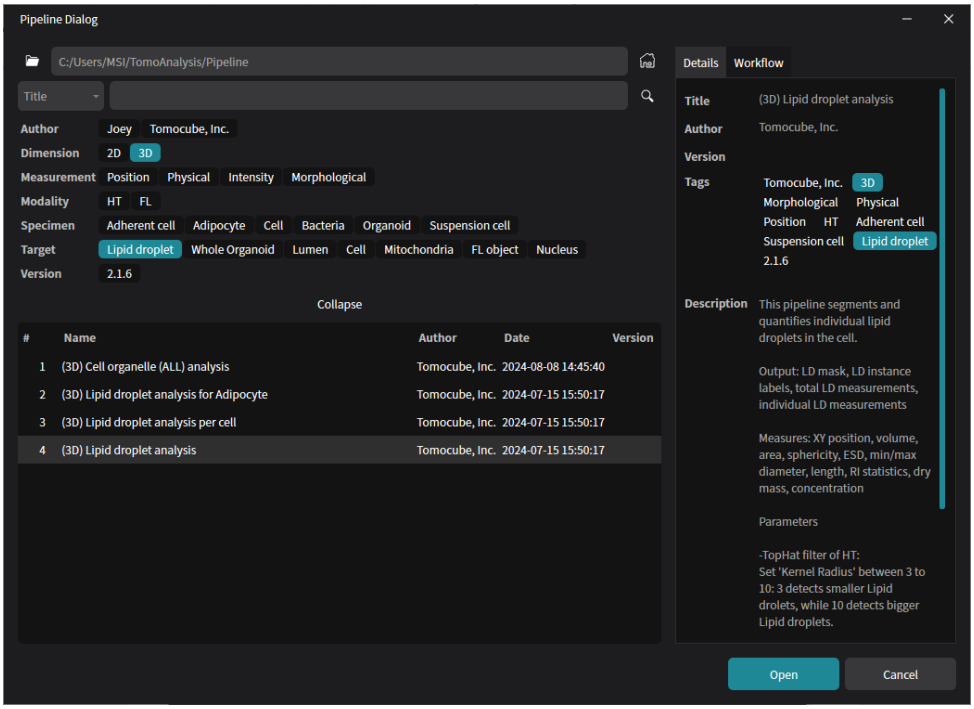
button to open the **TCF Explorer** window.
2. In the **TCF Explorer** window, select the desired TCF files by clicking their thumbnails. Use **Ctrl** or **Shift** keys to select multiple files.
3. The user can also browse TCF files from a different folder by selecting a folder from the left panel.
4. After selecting the desired TCF files, click the **Open** button to register the files to the Data List in the main window.

5.5 Changing the Analysis Pipeline

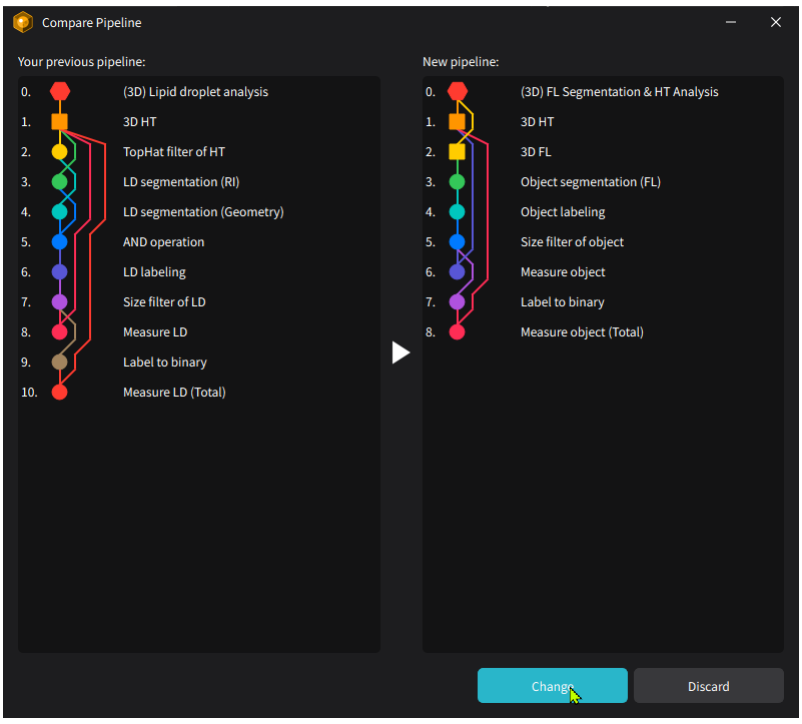
The analysis pipeline selected for the analysis project can be changed if needed. To change the pipeline:



1. Click the **Update Pipeline**  icon, or Right-click on the registered pipeline to open a pop-up menu and select the **Update Pipeline**.



2. The **Pipeline Dialog** window will open, allowing the selection of a new analysis pipeline.

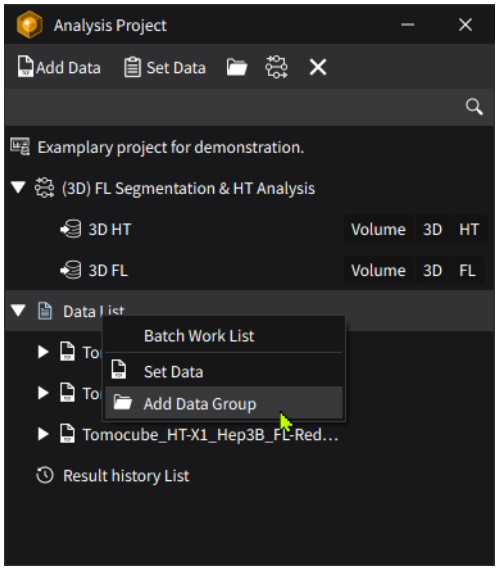


3. If a new pipeline is selected, the structure of the old and new pipelines will be compared for review. Click the **Change** button to apply and replace the pipeline for the analysis.

5.6 Organizing Data and Worksets

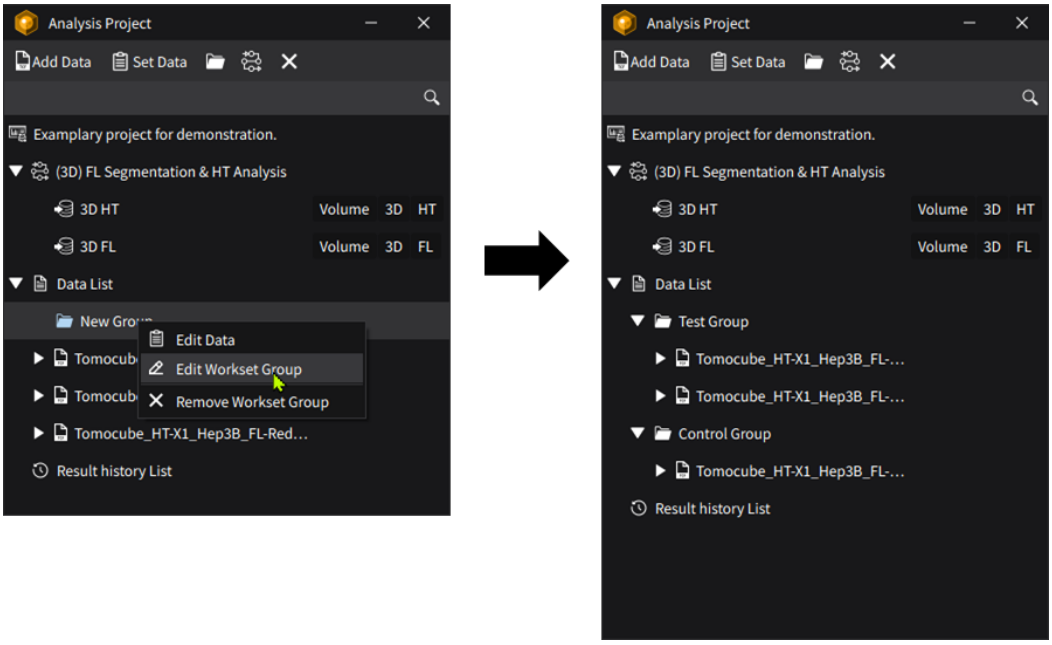
5.6.1. Workset Groups

In the **Analysis Perspective** window, TCF data files are registered in the **Data List** section. The TCF data can be grouped according to the experiment and acquisition setup. This group setting will be applied to the summary dashboard of analysis result.

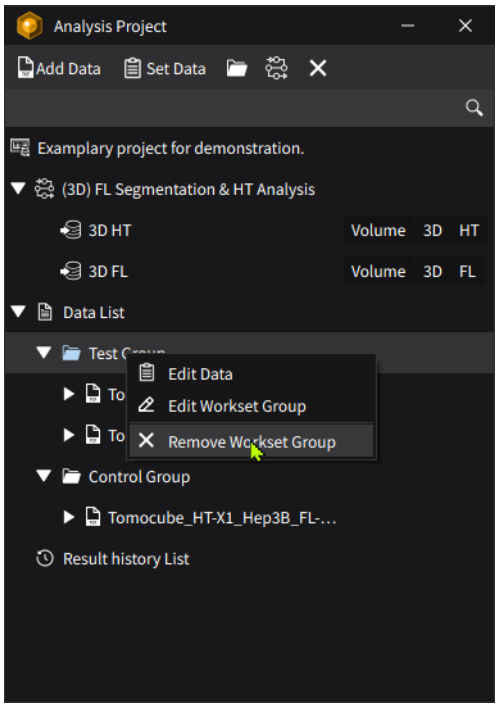


To create a group, click the **Add Data Group**  icon on the toolbar at the top of the **Analysis Project** panel.

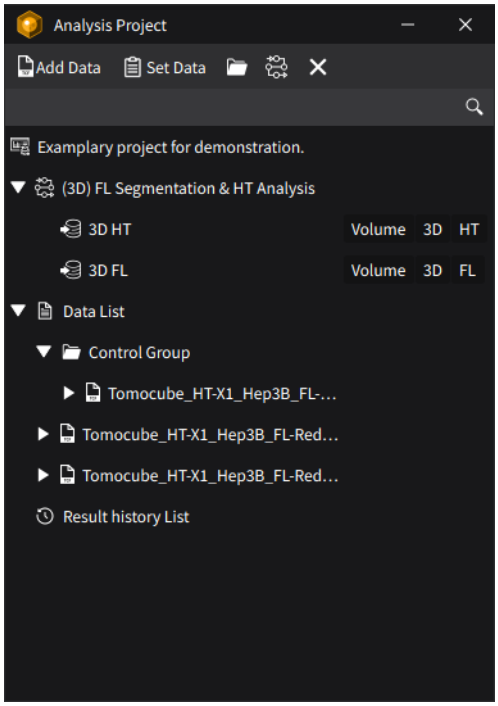
Or Right-click on the **Data List** in the **Analysis Project** panel and select **Add Data Group** for the same operation.



A new group will appear in the **Data List** section, and its name can be re-named by double-clicking on its name, or right-clicking on it and selecting **Edit Workset Group** option from the list.



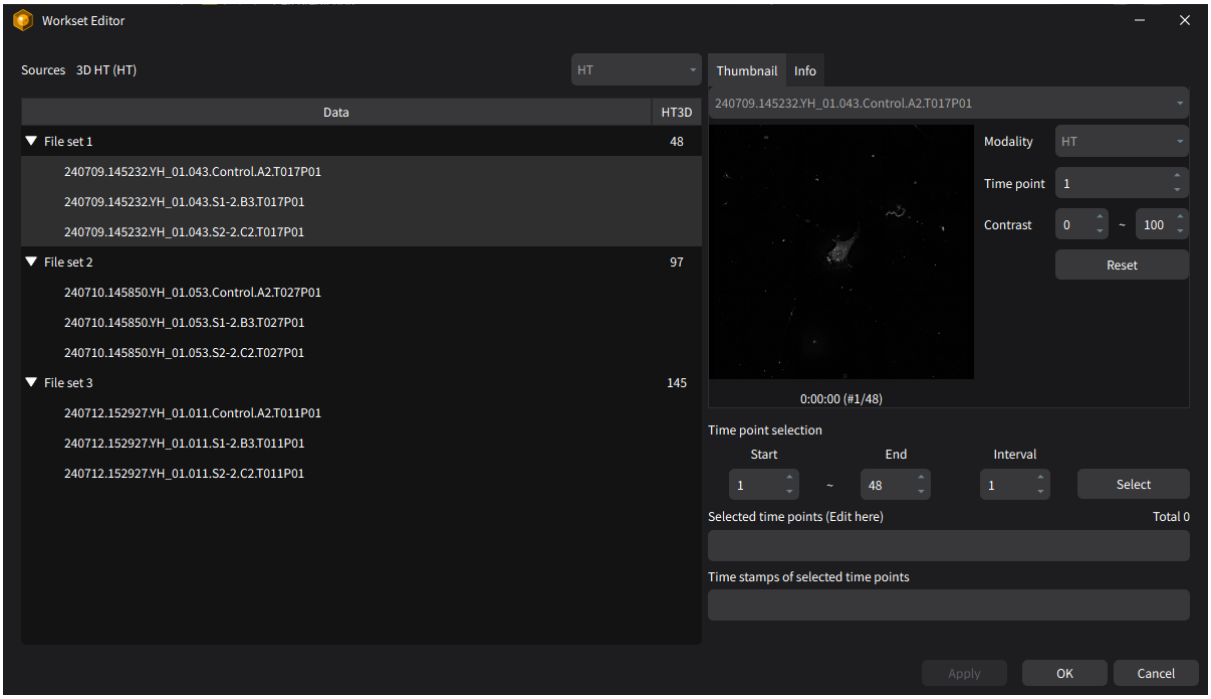
The data can be added in the group by selecting one or multiple data in the **Data List**, and drag & drop on the desired group.



To remove the workset group, right-click on the group name and select **Remove Workset Group**. The workset group will be deleted, and all TCF data files within the deleted group will be set as ungrouped.

5.6.2. Time point Selection in Timelapse TCF Data

For the timelapse data, all available time points will be added in the **Data List** as a default. Specific time points can be selected and used to reduce analysis time or run a test analysis. Use the **Workset Editor** to select the time points and channels.



1. To open the **Workset Editor**, click the **Set Data**  button located on the toolbar at the top of the **Analysis Project** panel.
2. The TCF data files in the **Data List** are now displayed on the left side of the **Workset Editor**. TCF files with the same data structure (e.g., X, Y, Z dimensions, modality combinations, and time points) are grouped as a **File set** in the **Workset Editor**.

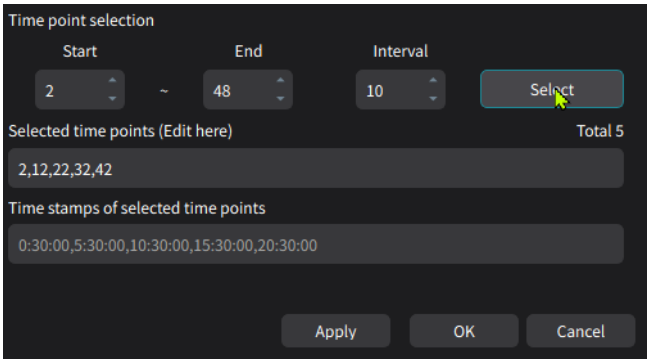
NOTE: These **File sets** are different from the **Data group.**

3. Select one or more files by clicking on them using **Ctrl** or **Shift** keys. Selecting a File set will select all files within that set.
4. When a TCF file is selected, its thumbnail and brief dimensional information are displayed on the right side of the window.
5. Designate time points by specifying the **Start**, **End**, and **Interval**. Click the **Select** button to populate the exact time points.

6. Review the selected time points in the **Selected time points** textbox. After confirming, click the **Apply** button to confirm the selection.
7. To update the time point selection in the main window, click the **OK** button at the bottom right of the **Workset Editor** window.

Select time points by interval in the range

When selecting the time point, the primary method is to define the start, end, and interval for time points.



Options	Description
Start	First time point to select. The start time point is always selected.
End	Last time point to select. The end time point may not be selected if the interval does not fit the last time point.
Interval	The interval for selecting time points from the Start time point.

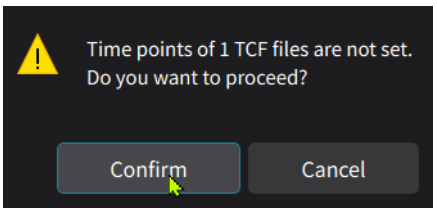
After assigning the **Start**, **End**, and **Interval**, click the **Select** button to generate the exact time points. The selected time points will be displayed in the **Selected time points** textbox, while the actual timestamps corresponding to selected time points will be shown below it. After verifying that the selected time points and timestamps are correct, click the **Apply** button to confirm the selection.

Once the selection of time points is applied, the TCF files updated with the selection will be marked with a check icon ☑ next to their file names.

Select time points by manual input

If specific time points that cannot be selected using a simple interval need to be chosen, the time point lists can edit the **Selected time points** textbox directly. To manually select the time points, enter the desired time points, separated by commas and hyphens (e.g., “1-4,7,9” will return a time point sequence with “1,2,3,4,7,9”). The **Timestamp** textbox will automatically update if the time point selection is valid. After verifying that the selected time points and timestamps are correct, click the **Apply** button to confirm the time point selection for the chosen TCF data files.

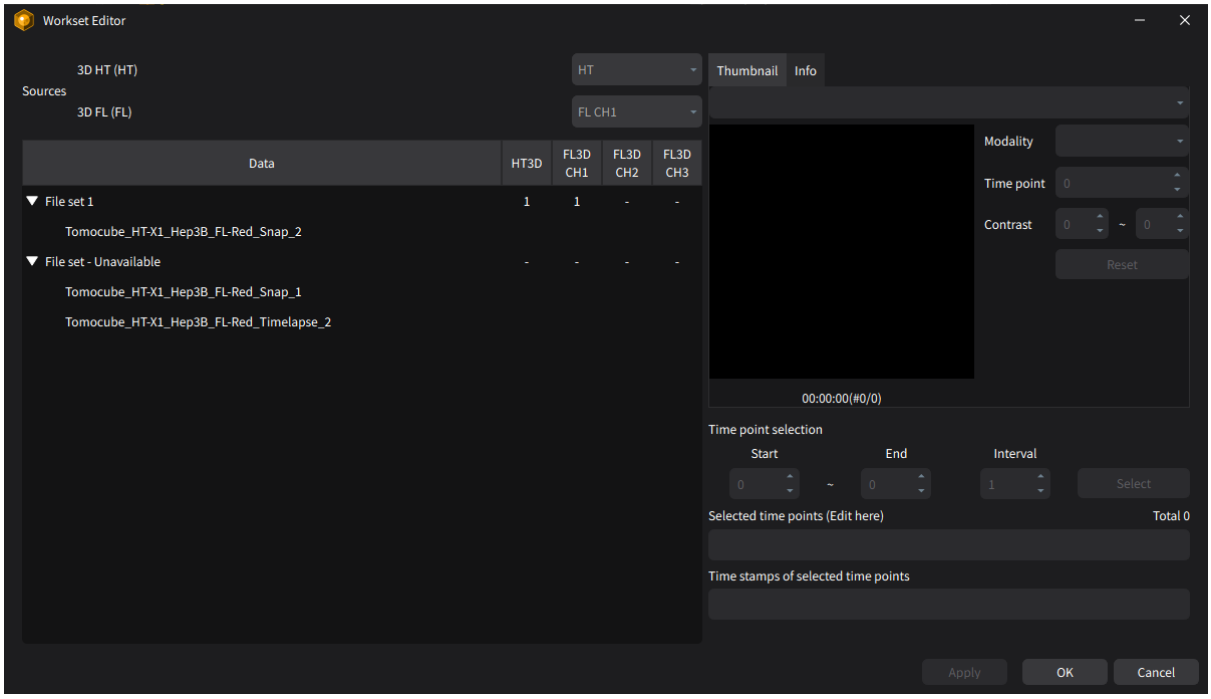
NOTE: Clicking **the Apply** button can only record changes temporarily. To update the **Data List,** Click the **OK** button. If the **Workset Editor** window is closed without clicking the **OK** button, the changes will not be applied to the **Data List.**

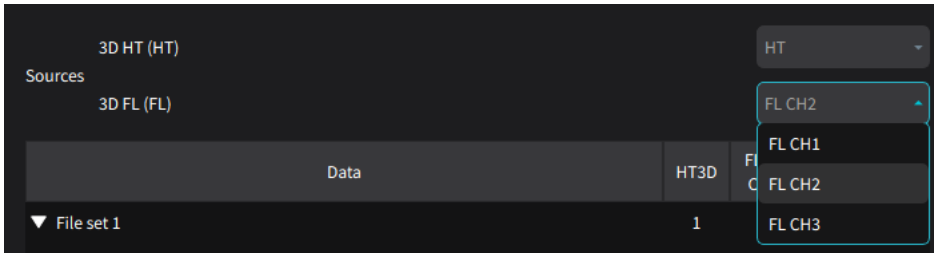


NOTE: Some of the TCF data files can be left without assigned timestamps for analysis. If this occurs, a notification will be displayed. The user can choose to leave them unassigned and click **Confirm**, or return to the **Workset Editor** by clicking **Cancel**.

5.6.3. Multiple Data Channels

Depending on the analysis pipeline, multiple data sources may need to be assigned. The **Workset Editor** displays the required data sources for the pipeline at the top left side of the panel.



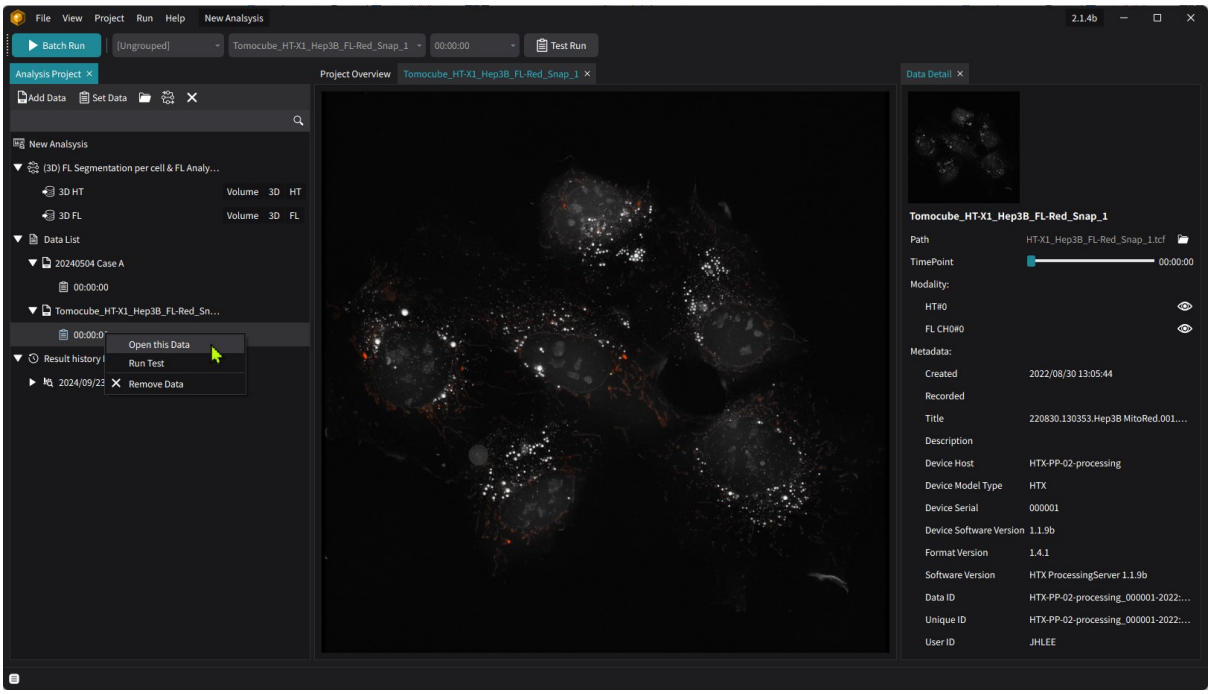


- If multiple data sources are required, the **Sources** section will show a row for each required data source, each corresponding to a specific data type (e.g., 3D HT or 3D FL).
- A valid data channel available from the TCF files must be assigned for each source using the dropdown menu.
- After assigning data channels, reassign the time points for each TCF file or set.
- Invalid TCF data files (e.g. TCF files that do not contain some of the required sources) will be grouped as Unavailable Sets and cannot be selected.

NOTE: Changing the source assignment after assigning some files will not automatically reset the assigned time points. Please reassign if needed.

5.6.4. Preview of Selected Workset

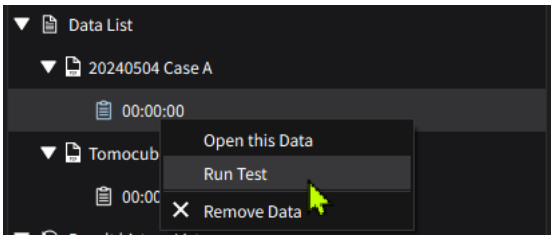
After selecting time points for the TCF files in the **Workset Editor**, the **Data List** will update to reflect these changes.



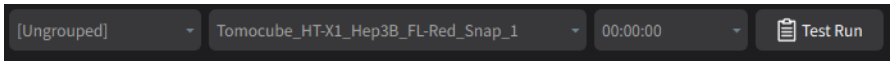
The tree structure of the **Data List** can be expanded to display the selected time points of the TCF files. Additionally, the thumbnail preview of the listed worksets can be provided by right-clicking on the time point in the **Data List** and selecting **Open this Data** will display the thumbnail and its detailed information on the right side of the main window.

5.7 Test Run Mode: Testing Pipeline with Selected Workset

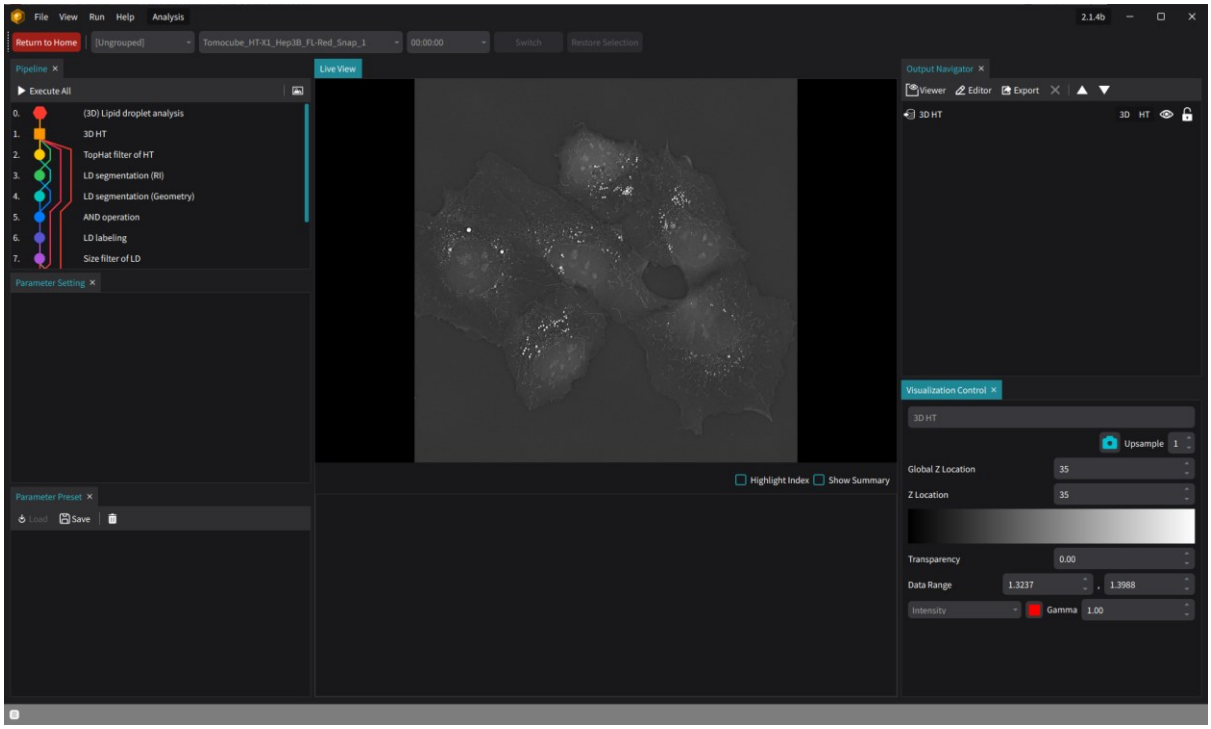
After the worksets are assigned in the **Data List**, users can test the compatibility and settings of the pipeline to find the appropriate parameter sets for analysis worksets using the **Test Run** mode. To activate the **Test Run** window, select one of the worksets in the **Data List**, right-click on it, and select the **Run Test** menu.



Test Run mode can also be activated via the navigation bar at the top of the main window by pressing the **Test Run** button.

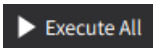


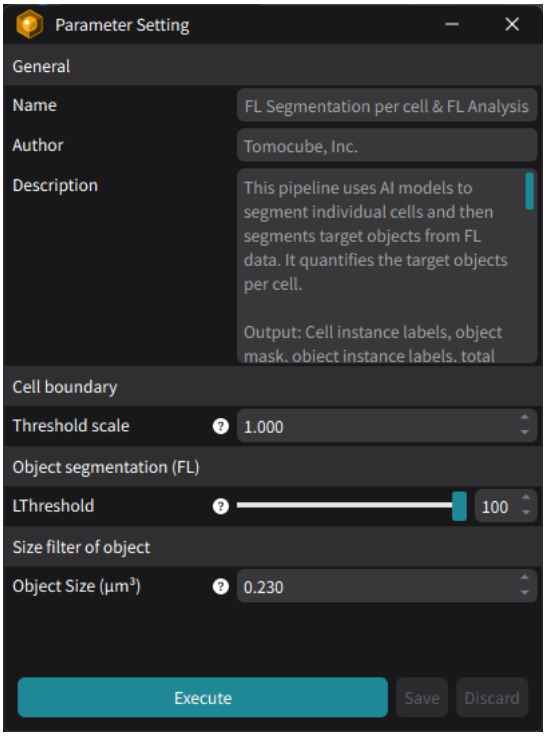
When the **Test Run** is activated, the window switches to the **Test Run** mode.



In the **Test Run** mode, the user can execute individual processes in the pipeline or run the entire pipeline at once.

Running the whole process at once

To run the entire pipeline without changing any parameters, click the **Execute All**  button at the top toolbar of the **Pipeline** panel. When clicked, the processes in the pipeline will be executed in sequence until the last process is completed. During execution, the output generated by each process will be displayed in the **Live View** panel, and the output names will be listed in the **Output Navigator** panel.



To adjust the pipeline parameters, select the pipeline name at the top of the **Pipeline** panel. When selected, the **Parameter Setting** panel will display the major parameters for running the pipeline as defined by the pipeline author. After adjusting the parameters, click the **Execute** button on the **Parameter Setting** tab, or **Execute All** at the top toolbar to run the pipeline.

Running one of the processes in the pipeline

In **Test Run** mode, individual processes in the pipeline can be executed separately to find the optimal parameter settings for each process. To do this, select a process in the pipeline to display its settings in the **Parameter Setting** panel.

The **Parameter Setting** panel consists of three sections.

Options	**Description**
Input	Displays the input data used by the process.
Output	Shows the output data produced by the process.
Parameters	Lists parameters that affect the output produced by the process.

In **Test Run** mode, the input and output sections are view-only, allowing understanding of the flow of data processing. However, the parameters can be adjusted to control the results produced by the process. For detailed instructions on how to adjust these parameters, refer to **Chapter 8. Processes.**

NOTE: Processes can be executed as many times as needed to find the optimal conditions if any parameter of upstream processes is changed.

Testing the Pipeline with Another Workset

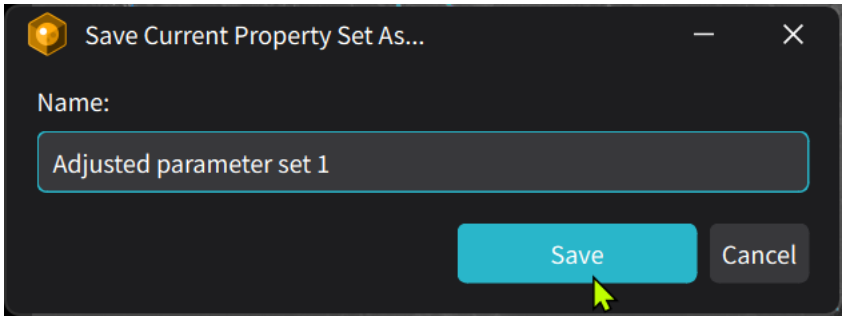
Since a particular parameter set may work well for one workset but not for others, it is recommended to test the parameter set with multiple worksets as representatives of the analysis data.

To switch the workset:

1. Use the navigation bar at the top of the window.
2. Select another workset from the drop-down menu and click the **Switch** button to change the workset for another test.

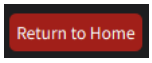
Saving the parameter set for batch run

After adjusting the pipeline parameters, the parameters can be saved as parameter preset for applying them in batch analysis. To do this, click the **Save** Save button in the **Parameter Preset** panel and assign a name to the parameter preset. The saved presets can be loaded back to pipeline using **the Load** Load button.



NOTE: Multiple parameter sets can be saved. Choose one of them during batch analysis.

Return to the main window

After saving parameter sets for batch analysis, return to the main window by clicking the **Return to Home** 

button.

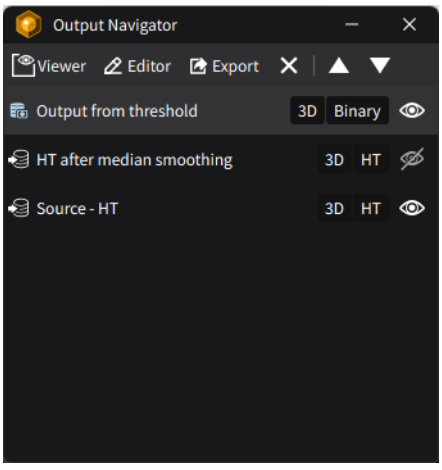
5.8 Adjusting Views

When testing various processes and their parameters in **Test Run** mode, it is necessary to evaluate the results by comparing them with the original source data or outputs from other processes. Since all image-type outputs, as well as sources, are displayed in the **Live View** panel, the contrast of each image output along with its order of appearance, can be adjusted.

Stacking order

The **Live View** panel displays all image outputs generated by the processes, with new image data stacked on top of previous data. As a result, previous image outputs will be hidden.

To make a hidden output visible again, change its stacking order in the **Output Navigator** panel.

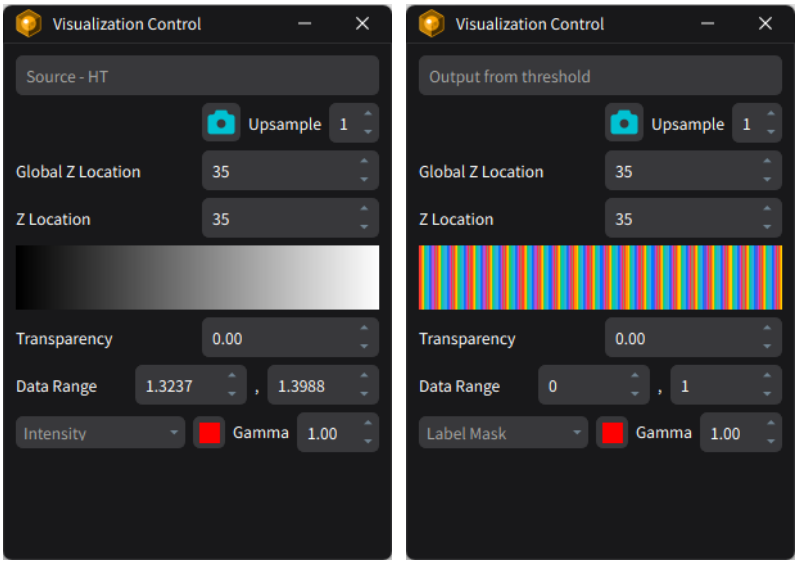


- To move an output upward and bring it above others, click the Up button, or drag & drop it above.
- To lower an output that is obscuring other layers, click the Down button or drag & drop it below.
- Show/hide an output from the **Live View** panel is possible by clicking the Show/Hide toggle button on the right side of each output name. When the **Show/Hide** icon is in the Hide state, the selected layer will be ignored by the **Live View** panel until the icon is toggled back to Show.

Image contrast

To enhance image contrast in the **Live View** panel:

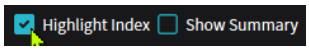
1. Select one of the outputs in the **Output Navigator** panel.
2. The **Visualization Control** panel will change based on the type of selected output (e.g., Image, Label, Mask).



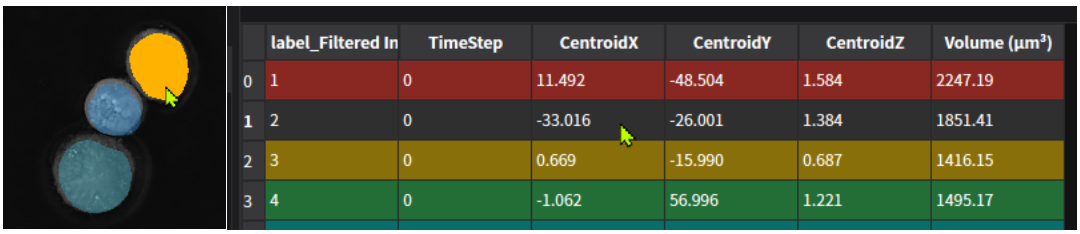
- The transparency of the selected output can be adjusted using the **Transparency** value, where 0 is completely opaque and 1 is fully transparent.
- Adjust the contrast by setting the lowest and highest values in the **Data Range** section, which are mapped to the intensity values of the chosen look-up table.
- Change the look-up table by selecting a color scheme, and use the **Gamma** value to fine-tune the intensity adjustments.

Highlighting Object

When measurement outputs are registered in the **Output Navigator** panel, it's possible to highlight corresponding objects in the **Live View** panel.

- To highlight an object, enable the **Highlight Index** 

option in the measurement tab. Select a row to be highlighted, and the corresponding object label will be emphasized while others are dimmed.
- Conversely, selecting an object label in the **Live View** panel will focus on the corresponding row in the measurement table.

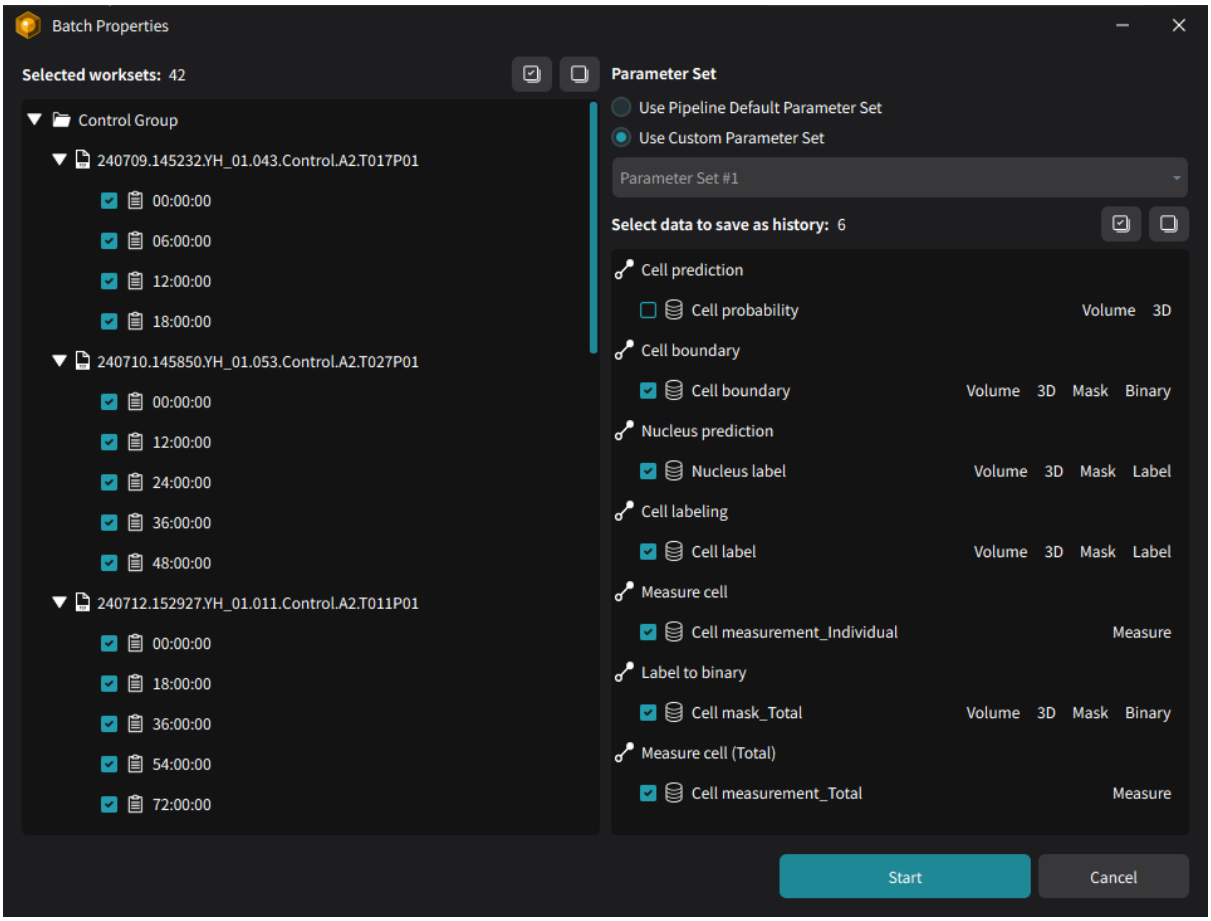


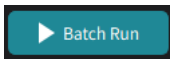
	label_Filtered In	TimeStep	CentroidX	CentroidY	CentroidZ	Volume (µm³)
0	1	0	11.492	-48.504	1.584	2247.19
1	2	0	-33.016	-26.001	1.384	1851.41
2	3	0	0.669	-15.990	0.687	1416.15
3	4	0	-1.062	56.996	1.221	1495.17

5.9 Batch Run Mode

5.9.1. Planning Batch Run

In **Batch Run** mode, TCF data is analyzed to produce results. To start a batch run:



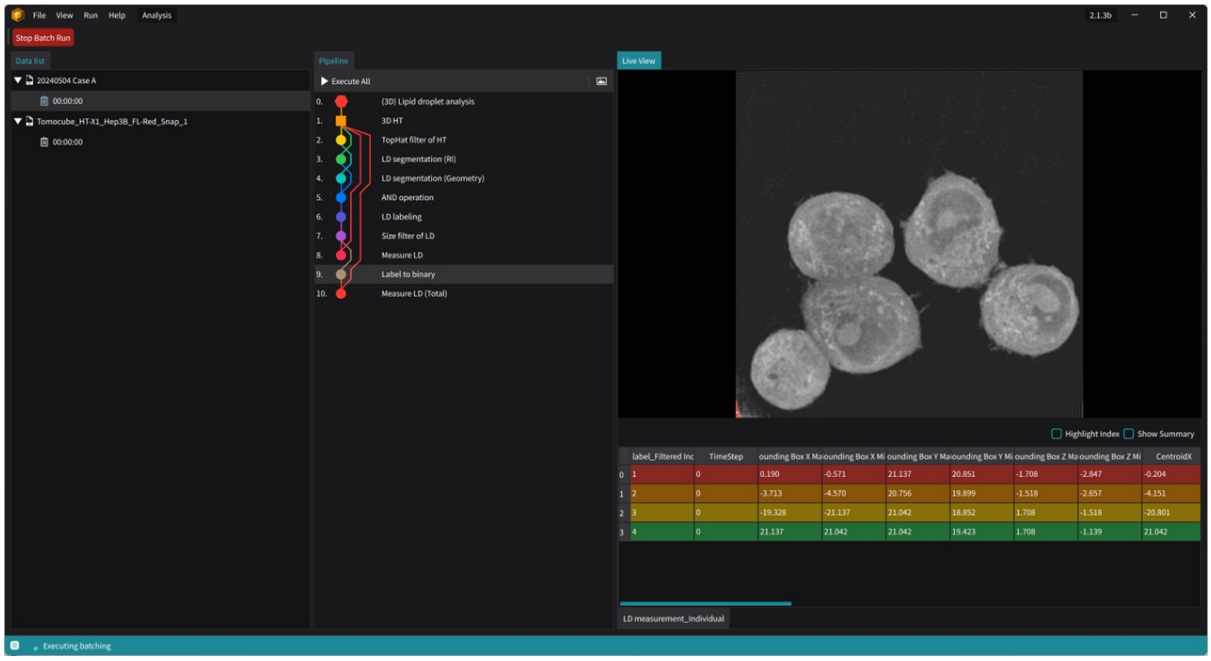
1. Click the **Batch Run** 

button to open the **Batch Properties** window.
2. On the left side of the window, select worksets to be analyzed.
3. On the right side, choose the parameter preset to apply for this batch run.
4. At the bottom, select the outputs to be saved as results.
5. Click the **Start** button to begin the batch analysis.

NOTE: If a parameter set was not saved during the test run, the **Use Customer Parameter Set** menu will be disabled. In this case, the batch run can be started with the default parameter, by selecting **Use Pipeline Default Parameter Set.**

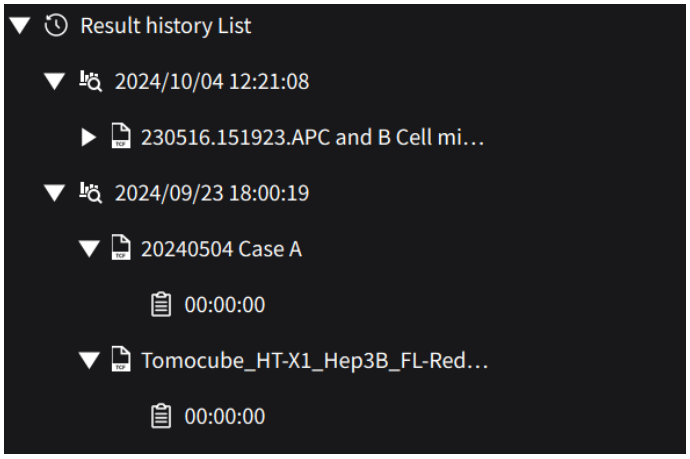
NOTE: By default, image data outputs are unchecked, while other types are checked due to storage limitations. Be cautious about the storage, when selecting image-type outputs (Outputs without Tags “Mask” or “Measure”).

5.9.2. Monitoring Batch Run Progress

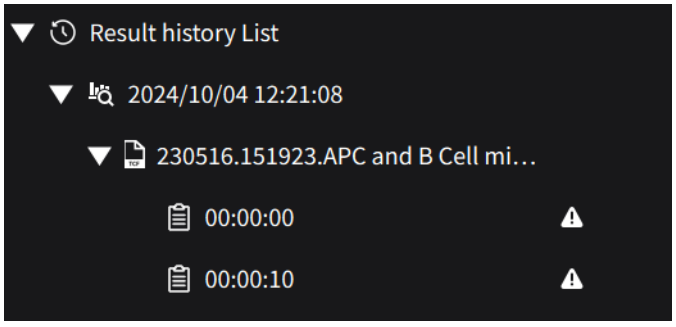


When the batch analysis starts, the window switches to the **Batch Analysis** screen, where the progress of the analysis can be monitored in real-time. During batch analysis, the **Live View** panel updates to show the latest outputs generated by the software.

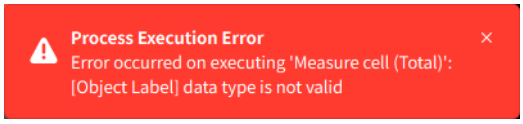
After the batch analysis is completed, the screen returns to the main window, and the results are registered as history records in the **Result history List.**

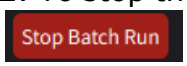


NOTE: If a workset in the result history was not properly analyzed during the batch run, it is marked with an exclamation mark.



NOTE: If any issues arise during batch analysis, an error message will be displayed to help identify the cause.



NOTE: To stop the batch analysis in the middle of execution, click the **Stop Batch Run** 

button. The batch process will stop shortly, and the screen will return to the main window. The stopped batch process can be revisited and resumed from the previous progress.

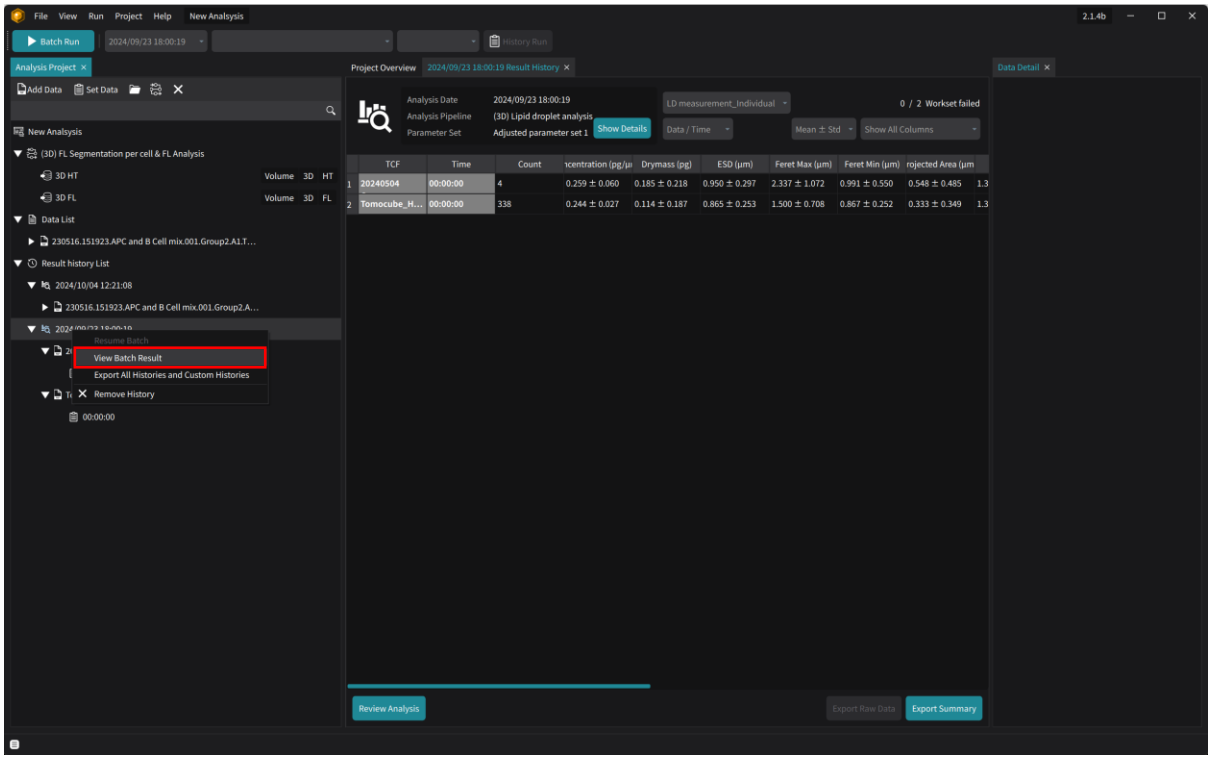
5.10 Review Mode: Investigating Analysis Results

5.10.1. Overview of Analysis Results in Dashboard

After the batch analysis is completed, the results are registered in the **Result History List** of the **Analysis Project** panel on the left side of the main window.

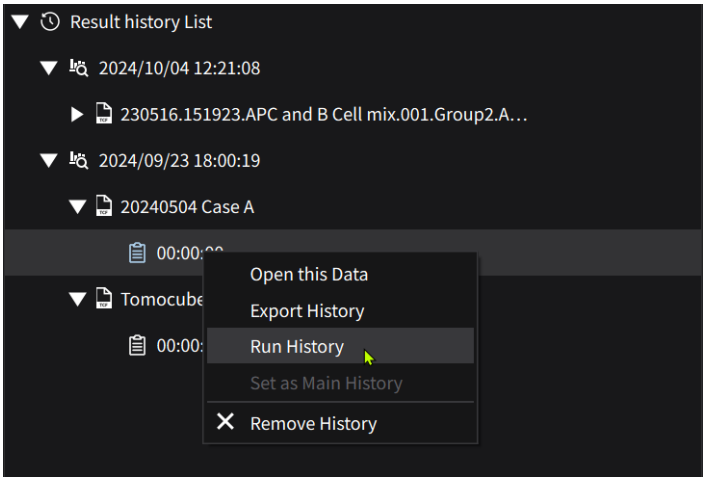
Since batch analysis can be run multiple times within a single analysis project, the **Result History List** can have multiple entries. Select any of these history records to review the results.

To review the summarized analysis results, right-click on the history record and select the **View Batch Result** menu to open the dashboard for analysis results.



The summary dashboard shows the summarized measurement results if the analysis pipeline produces measurement outputs. Refer to **Section 2.6.1. Dashboard** for the adjustment of the summary display options.

5.10.2. Opening a Workset in Analysis History



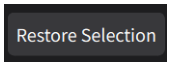
To review the results of individual worksets from batch analysis, select a workset from the **Result History List** and right-click on it to choose the **Run History** menu. This will open the **Review Mode** window, which has the same user interface as the **Test Run** mode.

Switching to another workset

To review the results of a different workset:

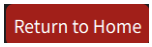
Features	Description
Batch history	Identifier for a batch analysis run by its execution time
TCF	TCF name of the workset to select
Timestamp	Timestamp of the workset to select

1. Use the navigation bar at the top of the window.
2. Select another workset using the drop-down menu.
3. Click the **Switch** 

button to switch to the new workset, or click the **Restore Selection** 

button to revert to the currently selected workset.

Returning to the main window

To return to the main window of the **Analysis Perspective**, click the **Return to Home** 

button.

5.10.3. Reviewing Intermediate Data

When reviewing analyzed results for a selected workset, the output data generated by the processes are displayed in the **Live View** panel and registered in the **Output Navigator**.

The intermediate outputs with other image data can be visually reviewed by adjusting the display options in the **Output Navigator** panel and the **Visualization Control** panel.

For more details on controlling display options, refer to **Chapter 5.8 Adjusting Views.**

5.10.4. Adjusting Process Parameters

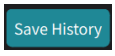
In **Review Mode**, re-analyzing the workset with adjusting process parameters is possible. This is to refine results for worksets that were not well-analyzed with a single fixed parameter set during batch analysis.

The method for adjusting parameters is the same as in the **Test Run** mode. Select the pipeline name in the **Pipeline** panel to adjust the main parameters for the pipeline or select individual processes to change their respective parameters.

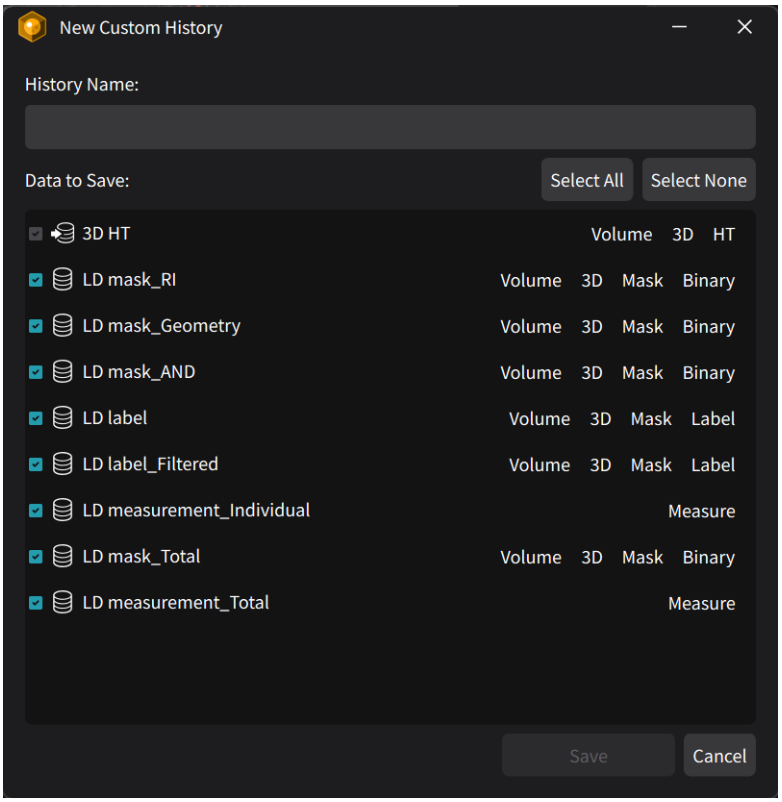
For more details, refer to **Chapter 5.7 Test Run Mode** for the details.

NOTE: If a process is executed after modifying its parameters, the output generated by the following process will become invalid, and a warning mark  will appear on the **Output Navigator**.

NOTE: The modified parameters in the Review mode can also be saved and applied for another batch analysis. Save the parameter set using the **Parameter Preset** window as described in **Test Run** mode.

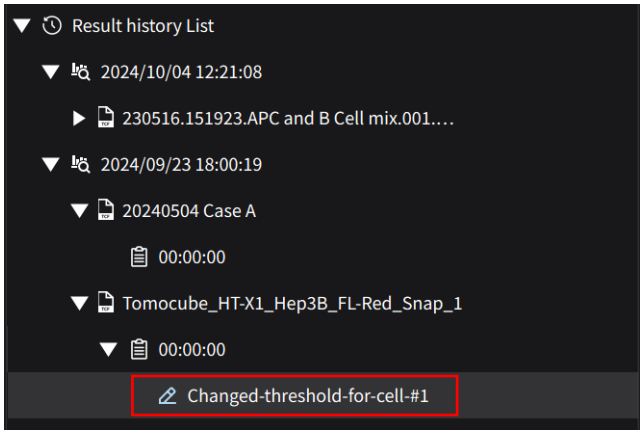
If the new analysis results for the workset are acceptable, save the results by clicking the **Save History** 

button at the top of the window, which will open the **New Custom History** window.



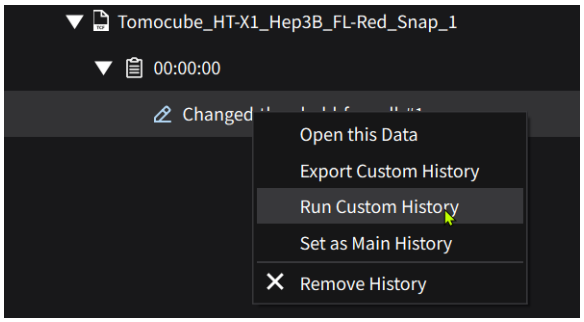
In the **New Custom History** window:

1. Select the output data to save in the custom history.
2. Enter a name in the **History Name** textbox to distinguish this revised result from the original.
3. After assigning a name, click the **Save** button to store the results.



The newly saved custom history will appear as a secondary analysis result for the original workset.

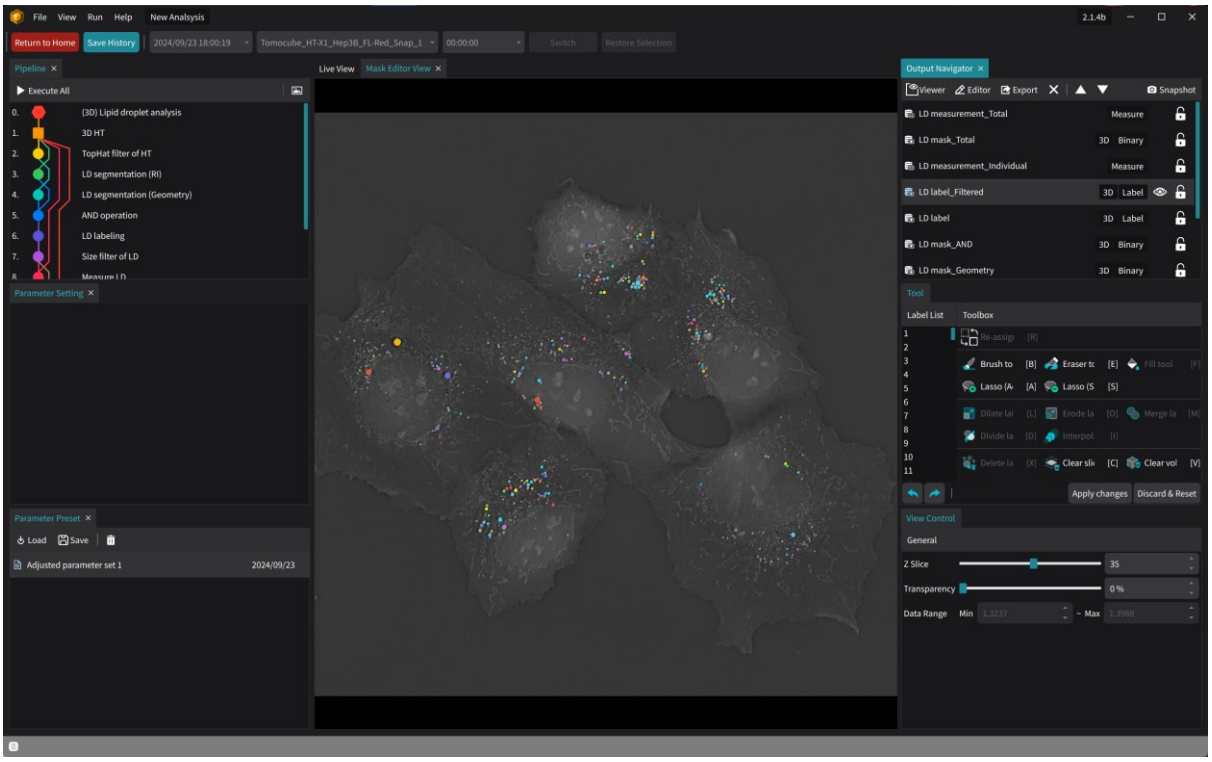
NOTE: To review custom history in **Review Mode**, right-click on custom history and select the **Run Custom History** menu.



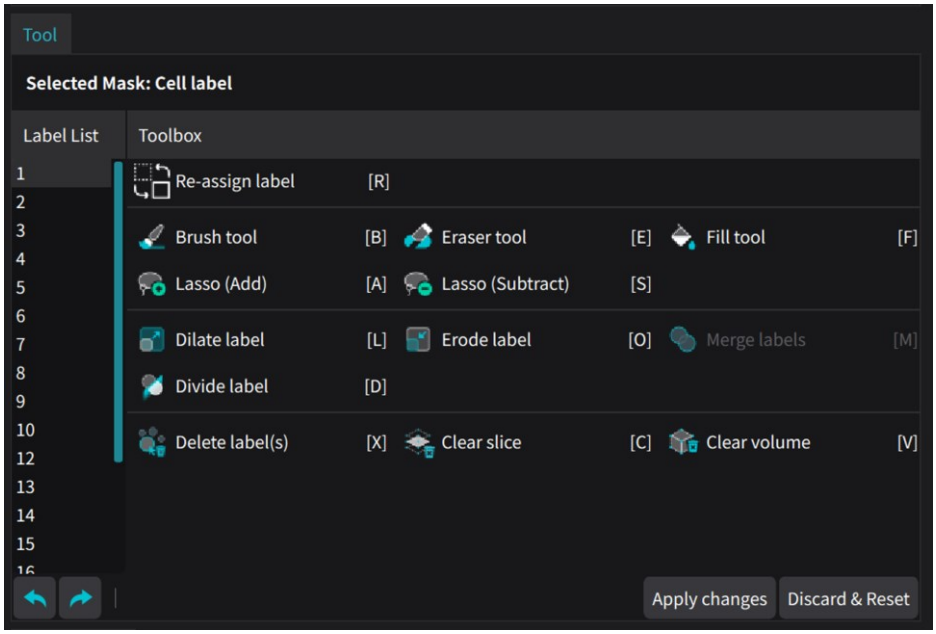
NOTE: To Apply the analysis result from the custom history, right-click on the custom history and select the **Set as Main History** menu.

5.10.5. Editing Mask and Label Data

During the review of analysis results, it may be necessary to manually edit masks that could not be segmented accurately by the processors. To edit masks:



1. Select the **Editor**  button on the toolbar of the **Output Navigator**.
2. When the **Mask Editor** opens, select the desired mask to edit from the **Output Navigator**.



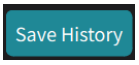
3. When a mask is selected, it is marked with the **Show** icon and displayed in the **Mask Editor** View panel. Use the interface in the **Tool** panel of the **Mask Editor** to edit the mask.

For detailed instructions on editing masks, refer to **Chapter 7. Editing Masks.**

Once editing is complete, transfer the changes to the analysis record by following these steps:

1. Press the **Apply Changes** button to save the edits in the **Output Navigator**. The edited mask will be marked with an asterisk (*) before its name in the **Output Navigator**.
2. Press the **Transfer** Transfer button to apply the mask changes to the **Live View**. This updates the data used by other processes for this workset. If multiple masks are edited, click the **Transfer All** Transfer All button to apply all changes at once.
3. Exit the **Mask Editor** by clicking the **Close** icon on the **Mask Editor View** panel.

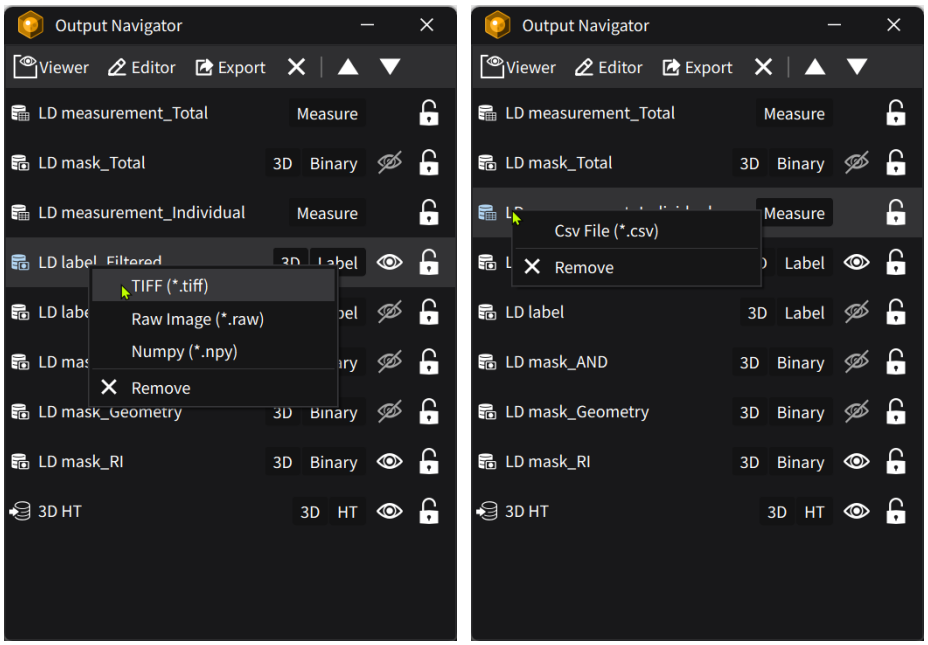
After transferring the changes, confirm if the updates are correctly applied in **Live View**. As the mask is changed, the output generated by subsequent processes that use the edited mask data will become invalid. These outputs will be marked with a warning icon in the **Output Navigator**. To make them valid again, execute the affected processes.

NOTE: If a mask is edited, save the results by clicking the **Save History** 

button at the top of the window. This will open the **New Custom History** window.

5.10.6. Exporting Intermediate Outputs

In **Review Mode**, all outputs registered in the **Output Navigator** can be exported as files and utilized in an external environment.



1. Right-click on the output name to export in the **Output Navigator**.
2. Choose an appropriate image format to save the selected output.

If the output data type is an image, label, or binary, the available formats are **TIFF**, **RAW**, or **NPY**.

For measurement data, the format will be **CSV**.

TIFF: Standard format for storing image data.

RAW: Format that stores original data without compression or conversion.

NPY: NumPy array format for storing data.

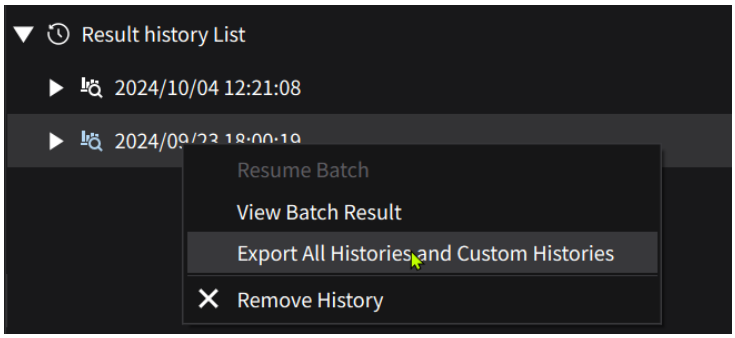
CSV: File format for storing tabular data.

5.10.7. Exporting Batch Analysis Results

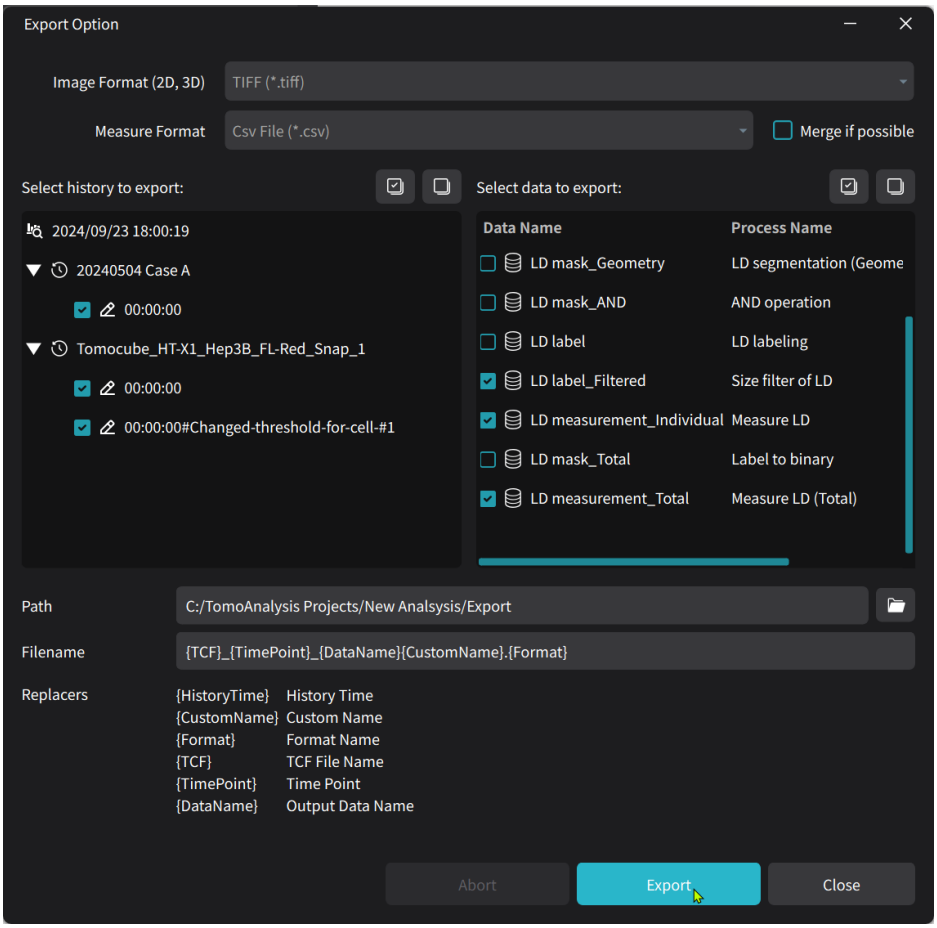
After batch analysis is completed, the analysis results can be exported as images or tables.

Exporting a set of batch analysis results

To export the results of a batch analysis set, right-click on the timestamp in the **Result History** list and select **Export All Histories and Custom Histories** to open the **Export Option** window.



In the **Export Option** window:



1. Select the data format for exporting image-type data and measurement data from the top section.
2. On the left side, choose the worksets for export.
3. On the right side, choose the output data for export.
4. At the bottom, specify the folder path for the export and set the filename rules for the exported data files.
5. After making the appropriate selections, click the Export button to export the selected analysis results. Progress will be shown accordingly.
6. Click the Close button to exit the window.

Chapter 6. Data Manager

6.1 Introduction

Data Manager is a tool designed to crop TCF files along the X, Y, and Z axes, as well as across time. It is effective for isolating regions of interest from large field-of-view (FOV) datasets. In addition to cropping in the XY plane, it allows for cropping in the Z direction, enabling the removal of irrelevant data and reducing file size by excluding unnecessary Z slices.

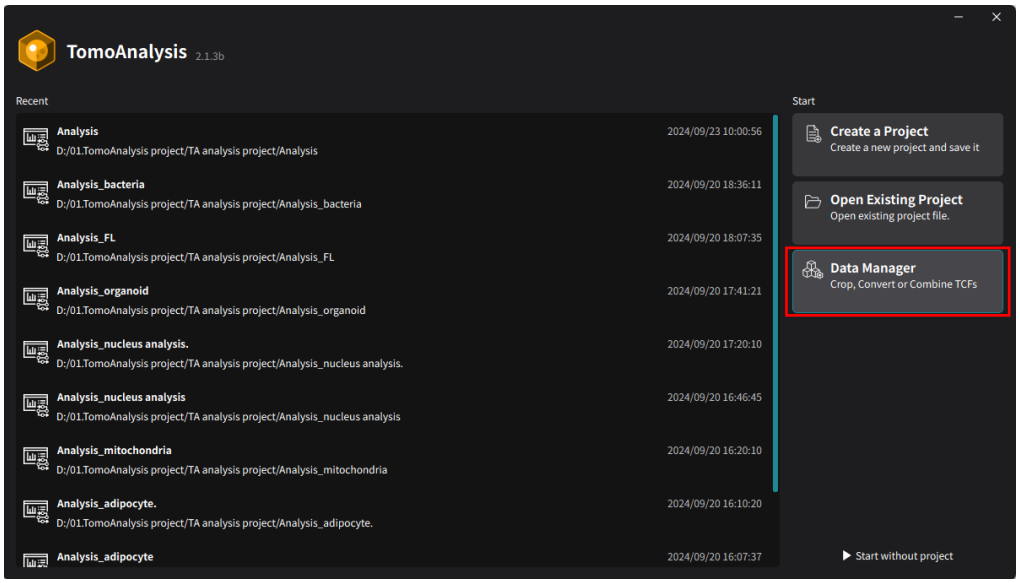
The tool also supports cropping in time, enabling the extraction and saving of only the time-lapse data where specific events occur. This feature enhances analysis by focusing on meaningful segments and simplifying file management.

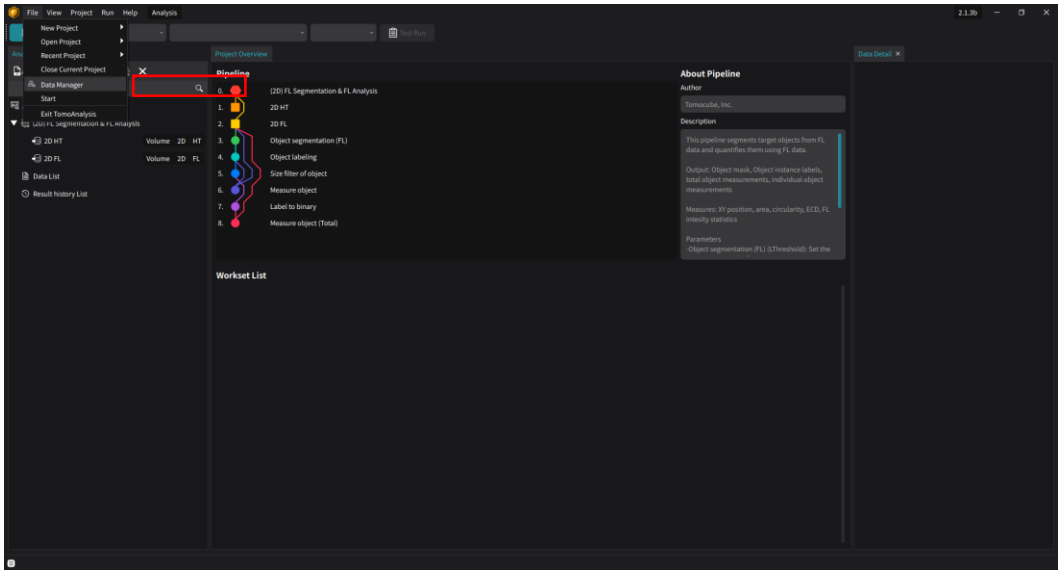
Multiple TCF files can be loaded into **Data Manager**, where the desired cropping area or time points can be defined and saved for each file, retaining only the specified regions or moments.

6.2 Data crop using Data Manager

6.2.1 Starting with Data Manager

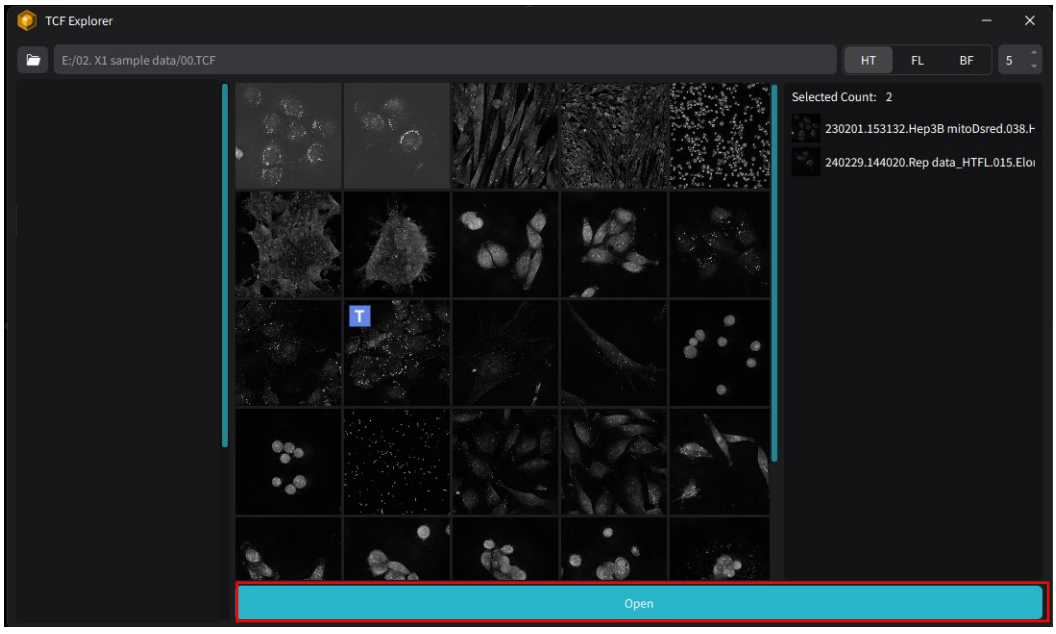
Launch **Data Manager** from the Start-up screen or access it from the File tab under the Analysis section.





6.2.2 Load/Unload data

Load TCFs using the **Add TCFs** or **Add Folder** button in the **Explorer** panel. When the Add TCFs button is selected, the **TCF Explorer** opens, allowing the selection of TCFs for use in the Data Manager while viewing thumbnails. Multiple files can be selected by holding the **Ctrl** or **Shift** keys, with the selected files displayed on the right side of the screen. After selecting the desired files, click the **Open** button at the bottom to load them into the Data Manager.

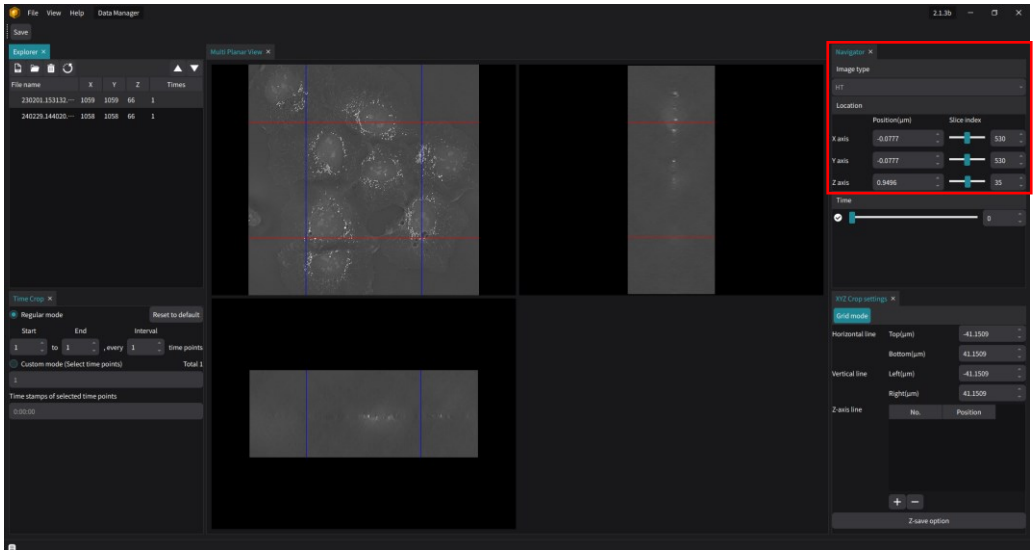


Opened TCFs are listed in the **Explorer** panel, along with their file information, including X, Y, and Z dimensions and time point numbers.

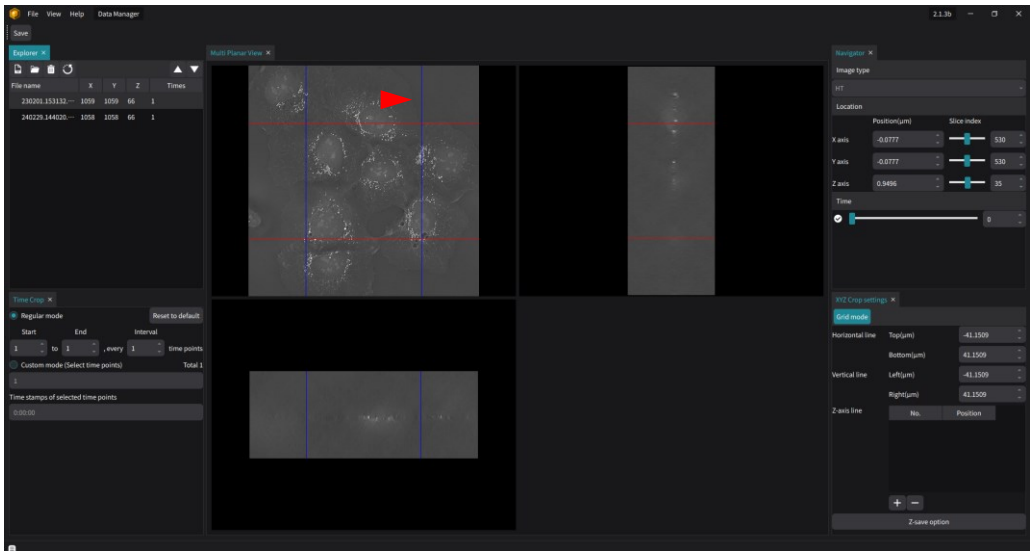
Select a file in the **Explorer** panel and click the **Remove** icon at the top to delete the selected file from the list. To delete all files from the list, use the **Remove all** button.

6.2.3 Navigating and Adjusting Views in Multi Planar View

When a file is selected from the list in the **Explorer** panel, the XY, YZ, and XZ planes of the selected file can be viewed in the **Multi Planar View**. The position can be adjusted using the mouse wheel, and the visible view can be modified by adjusting the position and slice index in the **Navigator**. Right-clicking with the mouse in the Multi Planar View will navigate the other views to the corresponding location. If the selected data includes the FL modality, the displayed modality in the **Multi Planar View** can be changed by using the dropdown menu under **Image type**.

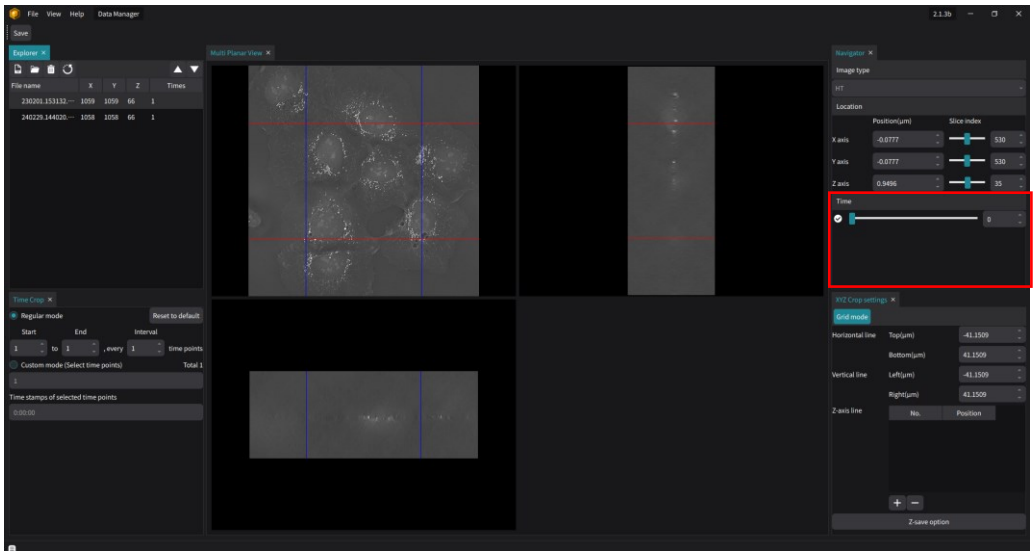


6.2.4 Configuring and Adjusting Grid Settings in Multi Planar View

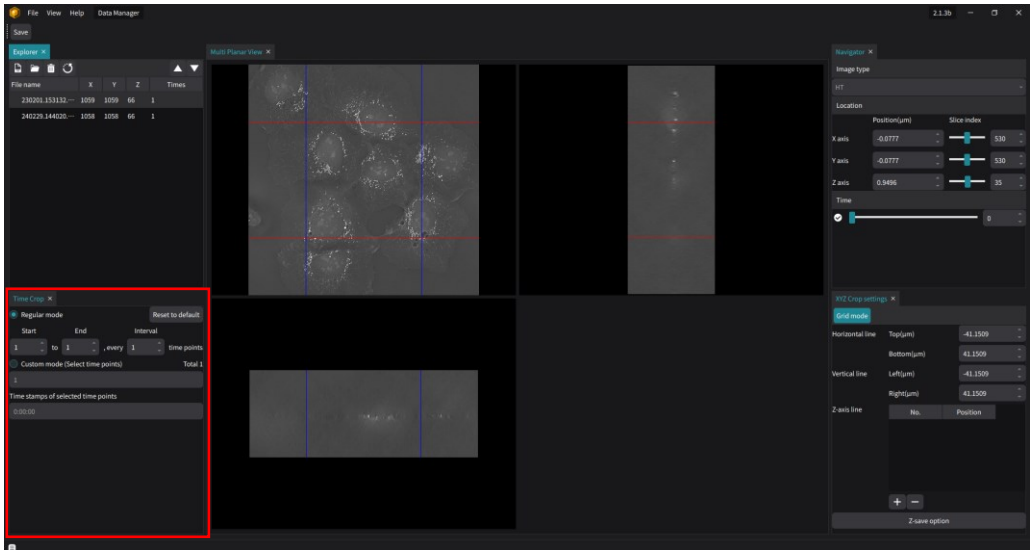


The red and blue lines displayed in the **Multi Planar View** represent the Horizontal and Vertical lines, respectively. These lines are settings for slicing the XY plane in **Grid mode** and can be adjusted by dragging them with the mouse or by entering values in the XYZ Crop Settings panel. Modifying the grid while viewing the **Multi Planar View** allows the selection of the region of interest within the entire TCF. To add a grid line along the Z-axis, click the '+' button below the Z-axis line table. This line serves as a guide for slicing the data in the Z direction. After adding the Z-axis line, use the **Z-save option** button to select and save the desired region. Each line in the Multi Planar View can be easily adjusted by dragging with the mouse to refine the grid.

6.2.5 Time Cropping for Time-Lapse Data



Time cropping can also be performed for time-lapse data. Whether the data is Time-lapse is indicated in the **Explorer** panel, with a Times value of 2 or more. By adjusting the slider in the Time section of the **Navigator**, images can be viewed at different time points. If the **Time Crop** setting is applied, the time points to be retained in the cropped file are marked with a check, while the points to be removed are marked with an X.



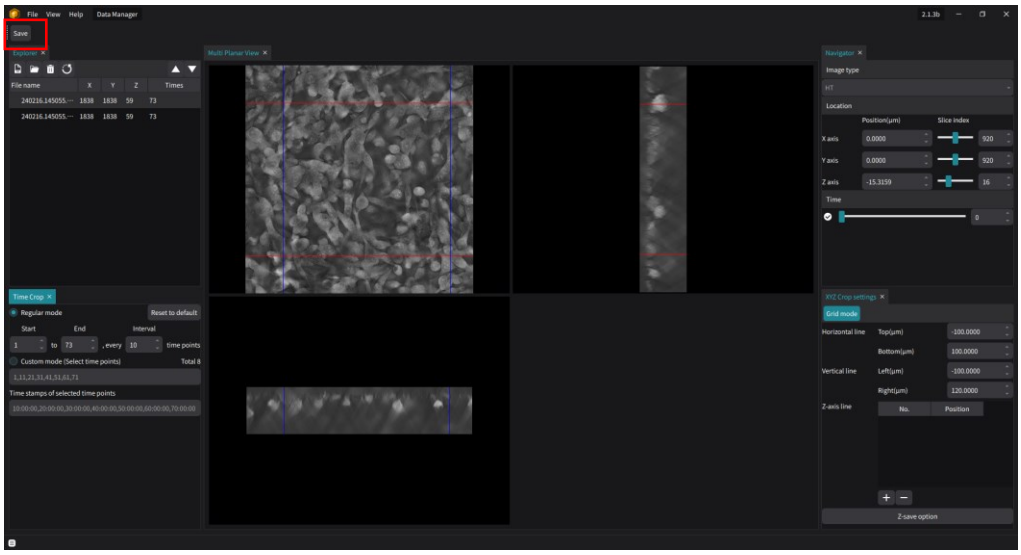
After selecting the time-lapse data, set the desired time points in the **Time Crop** panel. Start and End time points can be specified, and an interval can be set to create a new time-lapse file with a specific pattern. For irregular data generation, select the **Custom mode** to manually input time points.

NOTE: Cropping will be applied to all files in the Explorer list, so ensure that all files have the same time stamps.

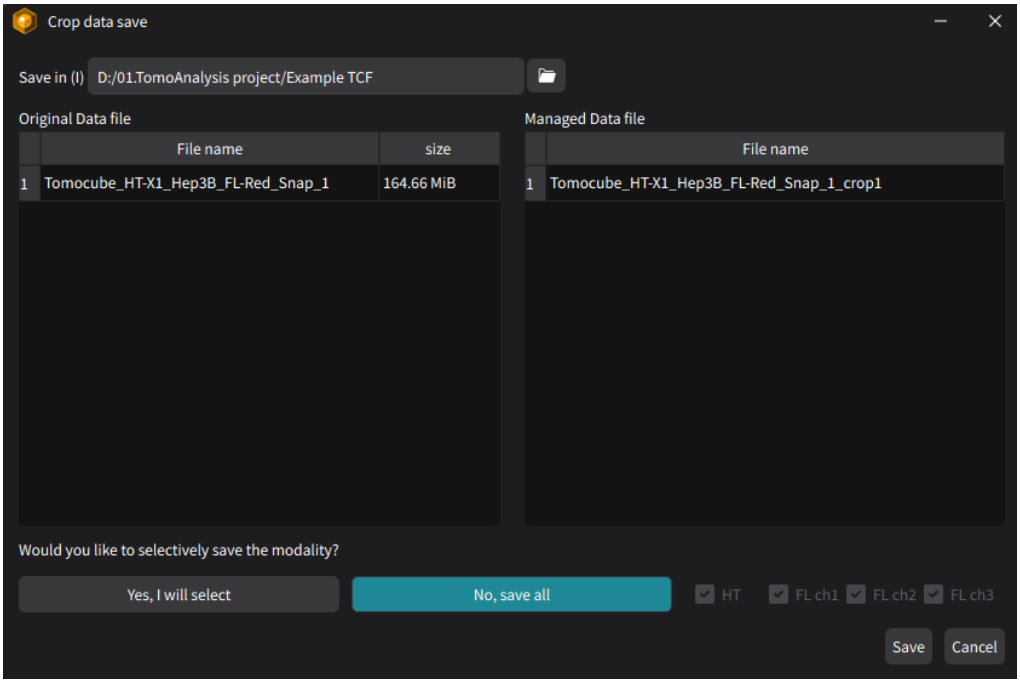
6.3 Save the managing files

6.3.1 Save cropped files

Click the **Save** button after crop settings.



The **Crop data save** window will be displayed.



In the **Crop Data Save** window, the list of original files and the data to be cropped can be reviewed. Use the 'Save in' section at the top to specify the folder where the cropped data will be saved. By clicking on the file name in the **Managed Data File** list, the file name can be modified, and the size of the cropped file can be checked. The modality to be saved during cropping can also be selected. By clicking **Yes, I will select**, the desired modalities can be chosen. If **No, save all** is selected, all modalities included in the original file will be saved. Once the save options are configured, click the **Save** button at the bottom to perform the crop operation.

NOTE: Errors may occur in processes that operate in 3D when applied to data with z-cropping.

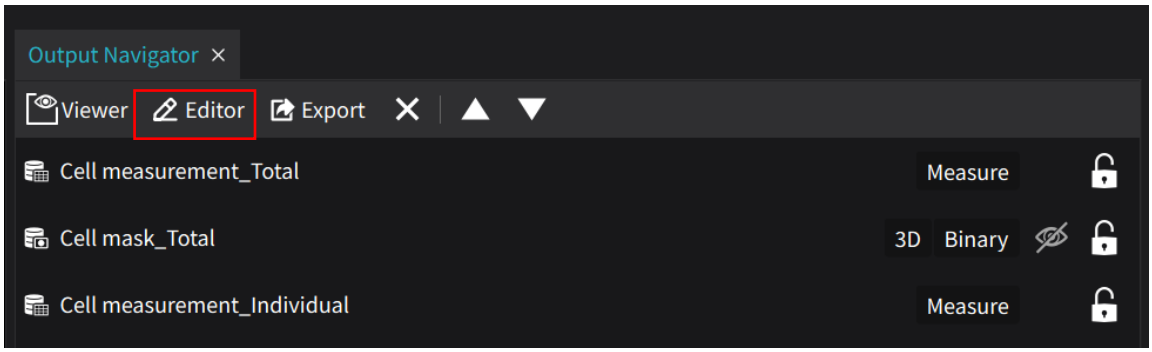
Chapter 7. Editing Masks

7.1 Introduction

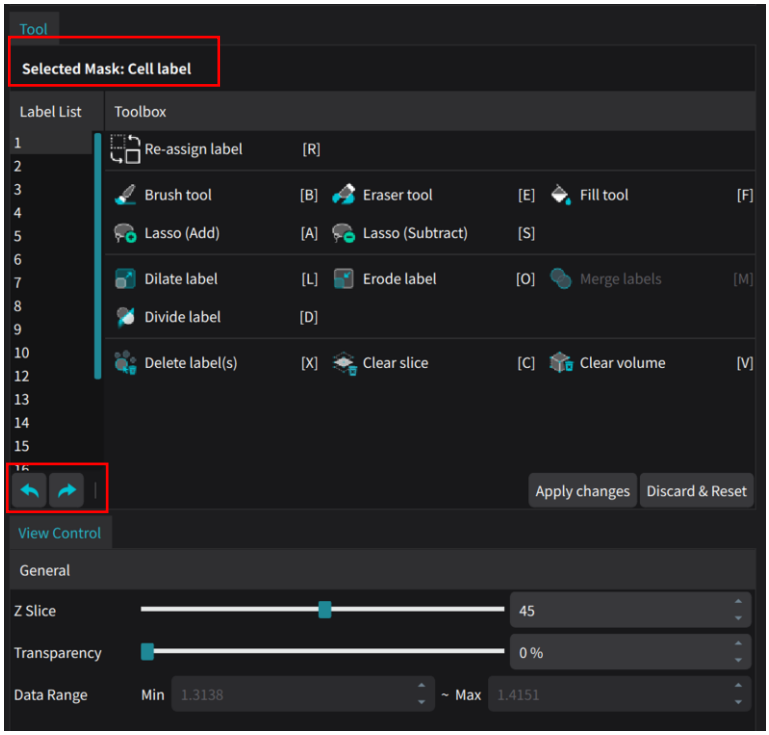
This chapter provides guidance on modifying existing masks in **TomoAnalysis** using the **Mask Editor**. The **Mask Editor** allows manual editing of automatically generated masks, which can be crucial when analysis results do not meet expectations. This chapter covers how to open the **Mask Editor**, provides examples of effective usage, and explains how to apply modification results of the mask back into the analysis process. For a detailed explanation of the **Mask Editor** user interface, please refer to **Chapter 2.4. Mask Editor.**

7.2 Opening the Mask Editor

The **Mask Editor** can be accessed from the **Pipeline Editor**, the **Test Run** window, or the **History Run** window in **Analysis Perspective**. To open the **Mask Editor**, click the **Editor** button at the top of the **Output Navigator** panel in any of these windows. If the intention is to manually modify the mask of an analysis already performed using automatic batch analysis, open the **Mask Editor** from the **History Run** window after the batch analysis is complete.



7.3 Basic Usage of the Mask Editor



Upon opening the **Mask Editor**, the first step is to select the output mask to be edited from the **Output Navigator**. Both binary and label-type output masks can be modified. Once a mask is selected, it can be confirmed at the top of the **Tool** panel.

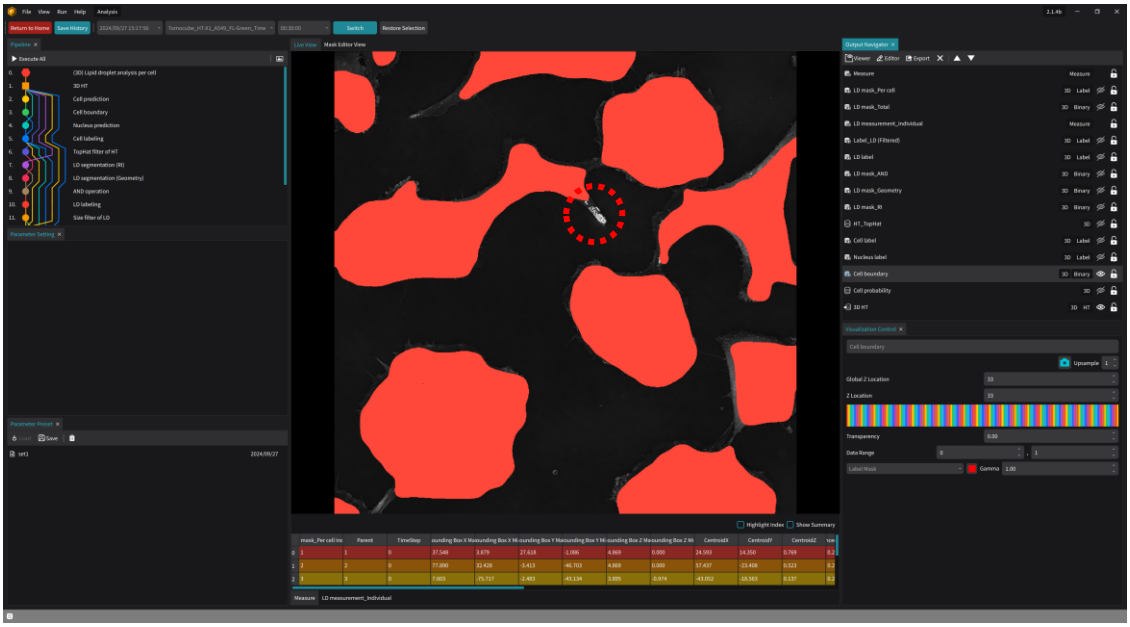
The **View Control** panel provides options to adjust the data range, transparency, and Z slice of the selected output. Adjusting the data range of reference images, such as HT or background images, and mask transparency is recommended to enhance contrast and facilitate editing. For 3D analyses, it is essential to move through different Z slices to verify and edit the mask accurately.

If an editing mistake occurs, the **Undo** and **Redo**  buttons at the bottom left of the **Tool** panel can be used to revert the last change. Note that these buttons only apply to the last action performed and only before the **Apply Changes** button is pressed.

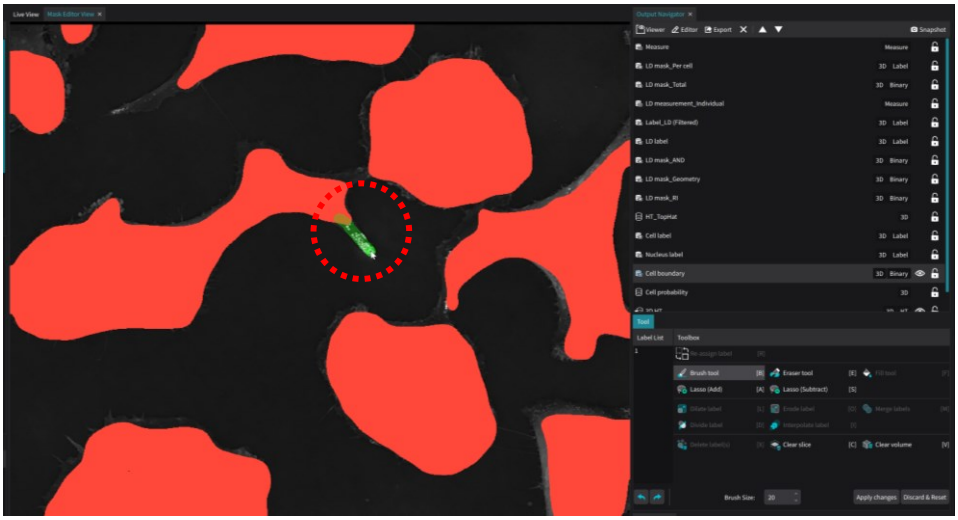
7.4 Editing Mask Regions Using 2D Tools

The 2D tools in the Mask Editor can be used to modify specific Z slices when the mask either fails to cover the entire region of interest or exceeds the intended area.

The example below shows a mask that does not fully cover the lipid droplets concentrated at the cell edge. To include this region in the analysis, the binary mask will be modified.



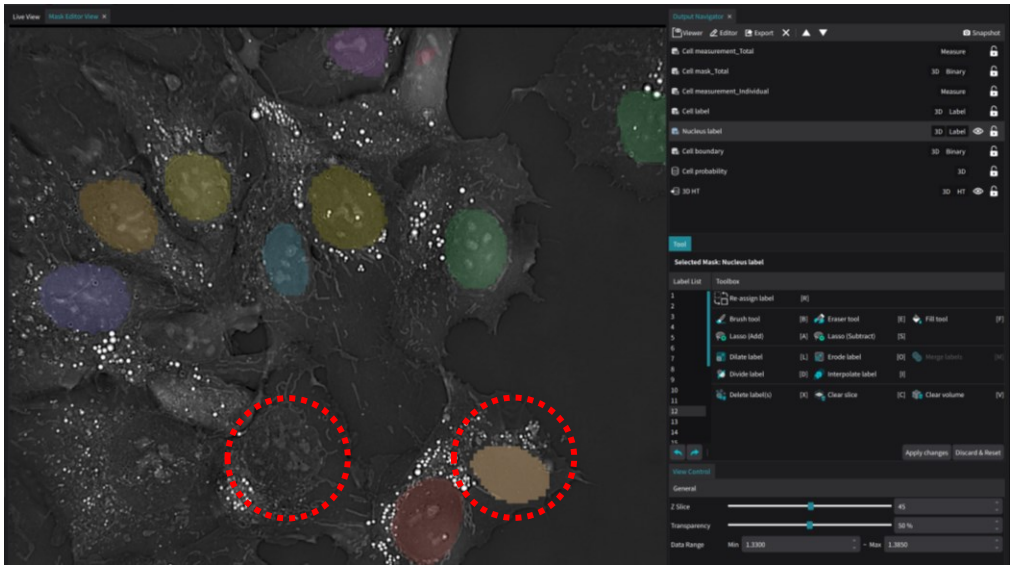
1. Open the **Mask Editor** and select the output mask to be modified from the **Output Navigator** panel.
2. Click on the label to be modified in the **Mask Editor View** panel or the **Label List** in the **Tool** panel.
3. Available 2D tools include **Brush Tool**, **Eraser Tool**, **Fill Tool**, **Lasso (Add)**, **Lasso (Subtract)**, and **Clear Slice**. To fill a missing region, use the **Brush Tool**, **Lasso (Add)**, or **Fill Tool**. Refer to **Chapter 2.4. Mask Editor** for descriptions of each tool.
4. For modifying a small area, as shown in the example, it is recommended to use the **Brush/Eraser Tool**. If the **Brush** or **Eraser** tool is selected, adjust the brush/eraser size, then use the mouse to draw on the **Mask Editor View** panel to add or erase the desired area.



5. For modifying a large area, **Lasso (Add/Subtract)** is recommended. Select **Lasso (Add)** or **Lasso (Subtract)** and use the mouse to draw on the **Mask Editor View** panel, adding or subtracting the specified region from the mask.

7.5 Adding or Deleting Labels

The example below shows the results of an AI model used to identify cell nuclei. In the mask, some non-nuclei regions are incorrectly labeled, while some nuclei lack labels. This can be easily corrected using the label addition/deletion feature in the **Mask Editor**.



1. Open the **Mask Editor** and select the output label mask to be modified from the **Output Navigator** panel.
2. Click on the label to be deleted in the **Mask Editor View** panel, then click the **Delete label(s)** tool in the **Toolbox**. Hold the **Ctrl** key to select multiple labels.
3. To add a label, select the **Brush Tool** or **Lasso (Add)**, and draw the region to be added in the **Mask Editor View** panel. Make sure no specific label is selected in the **Label List**.

7.6 Editing Mask Regions Using 3D Tools

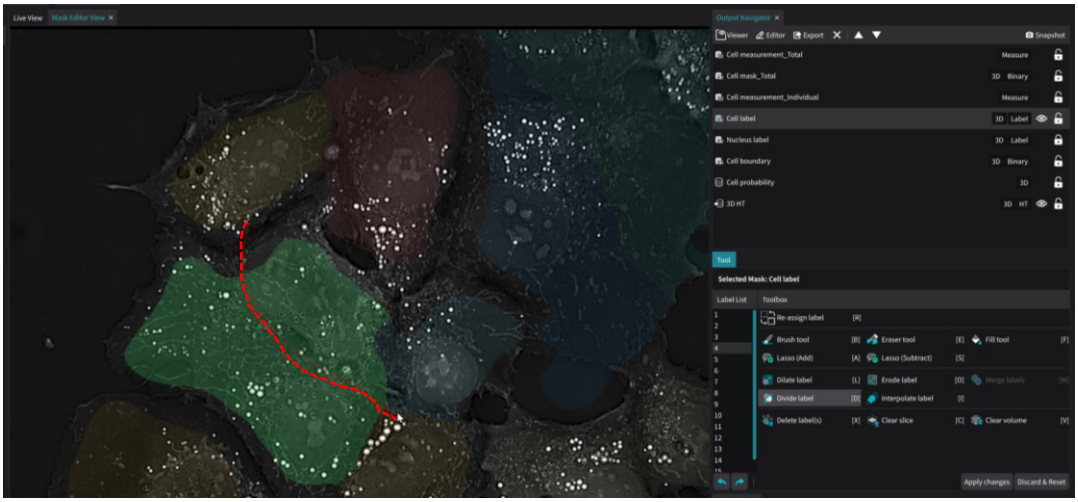
The 3D tools are useful for editing an entire label across multiple Z slices at once, making it easier to apply consistent changes throughout the depth of the mask.

1. Open the **Mask Editor** and select the output mask to be modified from the **Output Navigator** panel.
2. Click on the label to be modified in the **Mask Editor View** panel or the **Label List** in the **Tool** panel.
3. **Dilate/Erode label**: This tool is used to expand or shrink the volume of a label mask in the X, Y, and Z directions. First, select the label to modify, then click either the **Dilate Label** or **Erode Label** in the Toolbox. Set the **Dilate/Erode Radius**, which determines the number of pixels by which the label will be expanded or contracted along each axis, and then click the **Apply** button.

7.7 Merging or Dividing Labels

When a single cell is divided into multiple labels, or when two cells are mistakenly assigned to one label, the labels need to be merged or divided.

1. Open the **Mask Editor** and select the output mask to be modified from the **Output Navigator** panel.
2. **Merge labels**: Hold the **Ctrl** key and select all the labels to be merged. Once multiple labels are selected, click the **Merge labels** in the **Toolbox**.
3. **Divide label**: Select the label to be divided and click the **Divide label** in the **Toolbox.** In the **Mask Editor View**, draw a line along the boundary where the two objects should be separated. The label will be then divided into two.



4. When dividing a 3D label, the user needs to move through each Z slice and use the **Divide label** tool to draw a line on each slice. This will create multiple labels across the different Z slices. To create a mask for the 3D label, select all the labels from each Z slice and merge them together using the **Merge labels** tool.

7.8 Changing Label Numbers

After time-lapse analysis, the same cell may be assigned as different labels at different time points. To organize the analysis results, the label numbers should be unified. This can be done using the **Mask Editor**.

1. Open the **Mask Editor** and select the output label mask to be modified from the **Output Navigator** panel.
2. Click on the label whose number is to be changed in the **Mask Editor View** panel or the **Label List** in the **Tool** panel.
3. Click the **Re-assign label** button in the **Toolbox**. A **Re-assign label to:** number box will appear at the bottom of the **Tool** panel. Enter the desired number in this box, then click the **Apply** button.

7.9 Applying Edited Masks

Once mask editing is complete, they can be applied to the analysis process to view the updated results.

1. After completing the mask editing, click the **Apply Changes** button at the bottom of the **Tool** panel.
2. In the **Output Navigator** panel, a "*****" symbol will appear before the name of the edited output mask.
3. Click the **Transfer** button at the top of the **Output Navigator** panel, or if there are multiple edited output masks, click the **Transfer All** button.
4. Return to the **Live View**. As the edited mask is transferred to the original output list, all outputs based on this mask will become invalid.
5. Click the **Execute All** button at the top of the **Pipeline** panel to re-run all processes associated with the invalidated outputs. Updated analysis results using the edited masks will be generated.
6. Use the **Export** button at the top of the **Output Navigator** to save the results, or click **Save History** to save a new history.

Chapter 8. Processes

8.1 Introduction

TomoAnalysis performs data analysis using a series of processing steps, referred to as processes. Users can achieve the desired analysis results by combining these processes, and **TomoAnalysis** manages the combination as analysis pipelines.

Categories

The processes in **TomoAnalysis** are grouped into various categories, each serving a distinct role in image analysis:

1. **AI**: Employs advanced machine learning and deep-learning models for automatic segmentation, enabling accurate identification of target objects like cells and nuclei.
2. **Arithmetic**: Performs mathematical operations on images, allowing for pixel-wise adjustments that enhance image features or normalize intensity values.
3. **Edge Detection**: Identifies boundaries and significant intensity changes within images, aiding in contour detection and feature extraction.
4. **Filtering**: Enhances image quality by selectively removing noise, highlighting certain features, or applying custom modifications to the image data.
5. **Geometry**: Performs spatial transformations, such as translation, resizing, and slice extraction, to adjust image alignment, scale, and dimensional consistency.
6. **Import Files**: Integrates external data into the analysis workflow, allowing further processing of binary or label masks.
7. **Labeling**: Assigns unique identifiers to segmented objects and refines existing labels, ensuring consistent object identification in the image.
8. **Logic**: Applies logical operations on binary and label masks, enabling region selection, masking, or combining of different regions for analysis.
9. **Measurement**: Computes quantitative characteristics from segmented regions, providing insights into object size, shape, intensity, and physical properties.
10. **MorphOp**: Conducts morphological transformations, including erosion, dilation, opening, and closing, to refine the shapes and boundaries of segmented regions.

11. **Segmentation**: Separates target regions from the background using various techniques, creating distinct object masks based on intensity, texture, or shape.
12. **Smoothing**: Reduces noise in images while preserving critical structures, enhancing visual quality and preparing data for further analysis.

Input and Output Dimensions

Each process in **TomoAnalysis** produces output based on defined input data. Because the dimension of images can be either 2D or 3D, processes are categorized into three types according to the input dimension and how they process it:

- **Processor XY (2D)**: Applies its function to a 2D image in the XY direction, producing a 2D image as the output.
- **Processor XY (3D)**: Applies its function to every Z-slice of a 3D image independently, stacking them to generate a 3D image as the output.
- **Processor XYZ (3D)**: Applies its function in the XYZ direction across the entire 3D volume, producing a 3D image as the result.

8.2 AI

8.2.1. Cellpose segmentation

The **Cellpose segmentation** process allows users to perform segmentation on 2D images using the Cellpose deep-learning model. The process generates a label mask that represents segmented regions within the input image. This mask assigns labels to different objects or regions detected in the image, typically cells or nuclei.

Users can apply a pretrained model provided by Tomocube or select a custom model, with various parameters available to fine-tune the segmentation, including object size, thresholds, and edge exclusions.

Process Name	Input	Output	Filter Direction	Parameters
Cellpose segmentation XY (2D)	Image (2D)	Label (2D)	XY plane (2D)	Use Custom Model, Cellpose Version, Target Type, Model Version, Average Object Diameter, Minimum Object Diameter, Cell Boundary Threshold, Flow Threshold, Exclude on Edges

Parameter	Description
Use Custom Model	Enables the use of a custom model by specifying a file path for the model.
Cellpose Version	Selects the version of the Cellpose model to be used.
Target Type	Specifies the type of object to segment (e.g., cells, nuclei).
Model Version	Indicates the version of the pretrained model for segmentation.
Average object diameter (pixel)	Sets the expected average diameter of objects to improve segmentation accuracy.
Minimum object diameter (pixel)	Defines the minimum diameter of objects to detect, filtering out smaller regions.
Cell boundary threshold	Controls the sensitivity to object boundaries, refining delineation.
Flow threshold	Adjusts how the model separates overlapping or nearby objects based on flow dynamics.
Exclude on edges	Option to exclude objects that are partially touching the image edges from the segmentation results.

8.2.2. ilastik segmentation

The **ilastik segmentation** processes perform semantic segmentation on 2D or 3D images, designed to identify specific targets such as mitochondria. The output of these processes is a probability map indicating the likelihood of each pixel (2D) or voxel (3D) belonging to the target, with the output dimension matching the input.

Process Name	Input	Output	Filter Direction	Parameters
ilastik Segmentation XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Target Type, Model Version, Channel, Use Custom Model, Custom Model Path
ilastik Segmentation XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Target Type, Model Version, Channel, Use Custom Model, Custom Model Path
ilastik Segmentation XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Target Type, Model Version, Channel, Use Custom Model, Custom Model Path

Parameter	Description
Target Type	Specifies the type of object to segment (e.g., mitochondria, nuclei).
Model Version	Selects the version of the ilastik model used for segmentation.
Channel	Defines which channel(s) of the image to use for the segmentation task.
Use Custom Model	Enables the option to specify a custom model for segmentation.
Custom Model Path	(Hidden) Appears when Use Custom Model is selected, allowing the user to provide the path to the custom model.

8.2.3. Stardist Probability Map

The **Stardist Probability Map** processes are designed to generate probability maps for object segmentation, typically used for identifying star-convex shapes such as cells and nuclei. The output is a probability map indicating the likelihood of each pixel (2D) or voxel (3D) belonging to a segmented object, with dimensions matching the input.

Process Variation	Input	Output	Filter Direction	Parameters
Stardist probability map XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Target Type, Model Version, Use Custom Model, Custom Model Root Path, Downsampling Factor (XY), Execution Policy
Stardist probability map XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Target Type, Model Version, Use Custom Model, Custom Model Root Path, Downsampling Factor (XY), Execution Policy

Parameter	Description
Target Type	Specifies the type of object to segment (e.g., nuclei, star-convex objects).
Model Version	Selects the version of the Stardist model used for segmentation.
Use Custom Model	Enables the option to specify a custom model for segmentation.
Custom Model Root Path	(Hidden) Appears when Use Custom Model is selected, allowing the user to provide the path to the custom model.
Downsampling Factor (XY)	Adjusts the downsampling factor applied to the XY plane during processing, used to speed up execution.
Execution Policy	Specifies the execution policy, determining how the segmentation process is run (e.g., in parallel or sequentially).

8.2.4. Stardist Segmentation

The **Stardist Segmentation** processes provide segmentation based on star-convex shapes, such as cells and nuclei, using a Stardist model. The output is a segmented label mask with dimensions matching the input, either 2D or 3D, and it includes additional control over object detection thresholds for precise segmentation.

Process Name	Input	Output	Filter Direction	Parameters
Stardist Segmentation XY (2D)	Image (2D)	Label (2D)	XY plane (2D)	Target Type, Model Version, Use Custom Model, Custom Model Root Path, Downsampling Factor (XY), Execution Policy, Probability Threshold, NMS Threshold
Stardist Segmentation XYZ (3D)	Image (3D)	Label (3D)	Volume (3D)	Target Type, Model Version, Use Custom Model, Custom Model Root Path, Downsampling Factor (XY), Execution Policy, Probability Threshold, NMS Threshold

Parameter	Description
Target Type	Specifies the type of object to segment (e.g., nuclei, star-convex objects).
Model Version	Selects the version of the Stardist model used for segmentation.
Use Custom Model	Enables the option to specify a custom model for segmentation.
Custom Model Root Path	(Hidden) Appears when Use Custom Model is selected, allowing the user to provide the path to the custom model.
Downsampling Factor (XY)	Adjusts the downsampling factor applied to the XY plane during processing, used to speed up execution.
Execution Policy	Specifies the execution policy, determining how the segmentation process is run (e.g., in parallel or sequentially).

Probability Threshold	Controls the threshold for classifying pixels as part of an object based on the Stardist probability map.
NMS Threshold	Adjusts the Non-Maximum Suppression (NMS) threshold, which determines how tightly objects are separated during segmentation.

8.3 Arithmetic

8.3.1. Arithmetic Operation with Image

The **Arithmetic Operation with Image** process allows users to perform basic arithmetic operations using two 2D or 3D images. It enables pixel-wise operations such as addition, subtraction, multiplication, and more, across the entire image. The output retains the same dimensionality as the input image, either 2D or 3D.

Process Name	**Input**	**Output**	**Filter Direction**	**Parameters**
Arithmetic Operation with Image (2D)	Image (2D)	Image (2D)	XY plane (2D)	Arithmetic Operator: Add, Subtract, Multiply, Divide, Maximum, Minimum
Arithmetic Operation with Image (3D)	Image (3D)	Image (3D)	Volume (3D)	Arithmetic Operator: Add, Subtract, Multiply, Divide, Maximum, Minimum

Parameter	**Description**
Arithmetic Operator	Specifies the operation to perform on the image. Options: Add, Subtract, Multiply, Divide, Maximum, Minimum.

8.3.2. Arithmetic Operation with Value

The **Arithmetic Operation with Value** process allows users to perform arithmetic operations between an image (either 2D or 3D) and a scalar value. The operation is applied pixel-wise, with each pixel in the image being adjusted by the specified arithmetic operator and a coefficient. The output retains the same dimensionality as the input image.

Process Name	Input	Output	Filter Direction	Parameters
Arithmetic Operation with Value (2D)	Image (2D)	Image (2D)	XY plane (2D)	Arithmetic Operator, Coefficient
Arithmetic Operation with Value (3D)	Image (3D)	Image (3D)	Volume (3D)	Arithmetic Operator, Coefficient

Parameter	Description
Arithmetic Operator	Specifies the operation to perform between the image and the value. Options: Add, Subtract, Multiply, Divide, Maximum, Minimum.
Coefficient	The scalar value used in the arithmetic operation.

8.4 Edge Detection

8.4.1. Gradient

The **Gradient** processes are designed for edge detection in both 2D and 3D images. It applies gradient-based operators to detect edges in the image, producing a gradient map that highlights areas with significant intensity changes, which typically correspond to object boundaries. The output retains the same dimensionality as the input image.

Process Name	Input	Output	Filter Direction	Parameters
Gradient XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Gradient Operator, Gradient Mode, Smoothing Factor
Gradient XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Gradient Operator, Gradient Mode, Smoothing Factor
Gradient XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Gradient Operator, Gradient Mode, Smoothing Factor

Parameter	Description
Gradient Operator	Specifies the operator used for edge detection. Options: Canny-Deriche, Canny, Gaussian, Prewitt, Sobel.
Gradient Mode	Determines how the gradient is calculated. Options: Maximal amplitude, Euclidean amplitude.
Smoothing Factor	Controls the level of smoothing applied to the image before edge detection, affecting the sensitivity to noise.

8.4.2. Laplacian of Gaussian

The **Laplacian of Gaussian (LoG)** processes are used for edge detection by combining Gaussian smoothing with the Laplacian operator, enabling detection of object boundaries while reducing noise in 2D and 3D images. The output image highlights edges, and the dimensionality of the output matches the input.

Process Name	Input	Output	Filter Direction	Parameters
Laplacian of Gaussian XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Laplacian Kernel Radius, Precision, Gaussian SD X, Gaussian SD Y
Laplacian of Gaussian XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Laplacian Kernel Radius, Precision, Gaussian SD X, Gaussian SD Y
Laplacian of Gaussian XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Laplacian Kernel Radius, Precision, Gaussian SD X, Gaussian SD Y, Gaussian SD Z

Parameter	Description
Laplacian Kernel Radius	Specifies the radius of the Laplacian kernel, controlling the size of the neighborhood used for edge detection.
Precision	Determines the speed vs. accuracy of the processing. Options include settings like "Faster" for quicker processing.
Gaussian SD X	Standard deviation for the Gaussian filter in the X direction, controlling the amount of smoothing applied.
Gaussian SD Y	Standard deviation for the Gaussian filter in the Y direction, controlling the amount of smoothing applied.
Gaussian SD Z	(3D only) Standard deviation for the Gaussian filter in the Z direction, controlling the amount of smoothing in 3D images.

8.4.3. Laplacian

The **Laplacian** processes are used for edge detection in both 2D and 3D images. The method highlights regions of rapid intensity change, which typically correspond to edges or boundaries of objects within the image.

NOTE: The Laplacian method is sensitive to noise, which can lead to false edge detection. It is recommended to use the Laplacian of Gaussian process to reduce noise and improve edge detection accuracy.

Process Name	Input	Output	Filter Direction	Parameters
Laplacian XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Kernel Radius, Precision
Laplacian XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Kernel Radius, Precision
Laplacian XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Precision

Parameter	Description
Kernel Radius	Specifies the radius of the Laplacian kernel, controlling the size of the neighborhood used for edge detection.
Precision	Determines the speed vs. accuracy of the processing. Options include settings like "Faster" for quicker processing.

8.5 Filtering

8.5.1. Crop in Z Direction

The **Crop in Z Direction (3D)** process generates a mask that selects a specific region within a 3D image based on the designated Z-axis range. Rather than cropping the image data directly, the process creates a mask that highlights the area between the upper and lower Z locations, allowing for selective filtering or processing of this region within the 3D volume.

Process Name	Input	Output	Filter Direction	Parameters
Crop in Z Direction (3D)	Image (3D)	Image (3D)	Z-axis (3D)	Selection Method, Upper Z Location, Lower Z Location

Parameter	Description
Selection Method	Defines whether the cropping region is selected manually or automatically.
Upper Z Location	Specifies the upper boundary in the Z-axis for cropping when the selection method is manual.
Lower Z Location	Specifies the lower boundary in the Z-axis for cropping when the selection method is manual.

8.5.2. Discard Isolated Pixels

The **Discard Isolated Pixels** process removes isolated pixels or small clusters from a binary mask by evaluating the neighborhood around each pixel/voxel. For each pixel/voxel, the process counts the number of positive values within the defined neighborhood (based on the kernel size) and retains only those whose count exceeds a specified minimum threshold. This filtering can be applied to both 2D and 3D masks.

Process Name	Input	Output	Filter Direction	Parameters
Discard Isolated Pixels (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Kernel Size, Minimum Positive Pixels
Discard Isolated Pixels (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Size XY, Kernel Size Z, Minimum Positive Voxels

Parameter	Description
Kernel Size XY	Defines the size of the neighborhood in the XY plane used for counting positive values around each pixel/voxel.
Kernel Size Z	(3D only) Defines the size of the neighborhood in the Z direction for evaluating isolated voxels.
Minimum Positive Voxels (Pixels in 2D)	Specifies the minimum number of positive pixels/voxels required within the neighborhood to retain the region.

8.5.3. Discard Objects Touching Borders

The **Discard Objects Touching Borders** process removes objects that touch the borders of an image, whether the input is a binary mask or a labeled mask. The output retains the same type as the input (binary or labeled). This filtering is useful for excluding incomplete or partial objects that intersect the image edges. The process can be applied to both 2D and 3D masks, with the connectivity of neighboring pixels or voxels defined by the specified neighborhood setting.

Process Name	Input	Output	Filter Direction	Parameters
Discard Objects Touching Borders (2D)	Mask, Label (2D)	Mask, Label (2D)	XY plane (2D)	Neighborhood
Discard Objects Touching Borders (3D)	Mask, Label (3D)	Mask, Label (3D)	Volume (3D)	Neighborhood

Parameter	Description
Neighborhood	Defines the connectivity used to determine whether an object is touching the border. Options include different connectivity settings: 6-, 18-, or 26-neighborhoods.

8.5.4. Distance Map

The **Distance Map** processes calculate distance maps for either labels or binary masks in 2D or 3D. It computes the distance from each pixel/voxel to the nearest border of the labels or binary mask, generating an output image where intensity values represent these distances. When using **labels**, the boundaries between different labels are considered in finding the nearest border.

NOTE: The label image is reflected at the image boundary so that the pixels/voxels at the boundary are not treated as being on the border itself.

Process Name	Input	Output	Filter Direction	Parameters
Distance Map on Label (2D)	Label (2D)	Image (2D)	XY plane (2D)	Distance Mapping Target
Distance Map on Label (3D)	Label (3D)	Image (3D)	Volume (3D)	Distance Mapping Target, Custom Z Resolution, Z Resolution
Distance Map on Mask (2D)	Mask (2D)	Image (2D)	XY plane (2D)	Distance Mapping Target
Distance Map on Mask (3D)	Mask (3D)	Image (3D)	Volume (3D)	Distance Mapping Target, Custom Z Resolution, Z Resolution

Parameter	Description
Distance Mapping Target	Specifies how the distance map is calculated. **Foreground**: distance map on foreground, background set to 0 **Background**: distance map on background, foreground set to 0 **Signed**: positive distances for foreground, negative distances for background
Custom Z Resolution	Enables setting a custom Z-axis resolution for the distance calculation in 3D images.
Z Resolution	Defines the scaling factor in the Z direction for 3D distance mapping, influencing the scaling factor for distance computation.

8.5.5. Eigenvector of Hessian Matrix

The **Eigenvector of Hessian Matrix (3D)** process computes the eigenvalues and eigenvectors of the Hessian matrix for a 3D image. This analysis helps characterize the local structure of the image, useful for tasks such as edge detection or feature extraction. The process allows selecting specific eigenvalues or eigenvector components as the output.

Process Name	Input	Output	Filter Direction	Parameters
Eigenvector of Hessian Matrix (3D)	Image (3D)	Image (3D)	Volume (3D)	Standard Deviation X, Standard Deviation Y, Standard Deviation Z, Output Selection

Parameter	Description
Standard Deviation X	Sets the standard deviation of the Gaussian smoothing in the X direction.
Standard Deviation Y	Sets the standard deviation of the Gaussian smoothing in the Y direction.
Standard Deviation Z	Sets the standard deviation of the Gaussian smoothing in the Z direction.
Output Selection	Specifies the output to be generated. Options include: Eigenvalue: Largest, Eigenvalue: Medium, Eigenvalue: Smallest, and eigenvector components in X, Y, or Z directions for each eigenvalue (1, 2, or 3).

8.5.6. Filter Mask by Measure

NOTE: This process will be deprecated soon.

The **Filter Mask by Measure** processes filter objects within a label mask based on a specified measurement metric. It evaluates the chosen metric for each object in the mask using a reference image, allowing users to retain or discard objects based on their measurement values. The process can be applied to both 2D and 3D masks, with different metrics available for each.

Process Name	Input	Output	Filter Direction	Parameters
Filter Mask by Measure (2D)	Label (2D), Image (2D)	Label (2D)	XY plane (2D)	Metric, Ordering, Count
Filter Mask by Measure (3D)	Label (3D), Image (3D)	Label (3D)	Volume (3D)	Metric, Ordering, Count

Parameter	Description
Metric	Specifies the metric used for filtering objects. Options for 2D: **Surface Area, Mean Intensity, Sum Intensity, Circularity, Centroid X, Centroid Y.** Options for 3D: **Volume, Surface Area, Mean Intensity, Sum Intensity, Sphericity, Centroid X, Centroid Y, Centroid Z.**
Ordering	Determines whether to filter based on the top or bottom values according to the metric.
Count	Specifies the number of objects to retain based on the ordering criteria.

8.5.7. Filter Mask by Size

NOTE: This process will be deprecated soon.

The **Filter Mask by Size** processes filter labeled objects based on their size. They allow users to remove objects that do not meet a specified size threshold, retaining only those equal to or larger than the defined value. This filtering can be applied to both 2D and 3D labels, making it suitable for various segmentation tasks.

Process Name	Input	Output	Filter Direction	Parameters
Filter Mask by Size (2D)	Label (2D)	Label (2D)	XY plane (2D)	Object Size (μm²)
Filter Mask by Size (3D)	Label (3D)	Label (3D)	Volume (3D)	Object Size (μm³)

Parameter	Description
Object Size	Specifies the minimum size threshold for objects to be retained in the output label. For 2D images, the size is given in square micrometers (μm²), and for 3D images, in cubic micrometers (μm³).

8.5.8. Measure to Image

The **Measure to Image** process generates an output image by applying a measurement metric to labeled regions. A reference image is used to provide dimension information, ensuring consistency in resolution and spatial span when the mask and reference image differ in the XYZ directions. The output image represents the selected metric's values for each region in the labeled input. This process can be applied in both 2D and 3D, with various metrics available for different types of analysis.

Process Name	**Input**	**Output**	**Filter Direction**	**Parameters**
Measure to Image (2D)	Label (2D), Reference Image (2D)	Image (2D)	XY plane (2D)	Metric
Measure to Image (3D)	Label (3D), Reference Image (3D)	Image (3D)	Volume (3D)	Metric

Parameter	**Description**
Metric	Specifies the measurement metric applied to the labeled regions. Options for 2D: **Surface Area, Mean Intensity, Sum Intensity, Circularity, Centroid X, Centroid Y.** Options for 3D: **Volume, Surface Area, Mean Intensity, Sum Intensity, Sphericity, Centroid X, Centroid Y, Centroid Z.**

8.5.9. Normalize Image

The **Normalize Image** process scales the intensity values of an input image to a specified range. This normalization enhances the image by adjusting its pixel/voxel values to fit within the defined minimum and maximum target values, improving contrast or preparing the image for further analysis. The process can be applied to both 2D and 3D images.

Process Name	Input	Output	Filter Direction	Parameters
Normalize Image XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Target Min, Target Max
Normalize Image XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Target Min, Target Max

Parameter	Description
Target Min	Specifies the minimum intensity value in the normalized output image.
Target Max	Specifies the maximum intensity value in the normalized output image.

8.5.10. TopHat Filter

The **Top-Hat Filter** process enhances features in an image by subtracting a background estimated with morphological operations. It emphasizes small objects or details while suppressing large-scale variations. The filter can target either bright or dark objects and is applicable to both 2D and 3D images.

Process Name	Input	Output	Filter Direction	Parameters
Top-Hat Filter XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Kernel Radius, Object Lightness
Top-Hat Filter XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Kernel Radius, Object Lightness
Top-Hat Filter XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Object Lightness

Parameter	Description
Kernel Radius	Specifies the size of the neighborhood used for background estimation and filtering.
Object Lightness	Determines whether to filter bright objects on a dark background or dark objects on a bright background.

8.5.11. Variance Filter

The **Variance Filter** process calculates the local variance of pixel/voxel intensities within a specified neighborhood, enhancing regions of an image with high intensity variability. This filter is useful for detecting texture or fine details in the image. It can be applied to both 2D and 3D images, with options for specifying the neighborhood size.

Process Name	Input	Output	Filter Direction	Parameters
Variance Filter XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Kernel Radius
Variance Filter XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Kernel Radius
Variance Filter XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius

Parameter	Description
Kernel Radius	Defines the size of the neighborhood used for calculating the local variance.

8.6 Geometry

8.6.1.Image/Mask Translation

The **Translation** processes shift images, binary masks, or label masks by specified distances along the X, Y, and Z axes. This set of geometrical operations is useful for repositioning data or adjusting the alignment of features. The processes can be applied in both 2D and 3D, offering flexibility for various image and mask types.

NOTE: If the translation causes any part of the image or mask to move outside the field of view, the portion extending beyond the boundaries will be excluded from the output. For regions with no corresponding data due to the translation, these will be set to blank for binary or label masks, or to the minimum intensity value for image inputs.

Process Name	Input	Output	Filter Direction	Parameters
Binary Mask Translation (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Translation X, Translation Y
Binary Mask Translation (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Translation X, Translation Y, Translation Z
Image Translation (2D)	Image (2D)	Image (2D)	XY plane (2D)	Translation X, Translation Y
Image Translation (3D)	Image (3D)	Image (3D)	Volume (3D)	Translation X, Translation Y, Translation Z
Label Mask Translation (2D)	Label (2D)	Label (2D)	XY plane (2D)	Translation X, Translation Y
Label Mask Translation (3D)	Label (3D)	Label (3D)	Volume (3D)	Translation X, Translation Y, Translation Z

Parameter	Description
Translation X	Specifies the distance in pixels/voxels to shift the data along the X-axis.
Translation Y	Specifies the distance in pixels/voxels to shift the data along the Y-axis.
Translation Z	(3D only) Specifies the distance in voxels to shift the data along the Z-axis.

8.6.2. Extract Slice From Volume

The **Extract Slice From Volume** process extracts a single 2D slice from a 3D volumetric image. Users can specify the Z-index to manually select a slice or enable the automatic option, which selects the Z slice having the highest sum of refractive index (RI) gradient magnitude, effectively choosing the best slice based on image content. This allows for isolating a particular cross-section of the volume efficiently.

Process Name	Input	Output	Filter Direction	Parameters
Extract Slice From Volume	Volume (3D)	Image (2D)	Z-axis	Automatic, Z Index

Parameter	Description
Automatic	When enabled, automatically selects the slice with the maximal RI intensity from the 3D image.
Z Index	Specifies the index of the Z-plane to extract when Automatic mode is disabled.

8.6.3. Map Binary/Label Geometry

The **Map Geometry** process group adjusts the dimensions of a binary or label mask to match the dimensions of a specified input image. This transformation ensures that the mask aligns with the input image's spatial resolution and extent, making it suitable for further analysis or processing. The process can be applied to 3D data for both binary and label masks.

Process Name	Input	Output	Filter Direction	Parameters
Map Binary Geometry (3D)	Binary Mask (3D), Input Image	Mask (3D)	Volume (3D)	N/A
Map Label Geometry (3D)	Label (3D), Input Image	Mask (3D)	Volume (3D)	N/A

8.6.4. Resize Image

The **Resize Image** processes adjust the dimensions of an image to specified sizes along the X, Y, and Z axes. This geometrical transformation is useful for resampling images to different resolutions or fitting them to a desired size. The process can be applied to both 2D and 3D images, with different interpolation methods available to optimize the resizing for various types of data.

Process Name	Input	Output	Filter Direction	Parameters
Resize Image (2D)	Image (2D)	Image (2D)	XY plane (2D)	Size X, Size Y, Interpolation Type
Resize Image (3D)	Image (3D)	Image (3D)	Volume (3D)	Size X, Size Y, Size Z, Interpolation Type

Parameter	Description
Size X	Specifies the target size along the X-axis.
Size Y	Specifies the target size along the Y-axis.
Size Z	(3D only) Specifies the target size along the Z-axis.
Interpolation Type	Determines the method used for interpolating pixel/voxel values during resizing. Options include Nearest Neighbor, Linear, and Spline interpolation.

8.7 Import File

8.7.1. Import Tiff File

The **Import TIFF File as Binary/Label Mask** process allows users to import external TIFF files into the analysis workflow, either as binary masks or label masks. This process enables seamless integration of pre-existing segmentation results or masks from external tools into 2D or 3D analysis environments, or the exported segmentation produced by **TomoAnalysis**.

Process Name	Input	Output	Filter Direction	Parameters
Import TIFF File as Binary Mask (2D)	Image (2D)	Mask (2D)	XY Plane (2D)	File Path
Import TIFF File as Binary Mask (3D)	Image (3D)	Mask (3D)	Volume (3D)	File Path
Import TIFF File as Label Mask (2D)	Image (2D)	Label (2D)	XY Plane (2D)	File Path
Import TIFF File as Label Mask (3D)	Image (3D)	Label (3D)	Volume (3D)	File Path

Parameter	Description
File Path	Specifies the file path of the TIFF file to be imported. **NOTE**: The file path is determined by user input and does not automatically reflect the source information upon batch analysis.

8.8 Labeling

8.8.1. Assign Object Labels

The **Assign Object Labels** processes label connected components within a binary mask, assigning a unique label to each distinct object. This process is used for segmenting individual objects in an image based on connectivity criteria. It can be applied to both 2D and 3D masks, with options to define the neighborhood connectivity for determining object boundaries.

Process Name	Input	Output	Filter Direction	Parameters
Assign Object Labels (2D)	Mask (2D)	Label (2D)	XY plane (2D)	Neighborhood
Assign Object Labels (3D)	Mask (3D)	Label (3D)	Volume (3D)	Neighborhood

Parameter	Description
Neighborhood	Defines the connectivity criteria for determining whether neighboring pixels/voxels belong to the same object. Options include different connectivity settings, such as 4- or 8-neighborhood in 2D, and 6-, 18-, or 26-neighborhoods in 3D.

8.8.2. Reassign Labels

The **Reassign Labels** process reanalyzes the connectivity of labeled regions and assigns new labels to ensure consistent labeling based on the reassessed connectivity. This operation is useful for refining label assignments in cases where label continuity needs to be reassessed. The process can be applied to both 2D and 3D labeled images.

Process Name	Input	Output	Filter Direction	Parameters
Reassign Labels (2D)	Label (2D)	Label (2D)	XY plane (2D)	N/A
Reassign Labels (3D)	Label (3D)	Label (3D)	Volume (3D)	N/A

8.8.3. Reorder Labels

The **Reorder Labels** process adjusts the numbering of labeled regions in a label mask to follow a specific order, typically reassigning the labels sequentially. This operation is useful for standardizing label assignments, especially after modifications to the label mask or when label continuity needs to be re-established. The process can be applied to both 2D and 3D labeled images.

Process Name	Input	Output	Filter Direction	Parameters
Reorder Labels (2D)	Label (2D)	Label (2D)	XY plane (2D)	N/A
Reorder Labels (3D)	Label (3D)	Label (3D)	Volume (3D)	N/A

8.9 Logic

8.9.1. Contains Binary

The **Contains Binary/Label** process determines whether the regions defined in an input mask or label overlap with the regions defined in a stencil mask. It generates an output mask or label that retains only the regions from the input that have overlap with the stencil mask. An object is retained even if it is not fully overlapped with the stencil mask, as long as there is some intersection. This logical operation can be applied to both binary masks and label masks in 2D and 3D.

Process Name	Input	Output	Filter Direction	Parameters
Contains Binary (2D)	Mask (2D), Stencil Mask (2D)	Mask (2D)	XY plane (2D)	N/A
Contains Binary (3D)	Mask (3D), Stencil Mask (3D)	Mask (3D)	Volume (3D)	N/A
Contains Label (2D)	Label (2D), Stencil Mask (2D)	Label (2D)	XY plane (2D)	N/A
Contains Label (3D)	Label (3D), Stencil Mask (3D)	Label (3D)	Volume (3D)	N/A

8.9.2. Masking Binary/Label/Image

The **Masking Binary/Label/Image** process performs pixel/voxel-wise masking of an input binary mask, label mask, or image using a stencil mask. It generates an output that retains only the overlapping regions between the input and the stencil mask. Unlike the **Contains** process, which retains entire regions based on overlap, the **Masking** process keeps only the parts that are directly overlapping with the stencil mask. This logical operation can be applied to binary masks, label masks, and images in both 2D and 3D.

Process Name	Input	Output	Filter Direction	Parameters
Masking Binary (2D)	Mask (2D), Stencil Mask (2D)	Mask (2D)	XY plane (2D)	N/A
Masking Binary (3D)	Mask (3D), Stencil Mask (3D)	Mask (3D)	Volume (3D)	N/A
Masking Image (2D)	Image (2D), Stencil Mask (2D)	Image (2D)	XY plane (2D)	N/A
Masking Image (3D)	Image (3D), Stencil Mask (3D)	Image (3D)	Volume (3D)	N/A
Masking Label (2D)	Label (2D), Stencil Mask (2D)	Label (2D)	XY plane (2D)	N/A
Masking Label (3D)	Label (3D), Stencil Mask (3D)	Label (3D)	Volume (3D)	N/A

8.9.3. Logic – Invert

The **Logic - Invert** process performs a logical inversion on the input binary mask, transforming all regions where the mask is true (1) to false (0), and vice versa. This operation is useful for creating the inverse of a binary mask, effectively swapping foreground and background regions. The process can be applied to both 2D and 3D binary masks.

Process Name	Input	Output	Filter Direction	Parameters
Logic - Invert (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	N/A
Logic - Invert (3D)	Mask (3D)	Mask (3D)	Volume (3D)	N/A

8.9.4. Logic Operation

The **Logic Operation** process performs a logical operation between two input binary masks, combining them according to the specified logical operator. This operation allows users to generate a new mask based on the relationship between the input masks, such as intersecting, uniting, or subtracting regions. The process can be applied to both 2D and 3D binary masks.

Process Name	Input	Output	Filter Direction	Parameters
Logic Operation (2D)	Mask 1 (2D), Mask 2 (2D)	Binary Mask (2D)	XY plane (2D)	Logical Operator
Logic Operation (3D)	Mask 1 (3D), Mask 2 (3D)	Binary Mask (3D)	Volume (3D)	Logical Operator

Parameter	Description
Logical Operator	Specifies the type of logical operation to perform. Options include AND, OR, XOR, and SUBTRACT for combining or modifying the input masks.

8.10 Measurement

8.10.1. Measure Selective Features with Relation

The **Measure Selective Features with Relation** process calculates a variety of morphological, positional, physical, and intensity-related features for objects in 2D or 3D images. The process can also take into account hierarchical relationships between objects by using optional parent labels. It supports detailed measurements including custom refractive index (RI) settings and various physical properties.

Process Name	Input	Output	Filter Direction	Parameters
Measure Selective Features with Relation (2D)	Image (2D), Label (2D), Parent Label (2D, Optional)	Measurement Table	XY plane (2D)	Custom RI, Position, Morphology, Physical, Intensity, Parent Selection Criteria
Measure Selective Features with Relation (3D)	Image (3D), Label (3D), Parent Label (3D, Optional)	Measurement Table	Volume (3D)	Custom RI, Position, Morphology, Physical, Intensity, Parent Selection Criteria

Parameter	Description
Custom RI	Enables the use of a custom refractive index for measurements.
RII	Specifies the type of refractive index interaction to consider, such as Protein or Nucleic Acid.
RII Value	Defines the specific refractive index value for use in calculations.
Parent Selection Criteria	Determines the criteria used to relate objects to parent labels, such as Best Match or Nearest Neighbor.
Position	Enables calculation of positional features, including **Centroid** and **Bounding Box** (BBox).
Morphology	Includes measurements such as **Volume**, **Filled Volume**, **Surface Area**, **Projected Area**, **Sphericity**, **ESD** (Equivalent Spherical Diameter), **Feret Diameter** (Max/Min), and **Skeleton Length** for 3D and **Area, Circularity, ECD** (Equivalent circular diameter)**, Feret Diameter** (Max/Min)**,** and **Skeleton Length** for 2D.
Physical	Involves physical properties, such as **RI Mean, RI Min, RI Max, RI Std, Range of RI, Dry mass,** and **Concentration** for 3D and **RI Mean, RI Min, RI Max, RI Std**, and **Range of RI** for 2D.
Intensity	Includes intensity-related measurements such as **Intensity Mean, Intensity Sum, Intensity Min, Intensity Max, Intensity Std,** and **Range of Intensity.**

8.10.2. Global Measure

The **Global Measure** process calculates global properties of the input label mask, treating all labels as a single entity. This effectively converts the label mask into a binary mask for measurement purposes. The process computes various morphological, intensity, and physical features for the entire region covered by the input labels in 2D or 3D images. It also supports custom refractive index (RI) settings for physical measurements.

Process Name	**Input**	**Output**	**Filter Direction**	**Parameters**
Global Measure (2D)	Image (2D), Label Mask	Measurement Table	XY plane (2D)	Custom RI, Measurement Features
Global Measure (3D)	Image (3D), Label Mask	Measurement Table	Volume (3D)	Custom RI, Measurement Features

Parameter	**Description**
Custom RI	Enables the use of a custom refractive index for measurements.
RII	Specifies the type of refractive index interaction to consider, such as Protein or Nucleic Acid.
RII Value	Defines the specific refractive index value for use in calculations.
Measurement Features	Enables selection of features to measure, including **Volume, Surface Area, Projected Area, Mean Intensity, Sum of Intensities, Mean RI, Dry mass, Concentration,** and **Centroid Coordinates (X, Y, Z)** for 3D and **Surface Area, Mean Intensity, Mean RI, Circularity,** and **Centroid Coordinates (X, Y)** for 2D**.**

8.11 MorphOp

8.11.1. Opening/Closing

The **Opening/Closing** processes perform morphological transformations on binary masks using specified structuring elements. The operations include **Opening** (erosion followed by dilation) and **Closing** (dilation followed by erosion). These processes are used to remove small objects or fill small holes in binary masks. The operations can be applied in both 2D and 3D, with adjustable kernel size and connectivity settings. The border policy determines how the boundaries of the image are handled during the operation.

Process Name	Input	Output	Filter Direction	Parameters
Opening XY (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Kernel Radius, Neighborhood, Border Policy
Opening XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Neighborhood, Border Policy
Closing XY (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Kernel Radius, Neighborhood, Border Policy
Closing XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Neighborhood, Border Policy

Parameter	Description
Kernel Radius	Specifies the size of the structuring element used for the operation.
Neighborhood	Defines the connectivity for neighboring pixels/voxels. Options include 4- and 8-neighborhood for 2D, and 6-, 18-, and 26-neighborhood for 3D.
Border Policy	Determines how image boundaries are treated during the operation. Options include Ignore and Extended.

8.11.2. Ball Opening/Closing

The **Ball Opening/Closing** processes perform morphological transformations on binary masks using a spherical structuring element. The operations include **Ball Opening** (erosion followed by dilation) and **Ball Closing** (dilation followed by erosion) with a ball-shaped kernel. These operations are useful for smoothing object boundaries and filling holes in 3D data. The process can be applied to 3D binary masks, with options to adjust the kernel size and processing precision.

Process Name	Input	Output	Filter Direction	Parameters
Ball Opening XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Precision
Ball Closing XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Precision

Parameter	Description
Kernel Radius	Specifies the radius of the spherical structuring element used for the operation.
Precision	Determines the computational accuracy of the operation. Options include Faster and Higher precision settings.

8.11.3. Dilation/Erosion

The **Dilation/Erosion** processes perform fundamental morphological transformations on binary masks. The operations include **Dilation** (expanding object boundaries) and **Erosion** (shrinking object boundaries). These processes are useful for modifying the shape of objects, filling gaps, or removing small regions. The operations can be applied in both 2D and 3D, with adjustable kernel size and connectivity settings.

Process Name	Input	Output	Filter Direction	Parameters
Dilation XY (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Kernel Radius, Neighborhood
Dilation XY (3D)	Mask (3D)	Mask (3D)	XY slices (2D, per slice)	Kernel Radius, Neighborhood
Dilation XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Neighborhood
Erosion XY (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Kernel Radius, Neighborhood
Erosion XY (3D)	Mask (3D)	Mask (3D)	XY slices (2D, per slice)	Kernel Radius, Neighborhood
Erosion XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Kernel Radius, Neighborhood

Parameter	Description
Kernel Radius	Specifies the size of the structuring element used for the operation.
Neighborhood	Defines the connectivity for neighboring pixels/voxels. Options include 4- and 8-neighborhood for 2D, and 6-, 18-, and 26-neighborhood for 3D.

8.11.4. Fill Holes

The **Fill Holes** processes fill internal holes in binary masks, where a hole is defined as a region of background pixels/voxels that is entirely surrounded by foreground pixels/voxels. This operation is useful for closing gaps within objects in a binary mask. The process can be applied in both 2D and 3D, with different filtering directions: **XY plane (2D)**, **XY slices (3D)**, and **XYZ volume (3D)**.

Process Name	Input	Output	Filter Direction	Parameters
Fill Holes XY (2D)	Mask (2D)	Mask (2D)	XY plane (2D)	Neighborhood
Fill Holes XY (3D)	Mask (3D)	Mask (3D)	XY slices (2D, per slice)	Neighborhood
Fill Holes XYZ (3D)	Mask (3D)	Mask (3D)	Volume (3D)	Neighborhood

Parameter	Description
Neighborhood	Defines the connectivity for neighboring pixels/voxels to determine whether a hole should be filled. Options include 4- and 8-neighborhood for 2D, and 6-, 18-, and 26-neighborhood for 3D.

8.11.5. Centerline Line

The **Center Line** processes generate a path with a tree topology that approximates the shape of an object, maintaining the path equidistant from the boundaries. A Euclidean distance map ensures the path stays centered, and the algorithm enforces a loop-free tree structure, even if loops exist in the input.

The process iteratively searches for the longest branch to add to the tree, allowing for the detection of secondary branches based on a specified sensitivity parameter. This parameter controls when new branches are added to the tree, favoring branches that meet a length threshold.

Process Name	Input	Output	Filter Direction	Parameters
Center Line of Binary (3D)	Mask (3D)	Mask (3D)	Volume (3D)	New Branch Sensibility
Center Line of Label (3D)	Label (3D)	Label (3D)	Volume (3D)	New Branch Sensibility

Parameter	Description
New Branch Sensibility	Controls the sensitivity for detecting branches, with higher values making smaller branches more likely to be added.

8.11.6. Skeletonize

The **Skeletonize** processes reduce a shape in a binary or labeled mask to its skeletal form, preserving the object's connectivity while thinning it to a one-pixel/voxel-wide representation. The skeleton represents points where the distance to the object's boundary is minimized at two or more locations, resembling the central "spine" of the shape. This process is useful for shape analysis and feature extraction.

Process Name	Input	Output	Filter Direction	Parameters
Skeletonize Binary (3D)	Mask (3D)	Mask (3D)	Volume (3D)	N/A
Skeletonize Label (3D)	Label (3D)	Label (3D)	Volume (3D)	N/A

8.11.7. Grayscale Opening/Closing

The **Grayscale Opening/Closing** processes perform morphological transformations on grayscale images using a spherical structuring element. The operations include **Grayscale Opening** (erosion followed by dilation) and **Grayscale Closing** (dilation followed by erosion). These processes are used to smooth the intensity variations in grayscale images, such as removing small bright or dark artifacts. The process can be applied in 3D with adjustable kernel size and precision settings.

Process Name	Input	Output	Filter Direction	Parameters
Grayscale Opening XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Precision
Grayscale Closing XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Precision

Parameter	Description
Kernel Radius	Specifies the size of the structuring element used for the operation.
Precision	Determines the computational accuracy of the operation. Options include Faster and Higher precision settings.

8.11.8. Grayscale Erosion

The **Grayscale Erosion** processes perform grayscale erosion on images using a spherical structuring element. **Grayscale Erosion** shrinks brighter regions by reducing intensity values in areas with higher brightness, effectively darkening and minimizing features in the image. This operation is useful for enhancing boundaries and removing small bright artifacts in grayscale images.

Process Name	Input	Output	Filter Direction	Parameters
Grayscale Erosion XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Kernel Radius, Neighborhood
Grayscale Erosion XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Kernel Radius, Neighborhood
Grayscale Erosion XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Neighborhood

Parameter	Description
Kernel Radius	Specifies the size of the structuring element used for the operation.
Neighborhood	Defines the connectivity for neighboring pixels/voxels. Options include 4- and 8-neighborhood for 2D, and 6-, 18-, and 26-neighborhood for 3D.

8.12 Segmentation

8.12.1. Threshold

The **Threshold** processes segment image data by applying intensity-based thresholds to create a binary mask. It assigns pixels/voxels to the foreground if their intensity values fall within the specified range, otherwise assigning them to the background. The process allows for both lower and upper thresholding, and these thresholds can be independently enabled or disabled. The **Threshold** operation can be applied to both 2D and 3D images.

Process Name	Input	Output	Filter Direction	Parameters
Threshold (2D)	Image (2D)	Mask (2D)	XY plane (2D)	Disable Lower Threshold, LThreshold, Disable Upper Threshold, UThreshold
Threshold (3D)	Image (3D)	Mask (3D)	Volume (3D)	Disable Lower Threshold, LThreshold, Disable Upper Threshold, UThreshold

Parameter	Description
Disable Lower Threshold	Enables or disables the lower threshold setting. When enabled, pixels/voxels below LThreshold are set to background.
LThreshold	Specifies the lower threshold value for segmentation.
Disable Upper Threshold	Enables or disables the upper threshold setting. When enabled, pixels/voxels above UThreshold are set to background.
UThreshold	Specifies the upper threshold value for segmentation.

8.12.2. Automatic Threshold

The **Automatic Threshold** processes automatically determine intensity thresholds for segmenting image data based on various algorithms. It creates a binary mask by classifying pixels/voxels into foreground or background depending on their intensity relative to the computed threshold. Users can adjust parameters such as the algorithm, scaling factor, and offset to fine-tune the segmentation. This process can be applied to both 2D and 3D images.

Process Name	Input	Output	Filter Direction	Parameters
Automatic Threshold (2D)	Image (2D)	Mask (2D)	XY plane (2D)	Algorithm, Foreground, Threshold Scale, Threshold Offset
Automatic Threshold (3D)	Image (3D)	Mask (3D)	Volume (3D)	Algorithm, Foreground, Threshold Scale, Threshold Offset

Parameter	Description
Algorithm	Selects the thresholding algorithm (e.g., Entropy, Otsu, etc.) used to determine the threshold value.
Foreground	Specifies whether the foreground represents Bright or Dark regions in the image.
Threshold Scale	Scales the calculated threshold value to adjust the sensitivity of the segmentation.
Threshold Offset	Adds an offset to the calculated threshold value for fine-tuning the segmentation. **NOTE**: Threshold Offset is applied after Threshold Scale is multiplied to the calculated threshold value.

8.12.3. Regional Maxima

The **Regional Maxima** processes identify local maxima within grayscale images, where a local maximum is a pixel/voxel whose intensity is greater than or equal to that of its neighbors. The detected maxima are marked in the output mask, which can be used for further segmentation tasks. The process can be applied in both 2D and 3D, with adjustable neighborhood settings to define the connectivity for determining local maxima.

Process Name	Input	Output	Filter Direction	Parameters
Regional Maxima XY (2D)	Image (2D)	Mask (2D)	XY plane (2D)	Neighborhood
Regional Maxima XY (3D)	Image (3D)	Mask (3D)	XY slices (2D, per slice)	Neighborhood
Regional Maxima XYZ (3D)	Image (3D)	Mask (3D)	Volume (3D)	Neighborhood

Parameter	Description
Neighborhood	Defines the connectivity for neighboring pixels/voxels when identifying local maxima. Options include 4- and 8-neighborhood for 2D, and 6-, 18-, and 26-neighborhood for 3D.

8.12.4. Convert Label into Binary

The **Convert Label into Binary** processes transform a labeled mask into a binary mask, where all labeled regions are converted to foreground, and unlabeled regions are set to background. This operation is commonly used to simplify segmentation results for further processing. The process can be applied in both 2D and 3D.

Process Name	Input	Output	Filter Direction	Parameters
Convert Label into Binary (2D)	Label (2D)	Mask (2D)	XY plane (2D)	N/A
Convert Label into Binary (3D)	Label (3D)	Mask (3D)	Volume (3D)	N/A

8.12.5. Extend Mask from Seed

The **Extend Mask from Seed** processes generate an extended mask based on seed regions defined in the input label and a reference binary mask. The operation propagates the seed regions to the surrounding areas based on the reference binary mask, effectively growing the labeled regions. This process can be applied in both 2D and 3D, with an optional parameter to filter objects based on size.

Process Name	Input	Output	Filter Direction	Parameters
Extend Mask from Seed (2D)	Label Mask (2D), Binary Mask (2D)	Binary Mask (2D)	XY plane (2D)	Object Filter Size
Extend Mask from Seed (3D)	Label Mask (3D), Binary Mask (3D)	Binary Mask (3D)	Volume (3D)	Object Filter Size

Parameter	Description
Object Filter Size	Specifies the minimum size for objects to be retained in the extended mask. Objects smaller than this size will be filtered out.

8.13 Smoothing

8.13.1. Gaussian

The **Gaussian Smoothing** processes apply Gaussian blurring to an image, effectively reducing noise and smoothing out intensity variations. The operation uses a Gaussian kernel defined by its standard deviations along the X, Y, and Z directions, allowing for anisotropic smoothing. This process can be applied to both 2D and 3D images, with different filtering directions available.

Process Name	Input	Output	Filter Direction	Parameters
Gaussian XY (2D)	Image (2D)	Image (2D)	XY plane (2D)	Standard Deviation X, Standard Deviation Y
Gaussian XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Standard Deviation X, Standard Deviation Y
Gaussian XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Standard Deviation X, Standard Deviation Y, Standard Deviation Z

Parameter	Description
Standard Deviation X	Specifies the standard deviation of the Gaussian kernel in the X direction, controlling the amount of smoothing along that axis.
Standard Deviation Y	Specifies the standard deviation of the Gaussian kernel in the Y direction, controlling the amount of smoothing along that axis.
Standard Deviation Z	(3D only) Specifies the standard deviation of the Gaussian kernel in the Z direction, controlling the amount of smoothing along that axis.

8.13.2. Median

The **Median** processes reduce noise in image data by replacing each pixel/voxel's intensity with the median intensity value of neighboring pixels/voxels within a defined kernel. This operation is effective for preserving edges while smoothing out noise. The kernel shape and search mode can be customized for more refined control. The process can be applied in both 2D and 3D.

Process Name	Input	Output	Filter Direction	Parameters
Median (2D)	Image (2D)	Image (2D)	XY plane (2D)	Kernel Radius, Kernel Mode, Search Mode
Median XY (3D)	Image (3D)	Image (3D)	XY slices (2D, per slice)	Kernel Radius, Kernel Mode, Search Mode
Median XYZ (3D)	Image (3D)	Image (3D)	Volume (3D)	Kernel Radius, Kernel Mode, Search Mode

Parameter	Description
Kernel Radius	Specifies the radius of the filtering kernel.
Kernel Mode	Defines the shape of the kernel. Options include Cube and Sphere.
Search Mode	Determines the mode for finding the median value. Options include Automatic and Manual.

Chapter 9. Appendix

9.1 Glossary

Term	Description
TCF file	A file format used to store data captured by Tomocube's Holotomography (HT) systems, containing refractive index (RI) or fluorescence (FL) data used for analysis in TomoAnalysis.
Process	A single step within a pipeline, responsible for performing specific data processing tasks such as filtering or segmentation.
Pipeline	A sequence of processes in TomoAnalysis designed to perform various image processing tasks in a specific order.
Parameter	A variable that controls the behavior of a process within a pipeline, such as intensity threshold or segmentation method.
Analysis Project	An organizational unit in TomoAnalysis that gathers all relevant data, pipelines, and parameter presets to streamline the analysis process. Serves as the foundation for batch or individual analysis runs.
Workset	A unit of data used in an analysis project, where time points or channels from TCF files are selected for processing.
Workset group	A grouping of worksets based on their experimental setup or acquisition parameters, used for batch processing.
Mask	Binary data generated from image processing to segment or highlight regions of interest, such as cells or structures.
Label	A specific annotation assigned to different regions or objects in the image data, typically represented by unique numbers or colors.
Measurement data	Quantitative output data generated from the measurement process, represented in tabular format.
3D HT	3D holotomography data containing refractive index information.
2D HT	2D Maximum Intensity Projection (MIP) generated from 3D holotomography data, where the brightest voxel along the Z axis is projected.
3D FL	3D fluorescence data containing fluorescence intensity information.
2D FL	2D Maximum Intensity Projection (MIP) generated from 3D fluorescence data, where the brightest voxel along the Z axis is projected.

If you need further information,

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