LIM Tracker Quickstart Manual

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publication
LIM Tracker: a software package for cell tracking and analysis
with advanced interactivity. (2022).

Contents

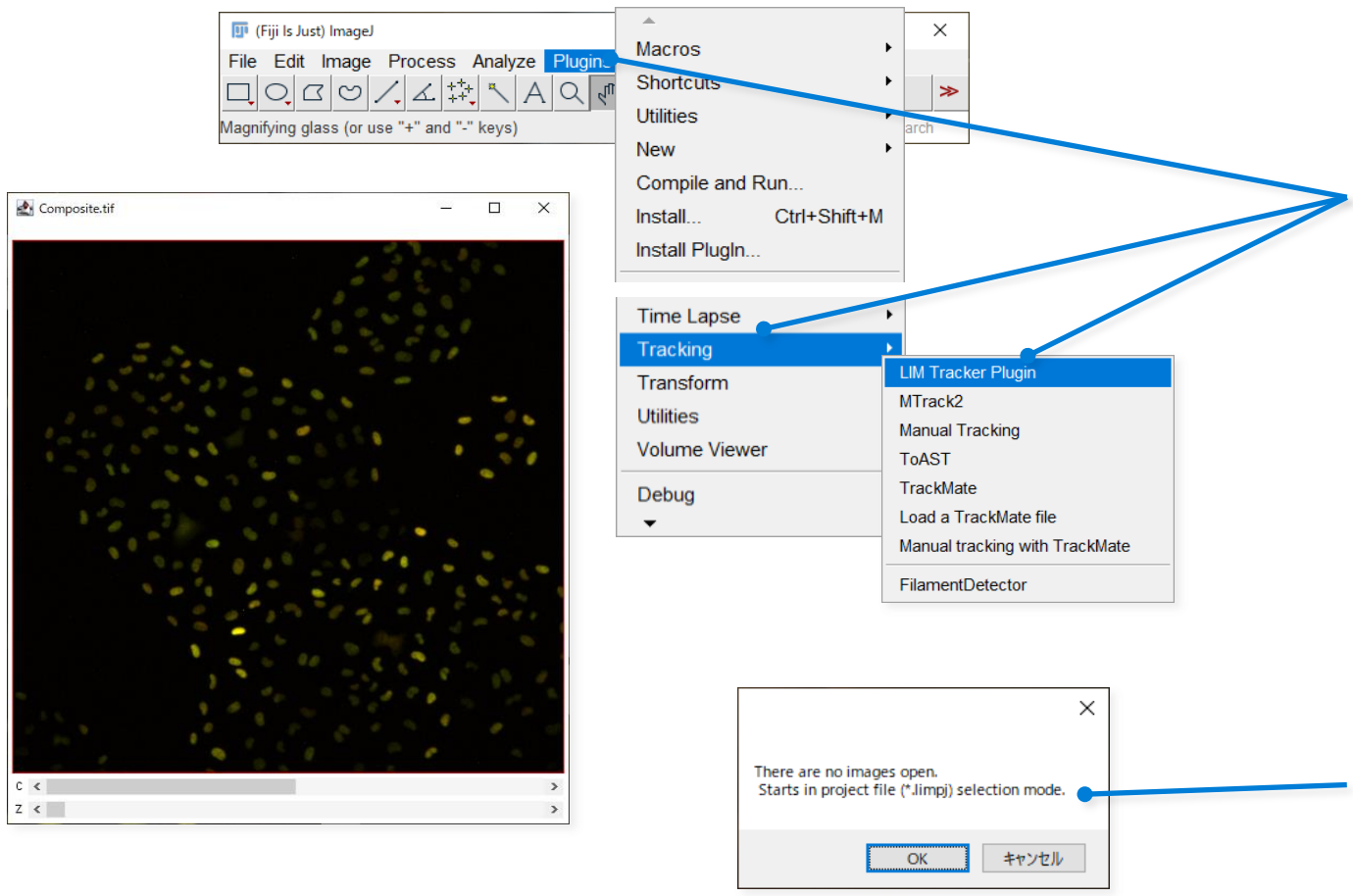
1. How to launch the plugin

You can start this plugin by loading an image into Fiji and selecting "Plugins/Tracking/LIM Tracker Plugin" in the menu.
8 or 16 bit grayscale images are supported. Multi-channel (composite) images with up to 5 channels are supported.
This plugin does not support the following image formats.

- *.RAW, *.ZIP, and *.TextImage.
- 24bit (RGB) image, 32bit image
- Tiff images with "slice labels" set as metadata
- Images loaded as Virtual Stack.

※ This plugin does not allow you to change the image to process from within the plugin.
If you want to load a different image, you need to close the plugin, select the image on Fiji, and then start the plugin again.

※ You cannot have multiple plugins running at the same time.



1 Launch Fiji and load the image.

2 Select "Plugins/Tracking/LIM Tracker Plugin" from the Fiji menu.

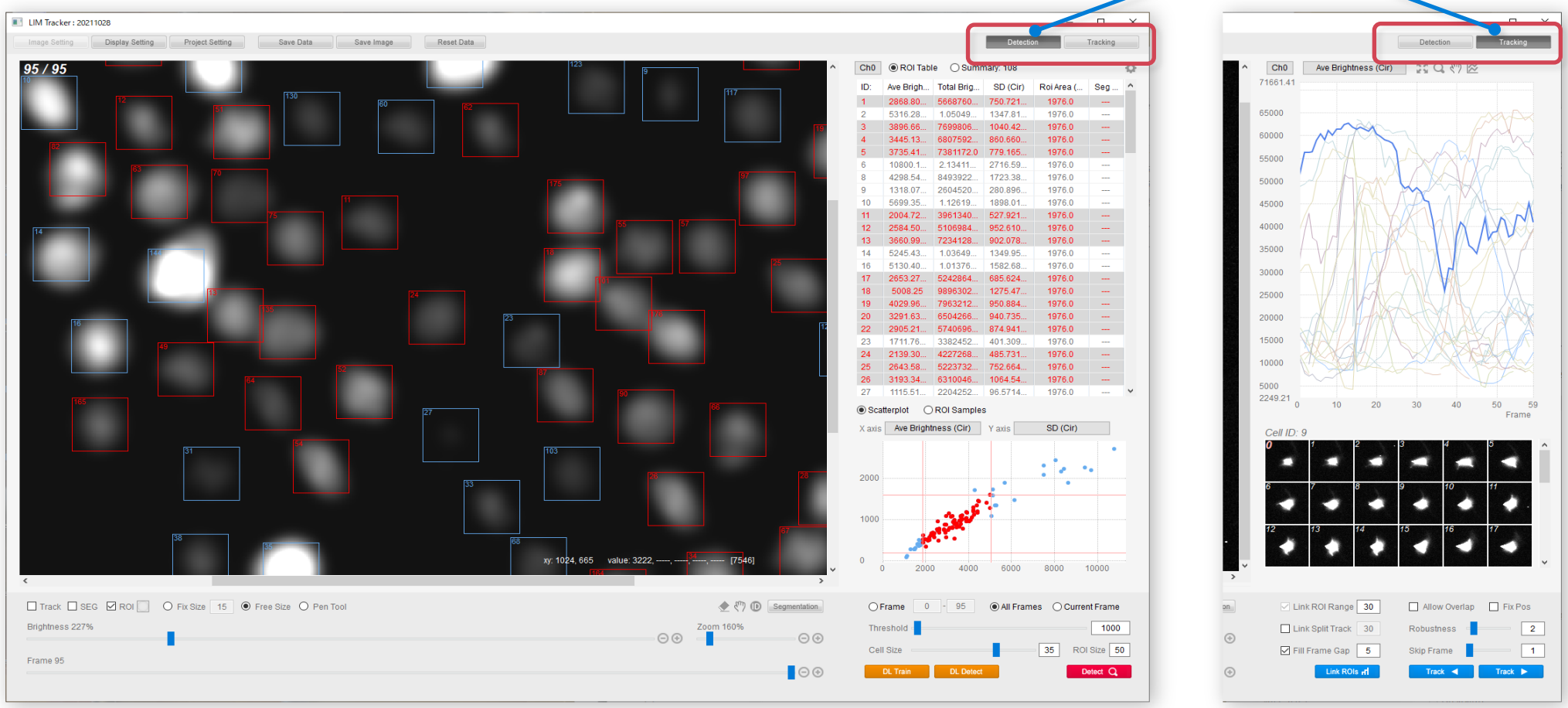
＞ This will launch the plugin.

※ The plugin will be launched in project file selection mode if you select the menu when no image is loaded (see Section 13).

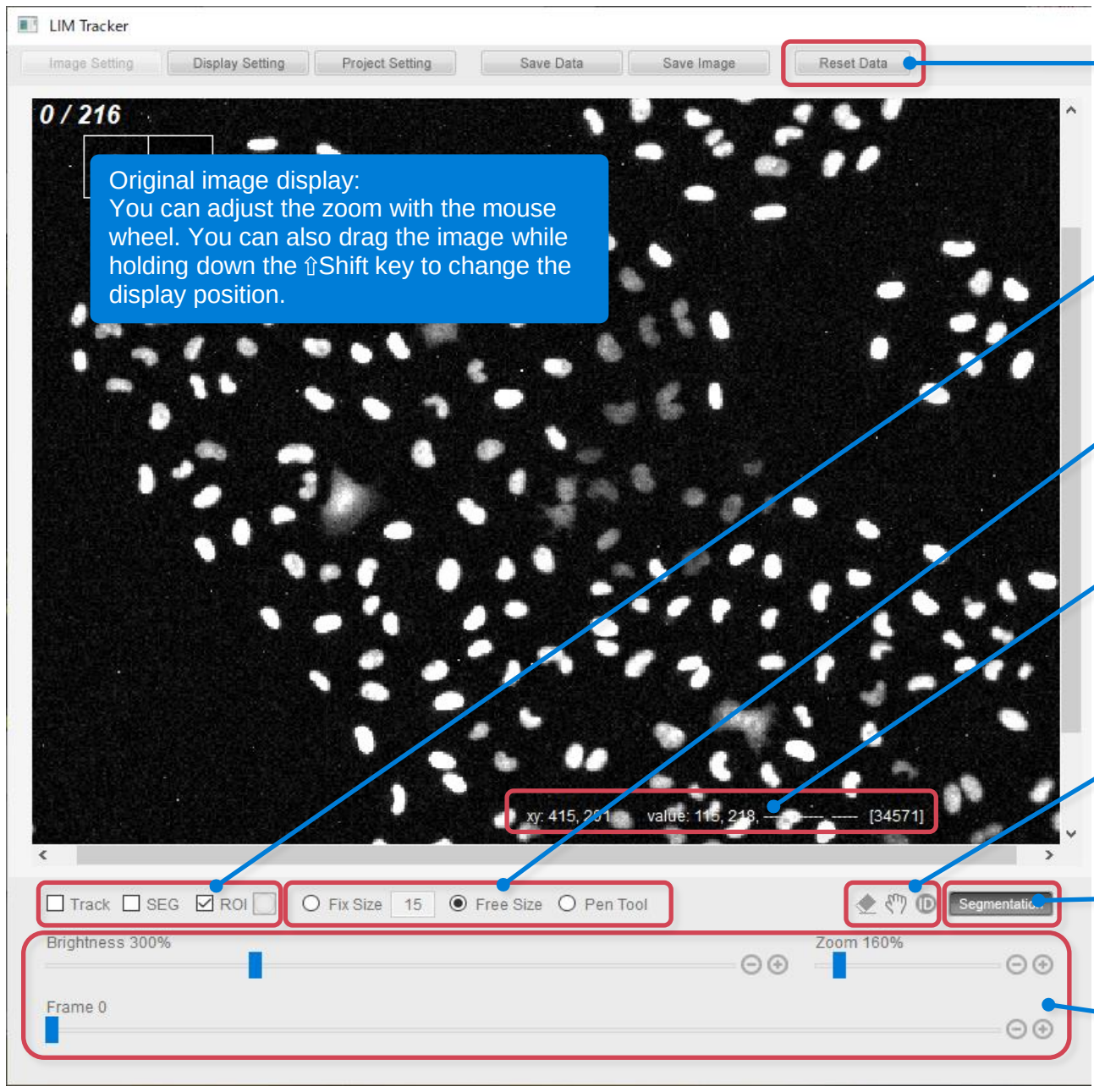
2. Detection mode and tracking mode

This plugin consists of "detection" mode and "tracking" mode and operates by switching between the two methods.

Switch between "detection" mode and "tracking" mode.



3. Basic operations



Reset button: Deletes all detection and tracking results.

Track: Toggles the tracking track display ON/OFF.
SEG: Toggles the region shape display ON/OFF.
ROI: Toggles the ROI display ON/OFF and the appearance of the ROI.

Fix Size: Switches the method of adding ROI.
Free Size: Switches the method of adding ROI.
Pen Tool: Switches the method for adding ROI.
(see Section 5.1)

Displays the cursor information (coordinates, pixel value, and apparent pixel value (values in [ ] are 16-bit equivalent values with brightness adjusted)).

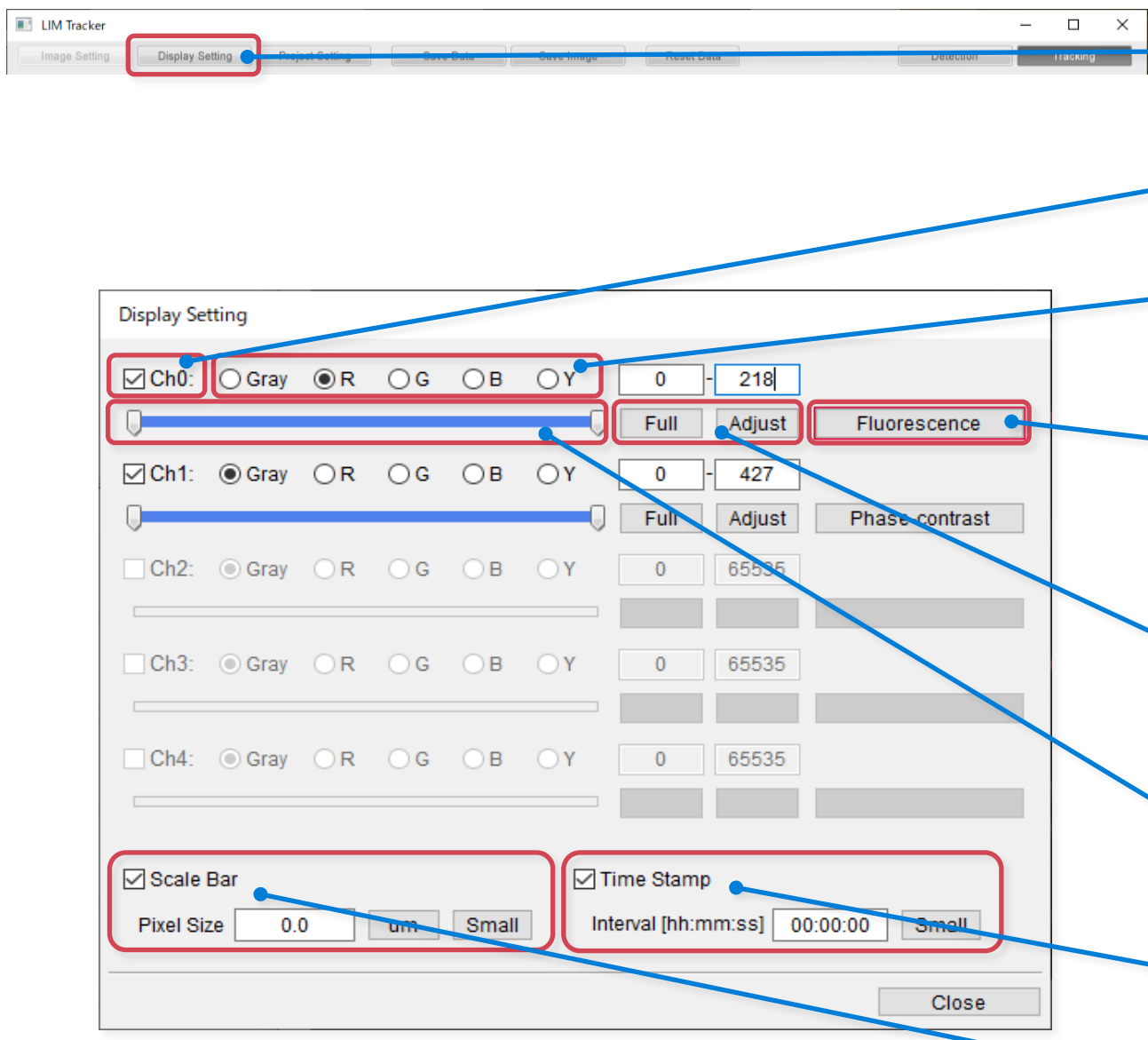
Remove Track icon: Opens the RemoveTrack dialog box. It allows you to delete trajectories less than a predetermined frame length.
Hand icon: When active, you can adjust the display position of the image by dragging the original image itself.
ID icon: Reassign the Cell ID numbers (see Section 5.1) of all cells a sequentially number from 1.

Segmentation button: Toggles the region shape acquisition mode ON/OFF (see Section 6).

Brightness slider: Adjusts the apparent brightness of the image.
Zoom slider: Adjusts the zoom of the image.
Frame slider: Changes the display frame.

4. Display setting

Allows you to set the brightness and color of each channel for image display.



1 Click the Display Setting button.
＞ The Display Setting dialog is displayed.

2 Click the Ch0 check box.
＞ Toggles the Channel0 image display ON/OFF.

3 Click the Color Select button.
＞ Switches the display color of Channel0.

4 Click the Modality Select button.
＞ Switch between "Fluorescence" and "Phase-contrast".

※ The channel selected for phase-contrast is excluded from the recognition process (non-DL, see Section 7).

5 Click the Full button or Adjust button.
＞ Full: Displays the image in 16-bit range (0-65535).
＞ Adjust: Detects the minimum and maximum pixel values and adjusts them to the 16-bit range for display.

※ Each time the Adjust button presses, the apparent width of the slider changes.

6 Adjust the brightness adjustment slider.
＞ Adjust the display brightness range.

7 Click the Time Stamp check box.
＞ Toggles the timestamp display ON/OFF.

8 Click the Scale Bar checkbox.
＞ Switches the scale bar display ON/OFF.

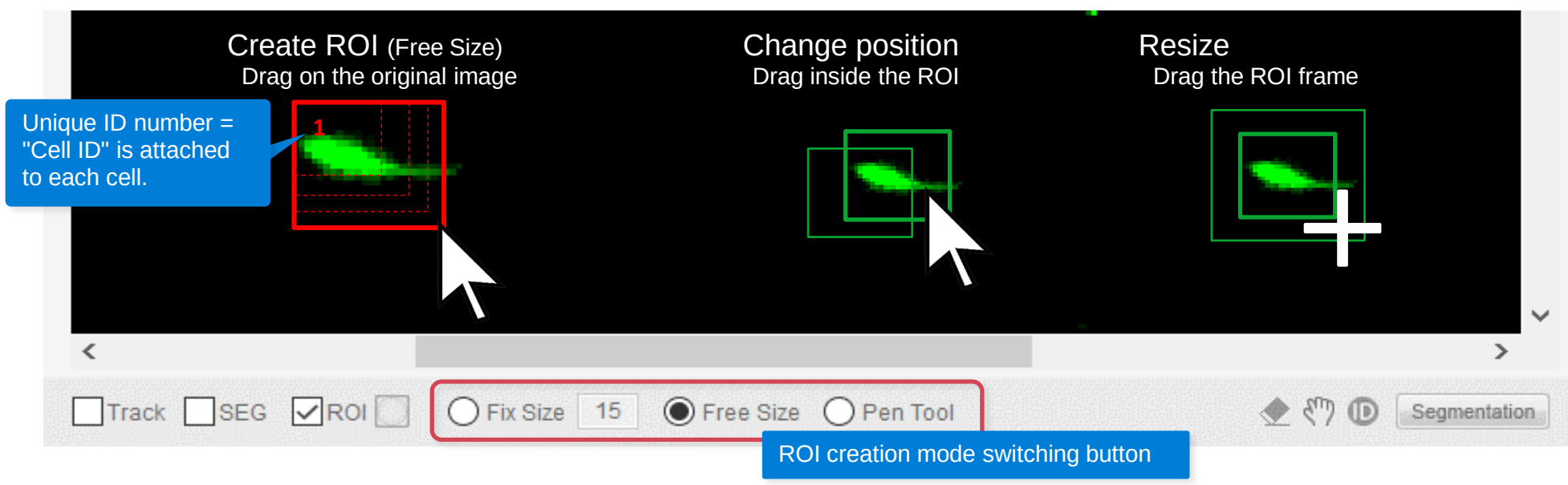
5. ROI operations and trajectory editing

ROI can be set at any position on the original image using the mouse.
You can also delete the ROI, edit the trajectory (connect, cut, split), and create a montage image from the right-click menu of the ROI.

5.1. ROI creation, position, size change

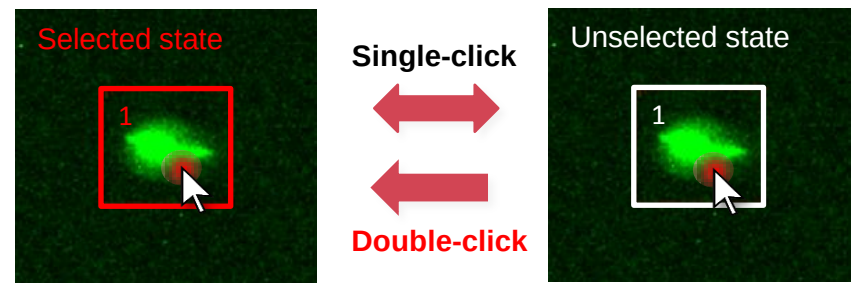
ROI can create on the original image at any time. You can also change the position and size of the ROI freely.
The three buttons at the bottom center of the original image allow you to switch between the following three ROI creation modes.

(1) **Fix Size**: A fixed-size ROI can be created by single-clicking on the original image to specify a numerical value.
(2) **Free Size**: ROI of any size can be created on the original image by mouse drag & drop operation.
(3) **Pen Tool**: ROI can be created by tracing the boundary of the target while holding down the left mouse button on the original image.



5.2. Single-click selection of ROI

Switch between selected (red frame) and unselected (white or blue frame) ROI.
In the cell tracking (sequential search-type method), the ROI in the selected (red frame) will be the target of tracking (see Section 9).



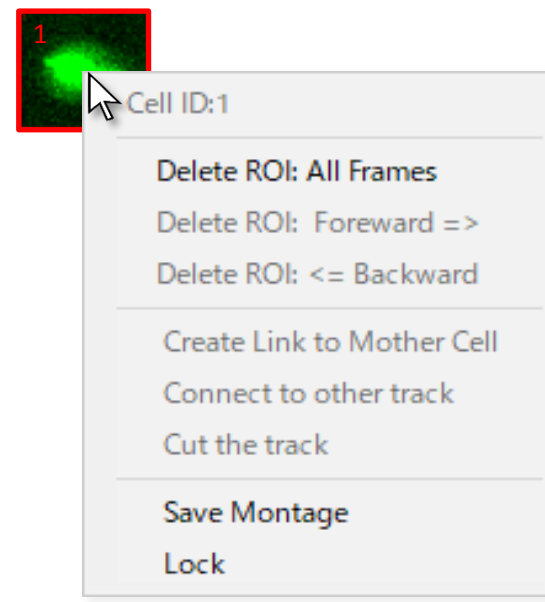
5.3. Double-click selection of ROI

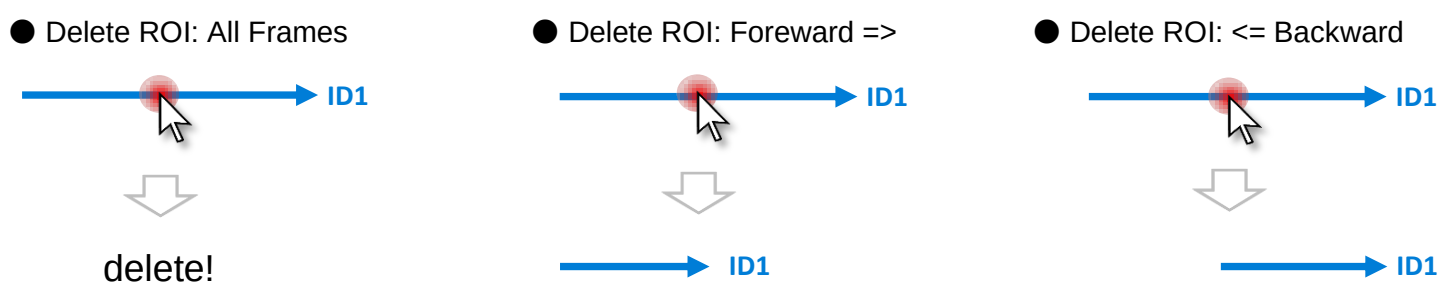
When there is an ROI that you want to focus on, double-clicking on the ROI will make only the relevant "ROI (trajectory)" selected (red frame).
Select the ROI (and the ROI with the same Cell ID) for the entire frame (red frame) and deselect all other ROIs (white or blue frame).
In the cell tracking (sequential search-type method), the ROI in the selected (red frame) will be the target of tracking (see Section 9).

5.4. Deleting ROI and editing trajectory by right-click menu

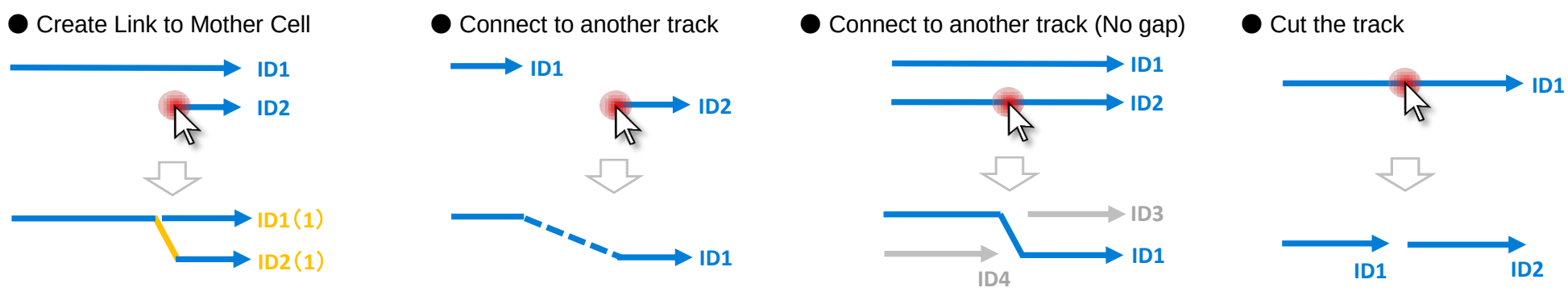
From the right-click menu of an ROI, you can delete the ROI, link mother-daughter cells, connect/cut trajectories, create montage images, and lock ROI operations.

(1) **Delete ROI: All Frames**: Delete ROIs with the same Cell ID across all frames.
(2) **Delete ROI: Foreward =>**: Deletes the ROIs for the frame after the frame is displayed (forward direction).
(3) **Delete ROI: <= Backward**: Deletes the ROIs for the frame before the frame is displayed (backward direction).





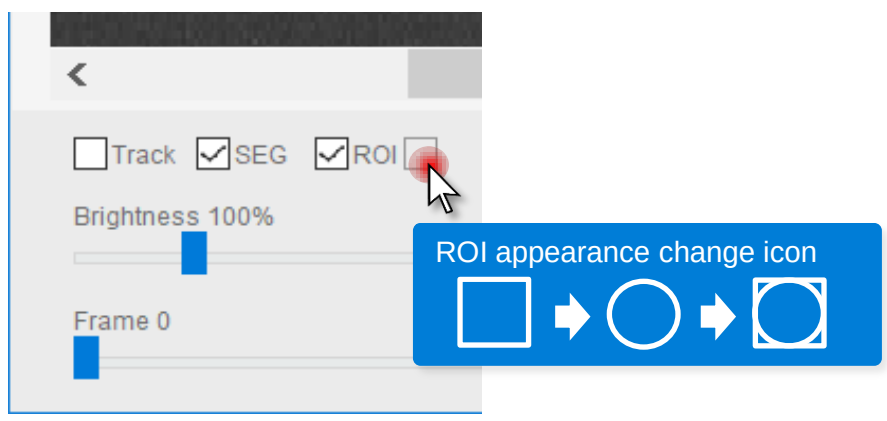
(4) **Create Link to Mother Cell**: Links cell divisions to ROI with different Cell ID that exist one frame before the currently displayed frame.
(5) **Connect to another track**: You can connect ROIs (trajectory) by specifying other ROIs that exist in the previous frame from the currently displayed frame.
(6) **Cut the track**: Set a new Cell ID for the ROI in the forward direction after the current frame, and split the trajectory in two.

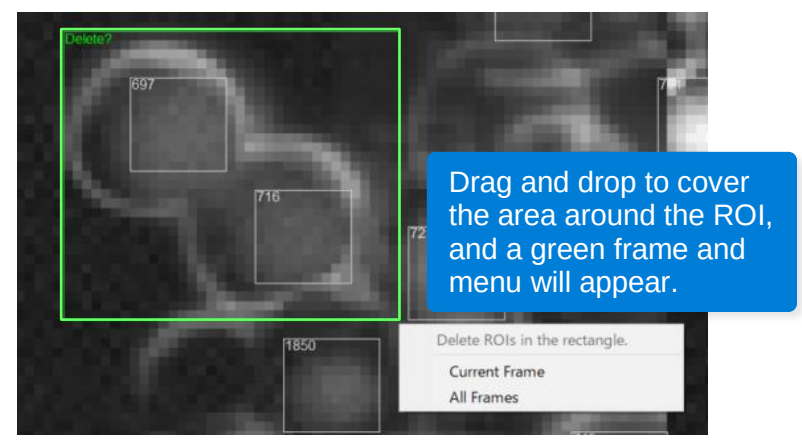


(7) **Save Montage**: Generates a montage image and a group of thumbnail images related to the relevant Cell ID and saves them in a file (see Section 15).
(8) **Lock or Unlock**: Locks or unlocks the operations related to the Cell ID.

5.5. Calculation of feature value

The ROI consists of a rectangle ☐ and a circle ○, and feature values such as average brightness are calculated based on the pixel values within each of the rectangle and circle.
You can change the apparent appearance by clicking the ROI appearance setting icon "☐" at the bottom left of the screen.
Features based on ☐ are labeled with "(Rect)", features based on ○ are labeled with "(Cir)", and features based on region shape are labeled with "(Seg)".





5.6. Deleting multiple ROIs at once

When "Free Size" is selected, drag and drop the ROI on the original image so that a green rectangle surrounds it, and the "Delete ROIs in the rectangle" menu will appear.
Select "Current Frame" from the menu to delete ROIs in the rectangle of the currently displayed frame.
Select "All Frames" from the menu to delete ROIs in the rectangle of all frames.

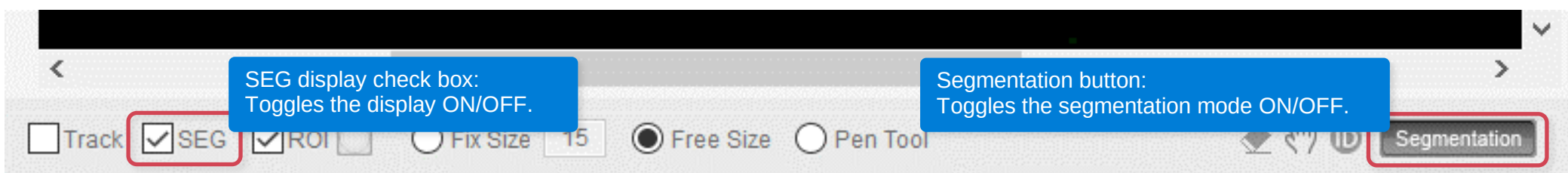
5.7. Connecting and linking trajectories by overlapping ROIs

By drag and drop the ROI onto another ROI with the mouse, a dialog box will appear, allowing you to connect trajectories or link cell divisions.

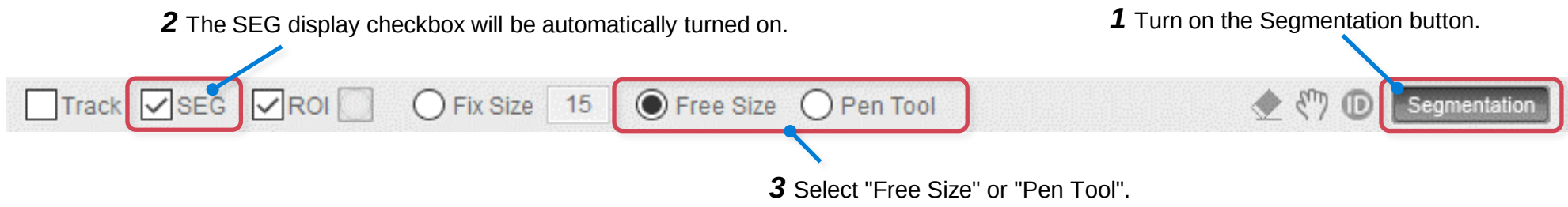
6. Segmentation mode (region shape acquisition)

When you add an ROI to the original image, you can choose whether or not to acquire the region shape by a segmentation process.
Clicking the "Segmentation button" at the bottom right of the original image display will toggle the "acquisition" of the region shape on and off.
The "Display" of the region shape can be turned on or off by clicking the SEG checkbox. The region shape is displayed as a yellow (when not selected) or red (when selected) semi-transparent area on the screen.

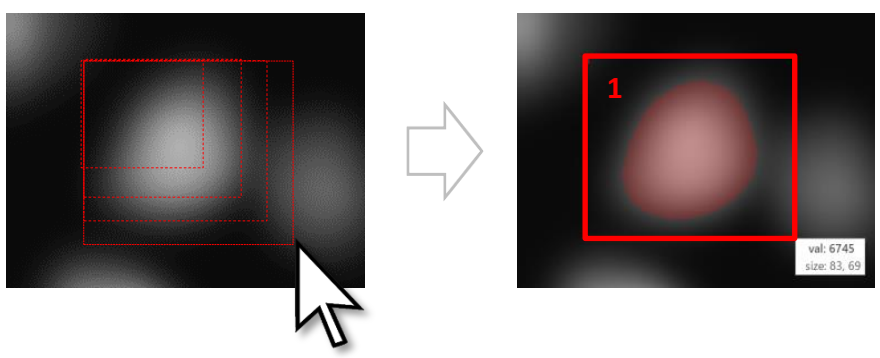
※ The region shape is automatically recognized (acquired) based on the local peak position and distribution of the luminance values in the original image. If the image does not have a brightness peak near the center of the cell, such as in a phase-contrast microscope image, the region shape may not be appropriately acquired, in which case set the Segmentation button to OFF.



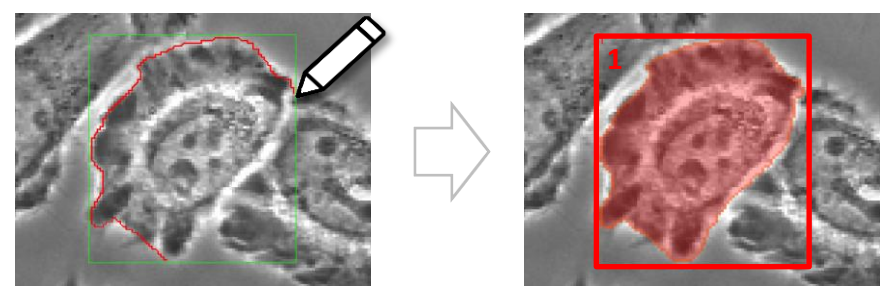
※ When creating ROI (see Section 5.1), the region shape can be acquired automatically.
(1) When "FreeSize" is selected: By D&D the mouse on the original image, the region shape can be set automatically at the same time as ROI creation.
(2) When "PenTool" is selected: The shape can be set freely by tracing the boundary of the target while holding down the left mouse button on the original image.



4-A When "Free Size" is selected, the region shape will be set automatically with mouse D&D.



4-B When "Pen Tool" is selected, it can set any shape by tracing the boundary while holding the left mouse button.

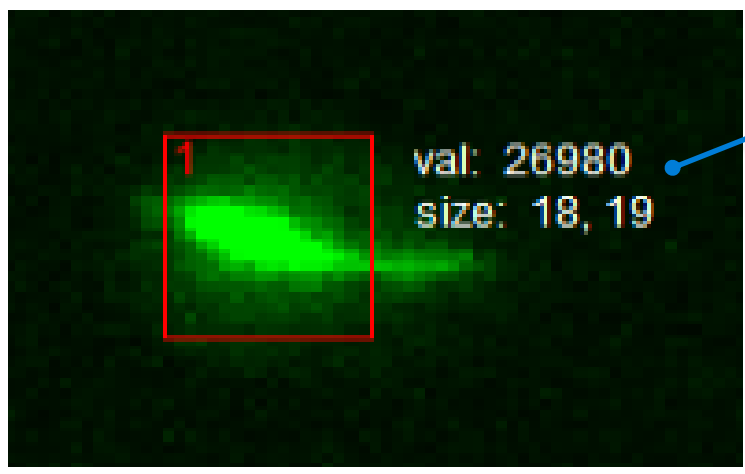


7. Cell Recognition

Automatically detects cells and displays ROIs on the original image.
The cell recognition process is based automatically on the local peak position and distribution of the luminance values in the original image.
This function performs the recognition process based on the brightness-adjusted pixel values displayed on the screen (see Section 4), not the actual pixel values.
(If you use the deep learning recognition function, see section 16.)

※ The recognition process may not work correctly for phase-contrast images and other images that do not have a brightness peak near the center of the cell.
※ The channel for which Display OFF is set in Display Settings (see Section 4) is excluded from the recognition process.
※ The channel for which "Phase-contrast" is set in the modality setting of the display settings (see Section 4) is excluded from the recognition process.

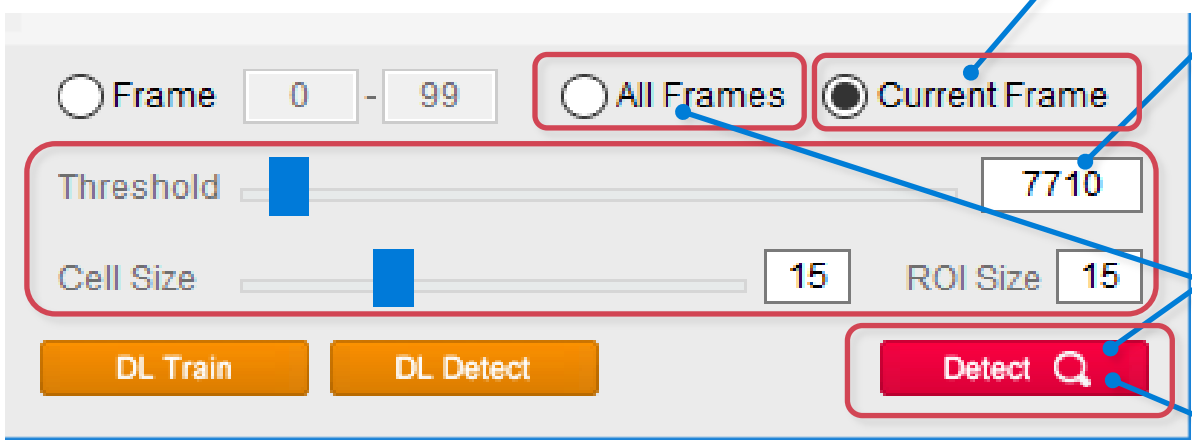
1 Select the Detection mode.



2 Get the parameter information.
＞ D&D an arbitrary target in the original image in FreeSize mode (see Section 5.1) to create ROI.
＞ The (apparent) average brightness and size will be displayed near the ROI. Use this information as a reference for the following parameter settings.

3 Set the target frame.
＞ First, select Current Frame. Only the currently displayed frame will be processed.

4 Set the recognition parameters.
＞ **Threshold**: Set the threshold value. Note that the threshold is not the actual pixel value but the apparent value adjusted for brightness.
＞ **Cell Size**: Set the size of the cell (diameter pix).Set the cell size referring to the brightness value and size in step "2" above.
＞ **ROI Size**: Set the size of the ROI generated after detection.

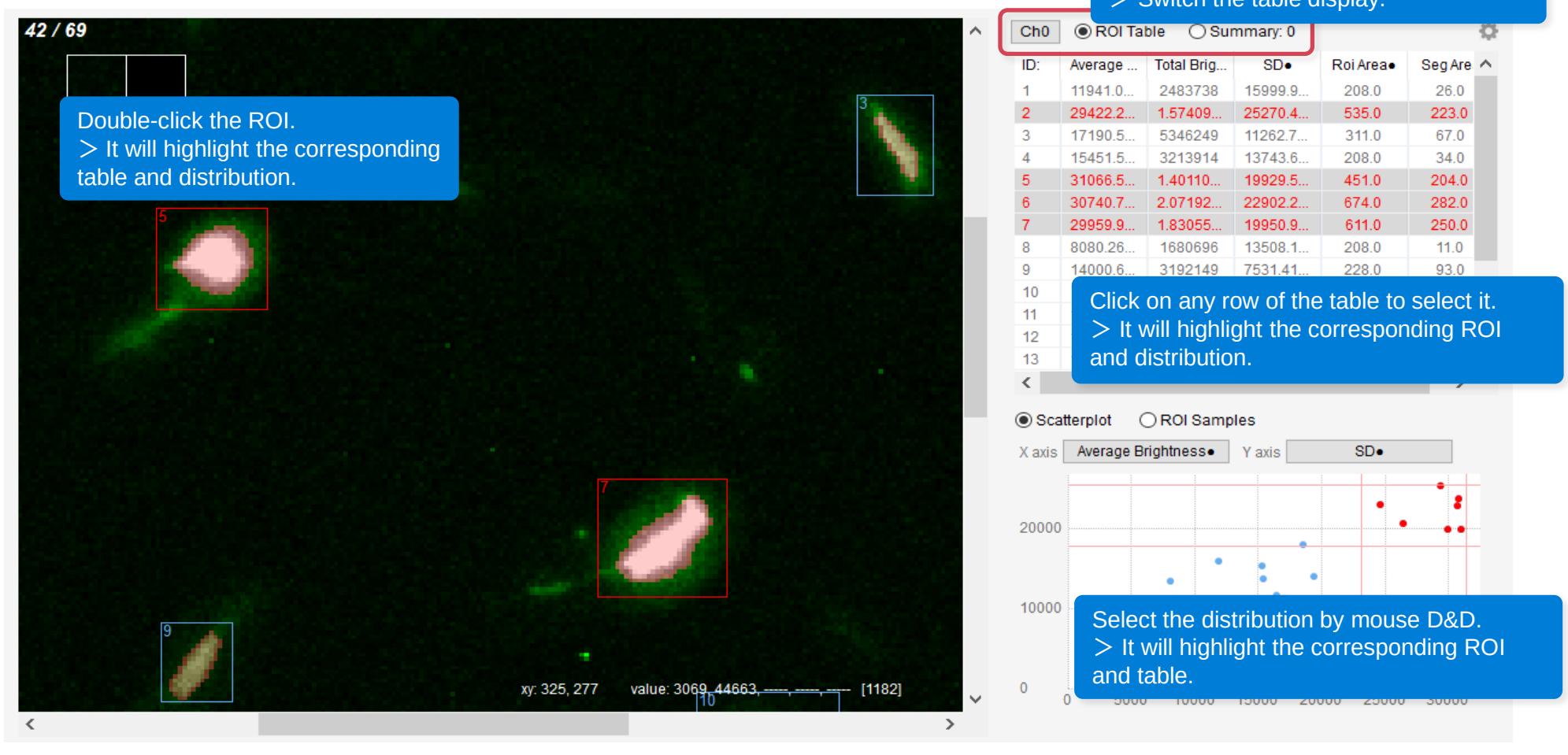


5 Click the Detect button.
＞ Start cell recognition process for the current frame.
＞ Generate ROIs for each recognized cell.
If there is a problem, repeat the step "4" and "5".

6 Set the target frame.
＞ If there is no problem, select All Frames.

7 Click the Detect button.
＞ All frames will be recognized with the same parameters.

● Recognition result display:
The recognition process generates ROIs.
Double-click an ROI to highlight the related item on the right side of the screen.

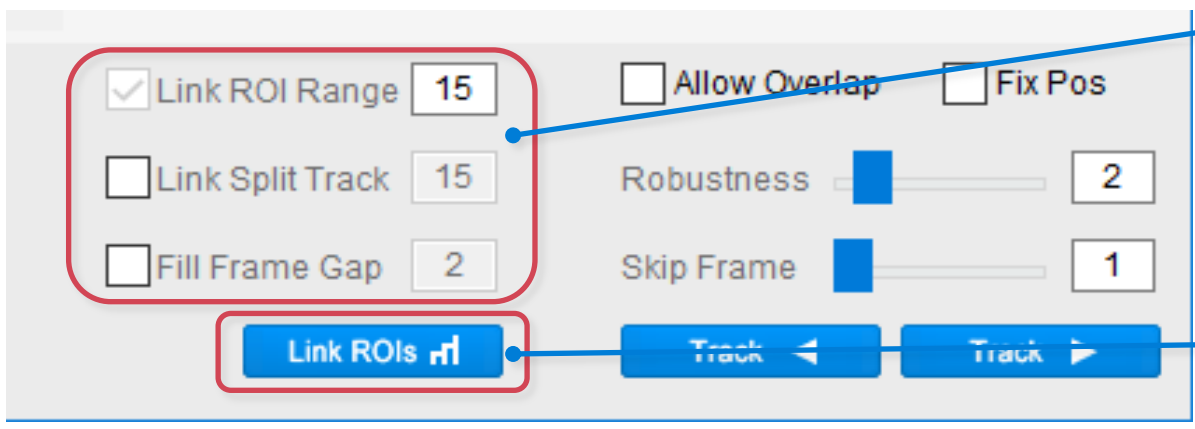


8. Cell Tracking / Link-type method

Associating all ROIs on all frames in a time series generates the cell trajectory.
ROIs must have been set beforehand by the cell recognition process (see Section 7) described above.

1 Select the Tracking mode.

※ The ROI must have been set beforehand by executing the cell recognition process (see Section 7). Manually set ROIs (see Section 5.1) are also acceptable.

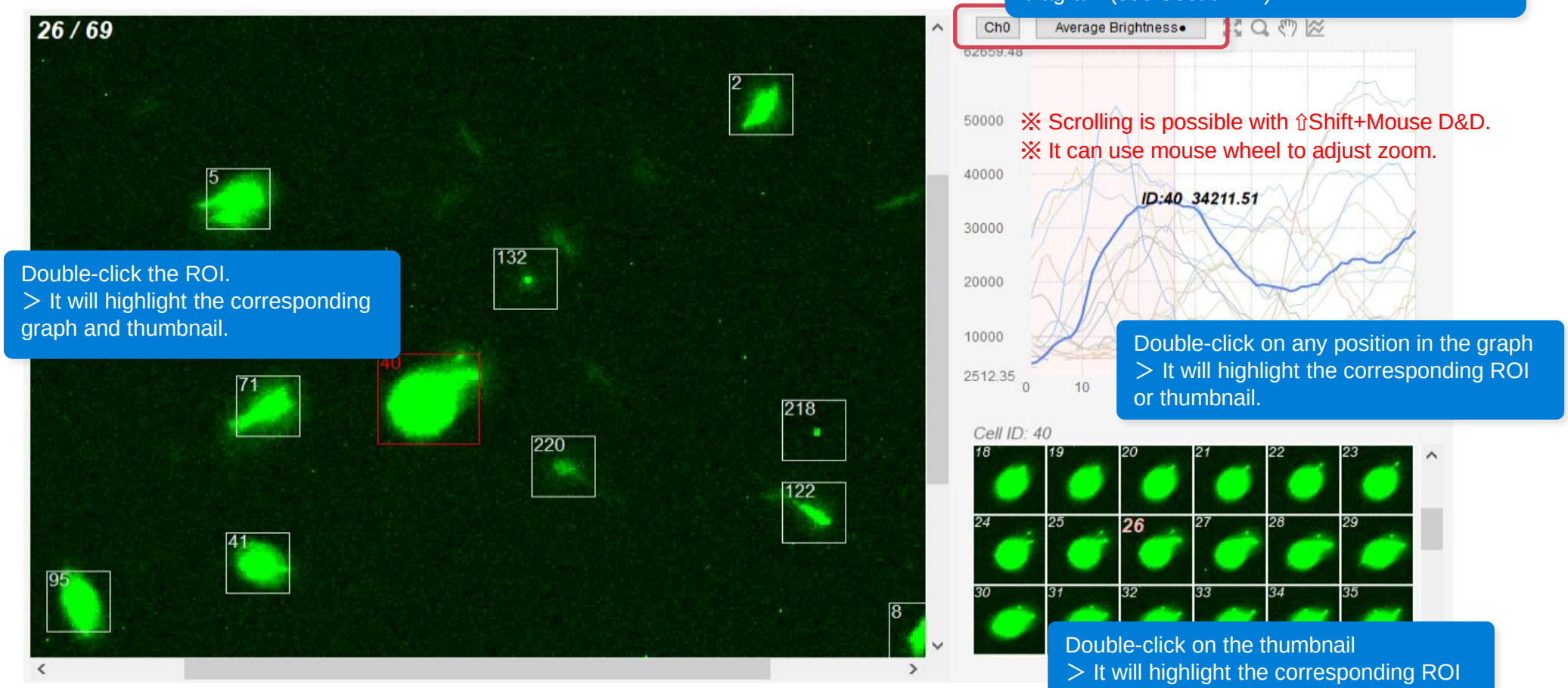


2 Set the tracking parameters.
> **Link ROI Range**: Sets the threshold value for the cell migration distance (pix) between one frame and the next.
> **Link Split Track**: Detects cell division (split trajectory). Set the maximum distance (pix) between split trajectories.
> **Fill Frame Gap**: Automatically fills in gaps between trajectories. Set the maximum number of frames that will be considered a gap.

3 Click the LinkROIs button.
> Perform cell tracking process.
> All ROIs are associated. The Cell ID of each ROI will be changed so that the same Cell ID is set for the same cell.

● Tracking result display:
Double-click an ROI to highlight the related item on the right side of the screen.

Click the Select Ch button or the Select Feature button.
> Switch the graph display.
> Select "Cell Lineage" to display a cell lineage diagram (see Section 12).



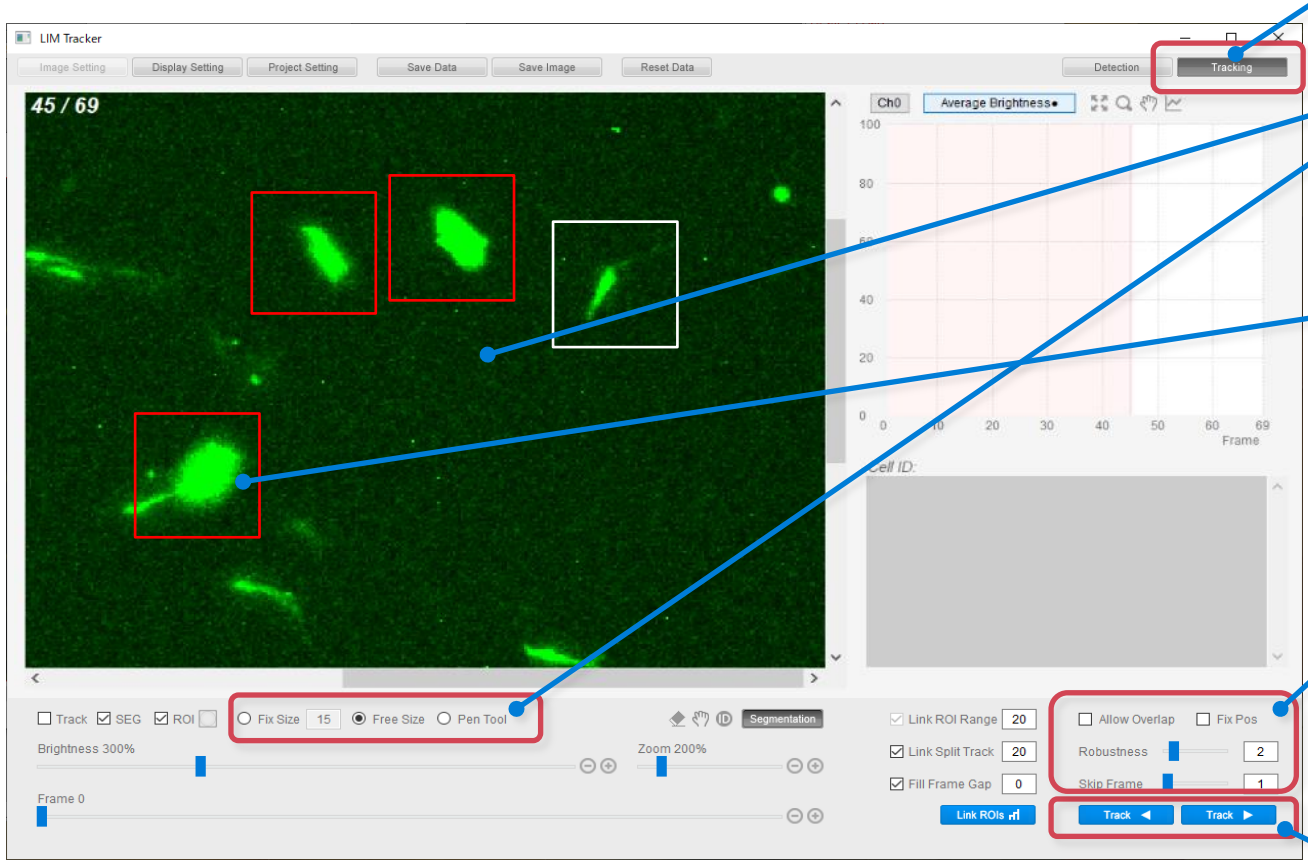
Double-click the ROI.
> It will highlight the corresponding graph and thumbnail.

Double-click on any position in the graph
> It will highlight the corresponding ROI or thumbnail.

Double-click on the thumbnail
> It will highlight the corresponding ROI or graph.

9. Cell Tracking / Sequential search-type method

Performs forward or backward tracking of any ROI in the original image.



1 Select the Tracking mode.

2 Create an ROI on the original image.
> FixSize: Create ROI by single mouse click.
> FreeSize: Create ROI by mouse D&D.
> PenTool: Create ROI by drawing a boundary line.

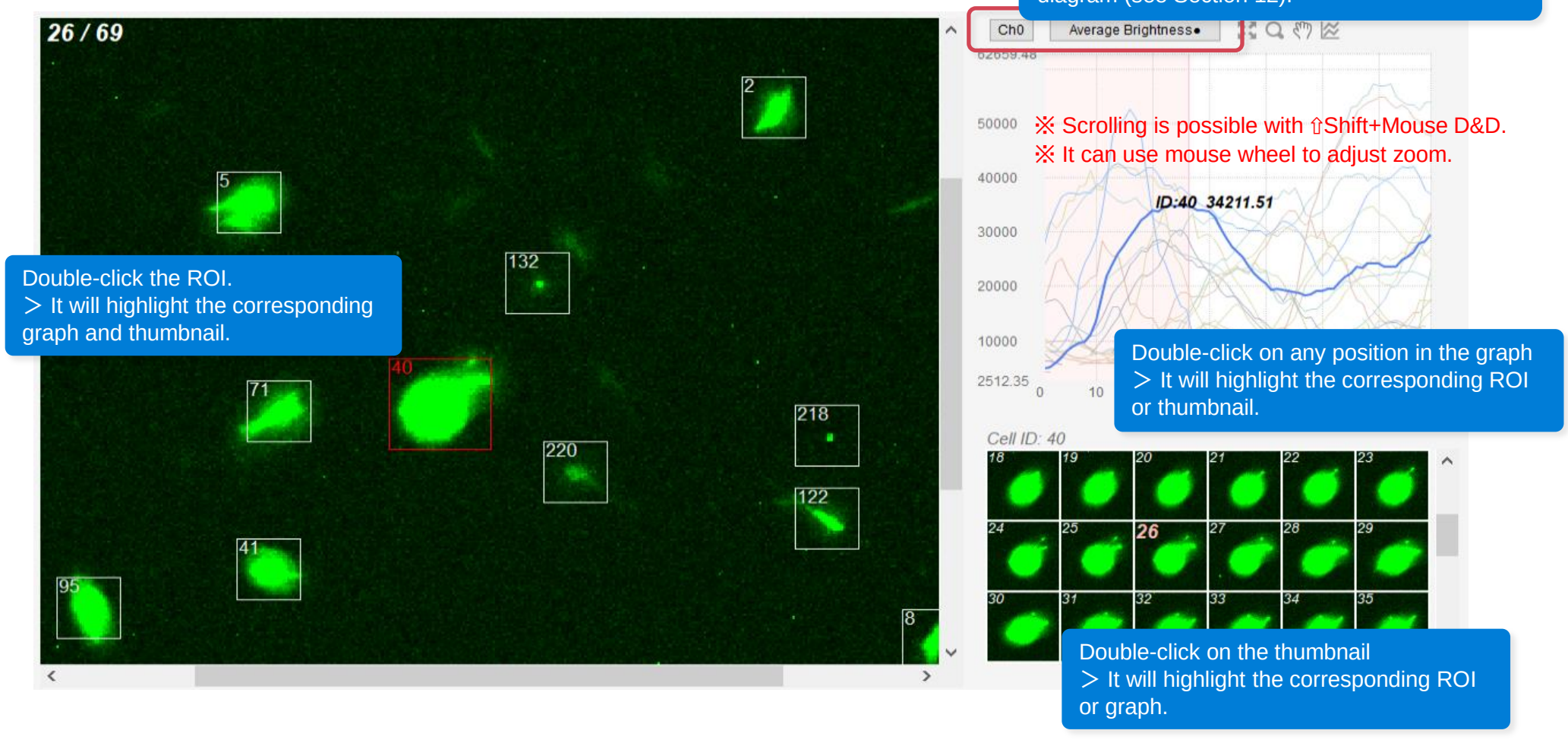
3 Select the ROI to track.
> Single click on the ROI: toggles between selected (red frame) and unselected (white frame).
> Double click on the ROI: Select "only" the relevant ROI (red frame).

4 Set the tracking parameters.
> **Allow Overlap**: Allows overlap with existing ROIs when tracking.
> **Fix Pos**: Create ROIs in the next or previous frame without changing the ROI position.
> **Robustness**: Adjusts the fineness of the search.
> **Skip Frame**: Skip frames to speed up the process.

5 Click the "Track" button (or press the [A] or [S] key).
> Performs forward or backward tracking for the selected ROI (red box).

※ By using keyboard shortcuts ([A], [S], [Z], [X], [Q], [W]) in combination with manual ROI repositioning using the mouse, you can efficiently track targets that are difficult to track and often misaligned (see Section 11).

● Tracking result display:
Double-click an ROI to highlight the related item on the right side of the screen.



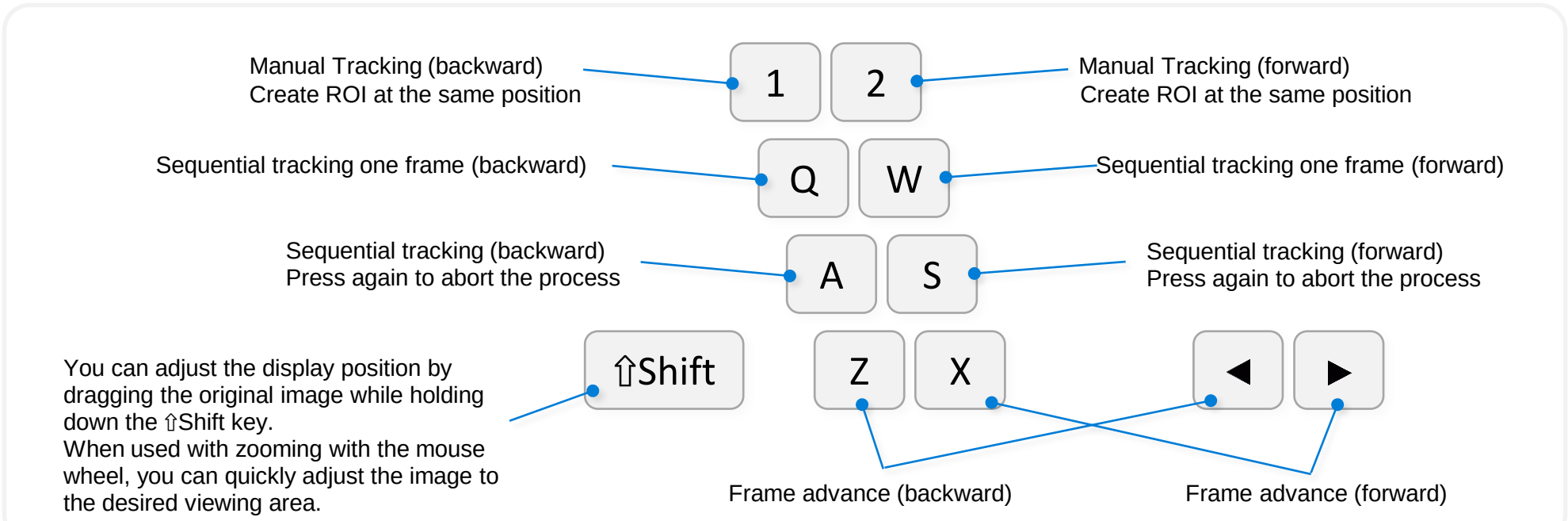
10. Manual Tracking

By pressing the [1] key (or [2] key) on the keyboard to the selected ROI (red frame), you can create a new ROI at the same position on the next (or previous) frame.
It can perform manual tracking by creating ROIs sequentially and correcting the positional deviation from the target with the mouse.
(see Section 11 for details)

11. Keyboard shortcuts

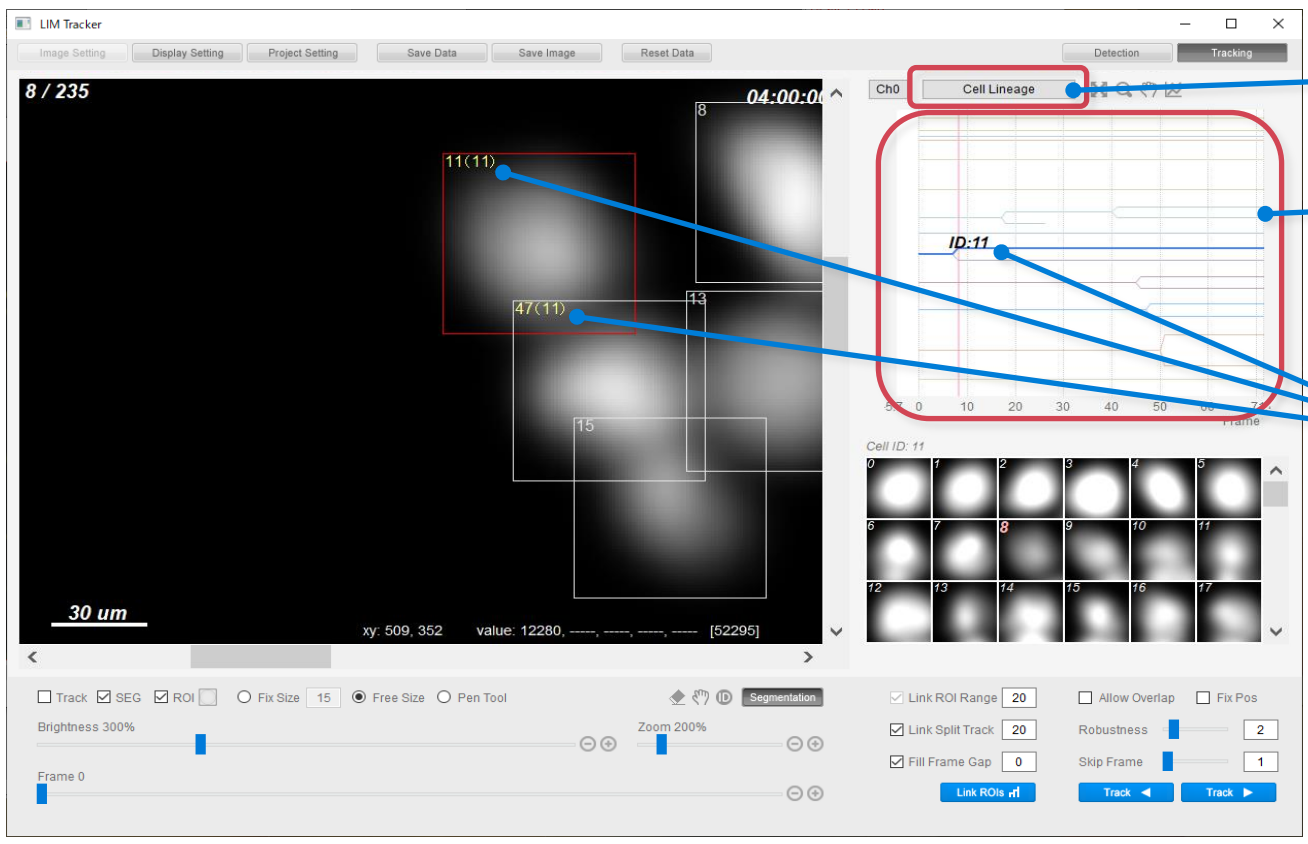
By using the arrow keys and Shift key together, you can check the image efficiently.
You can also use the [Z], [X], [A], [S], [Q], and [W] keys for Sequential search-type tracking (see Section 9) to work more efficiently.

※ [Ctrl] + [A]: Selects (red box) or deselects (white box) all ROIs in the image.
※ [Ctrl] + [D]: Delete all selected ROIs (red box) in the image.
※ Clicking on an ROI while holding down the spacebar: deletes the ROI.



12. Cell lineage display

Selecting the "Cell Lineage" menu in Tracking mode displays the cell lineage.
Cell division = mother cell and daughter cell linkage status reflected in real-time.



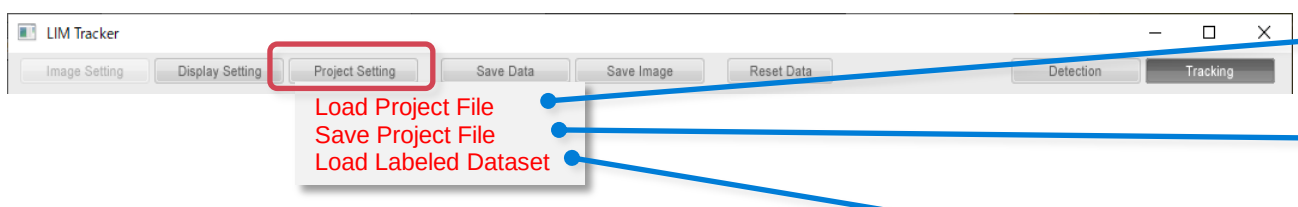
Select "Cell Lineage" menu to display the cell lineage diagram.

※ The cell lineage can be scrolled by holding down the Shift key and dragging the mouse. You can also use the mouse wheel to adjust the zoom.

Cell IDs immediately after division are shown in yellow.
Double-clicking on an ROI will highlight the lineage.
Double-clicking on a lineage will selectively highlight the ROI with the corresponding Cell ID.

13. Project setting (saving project file and restoration)

You can save the status of the work in progress to a project file. And restore the working state from a file.



Click the "Load Project File" menu and select a project file (*.limpj) to restore the past working state.

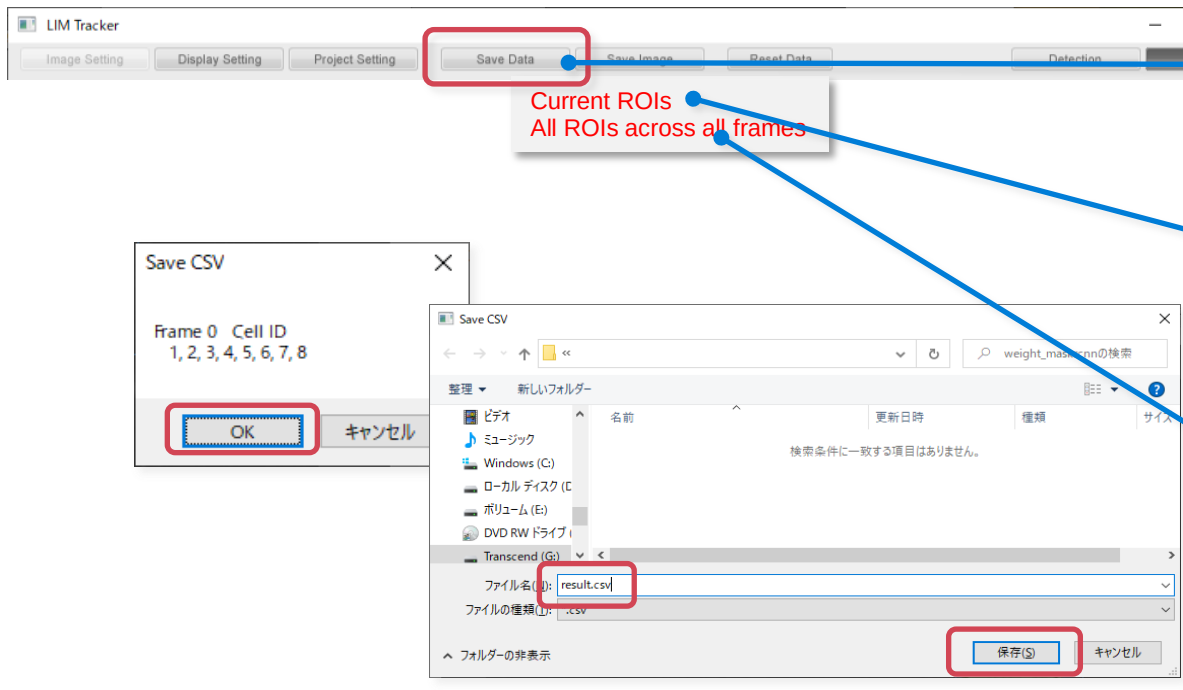
Click the "Save Project File" menu and enter a file name to save the current working state as a project file (*.limpj).

Click the "Load Labeled Dataset" menu and select a labeled image dataset to restore the ROI/region shape.

※ The original images (0000.png~) and the 16-bit labeled images (0000_masks.png~) must be saved in the dataset folder.

14. Save Data

Output numerical data such as feature values related to ROI to a CSV file.



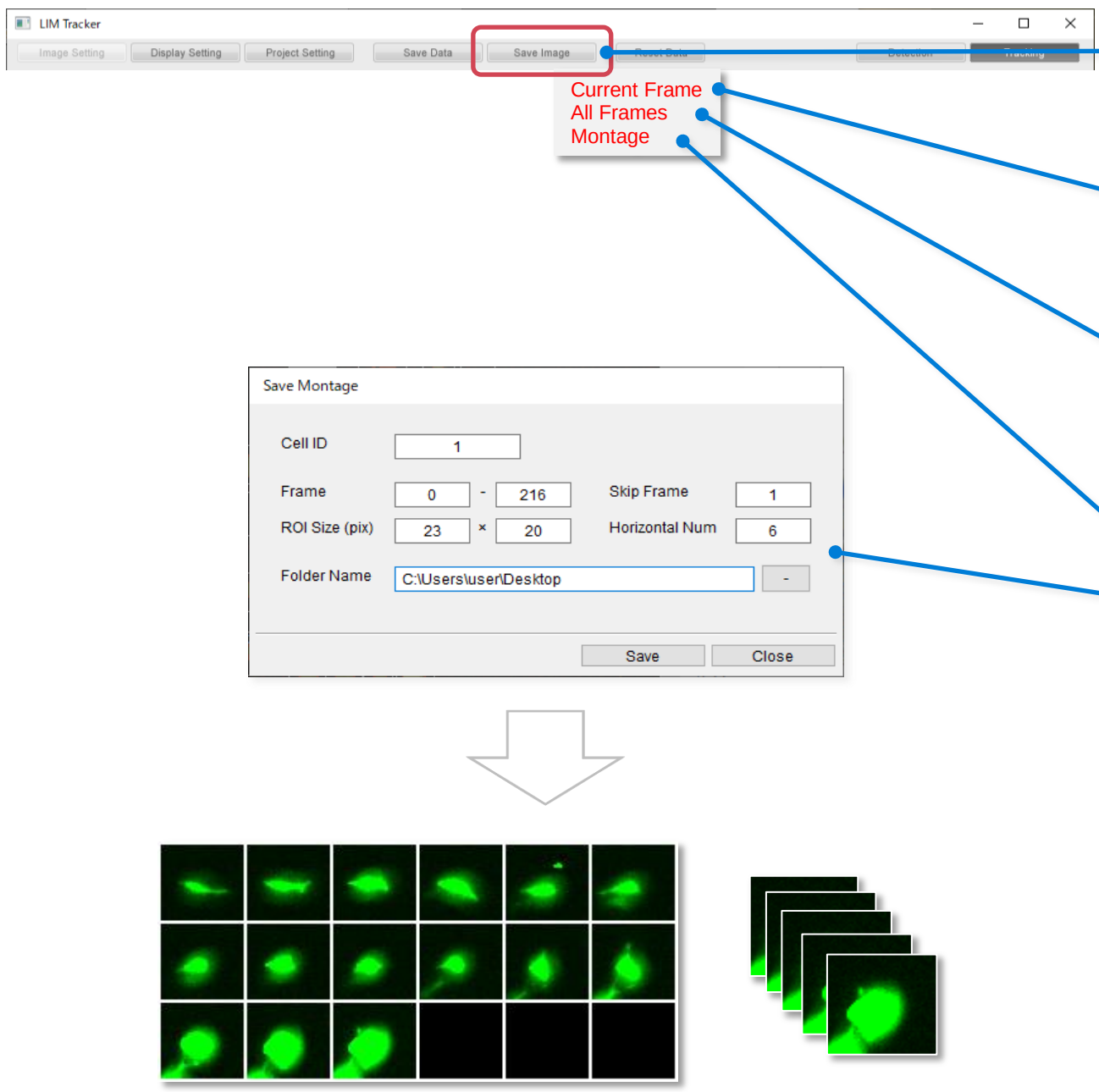
1 Click the SaveData button.
> The pull-down menu is displayed.

2-A Select the "Current ROIs" menu.
> A message box will appear.
> A file dialog box will appear. Enter a file name and click the Save button.
> The various feature values for the ROIs in the selected state (red frame) on the currently displayed original image will be saved as a CSV text file.

2-B Select the "All ROIs across all frames" menu.
> The File dialog box will appear. Enter a file name and click the Save button.
> All ROI information will be saved as a CSV text file.

15. Save Image

Output the tracking result images to a file.



1 Click the "Save Image" button.
> The menu "Current Frame", "All Frames" and "Montage" will be displayed.

2-A Select the "Current Frame" menu.
> The "Save File" dialog box appears. Enter a file name and click the Save button.
> It will save the tracking result image for the current frame.

2-B Select the "All Frames" menu.
> The "Select Folder" dialog box will appear. Select a folder in the dialog and click the OK button.
> It will save the result images in the selected folder.

2-C Select the "Montage" menu.
> The "Save Montage" dialog will appear. After setting the parameters, press the Save button.
> It will save the montage and thumbnail images (PNG and multi-page TIFF) in the specified folder.

※ Parameter Setting
> **Cell ID**: Sets the Cell ID of the ROI to be output.
> **Frame**: Sets the frame interval.
> **Skip Frame**: Sets the frame interval to skip. (When set to "1", no skipping is performed.)
> **ROI Size (pix)**: Sets the thumbnail image size.
> **Horizontal Num**: Sets the number of thumbnail images to be arranged horizontally in the montage image.
> **Folder Name**: Sets the folder path to output the images.

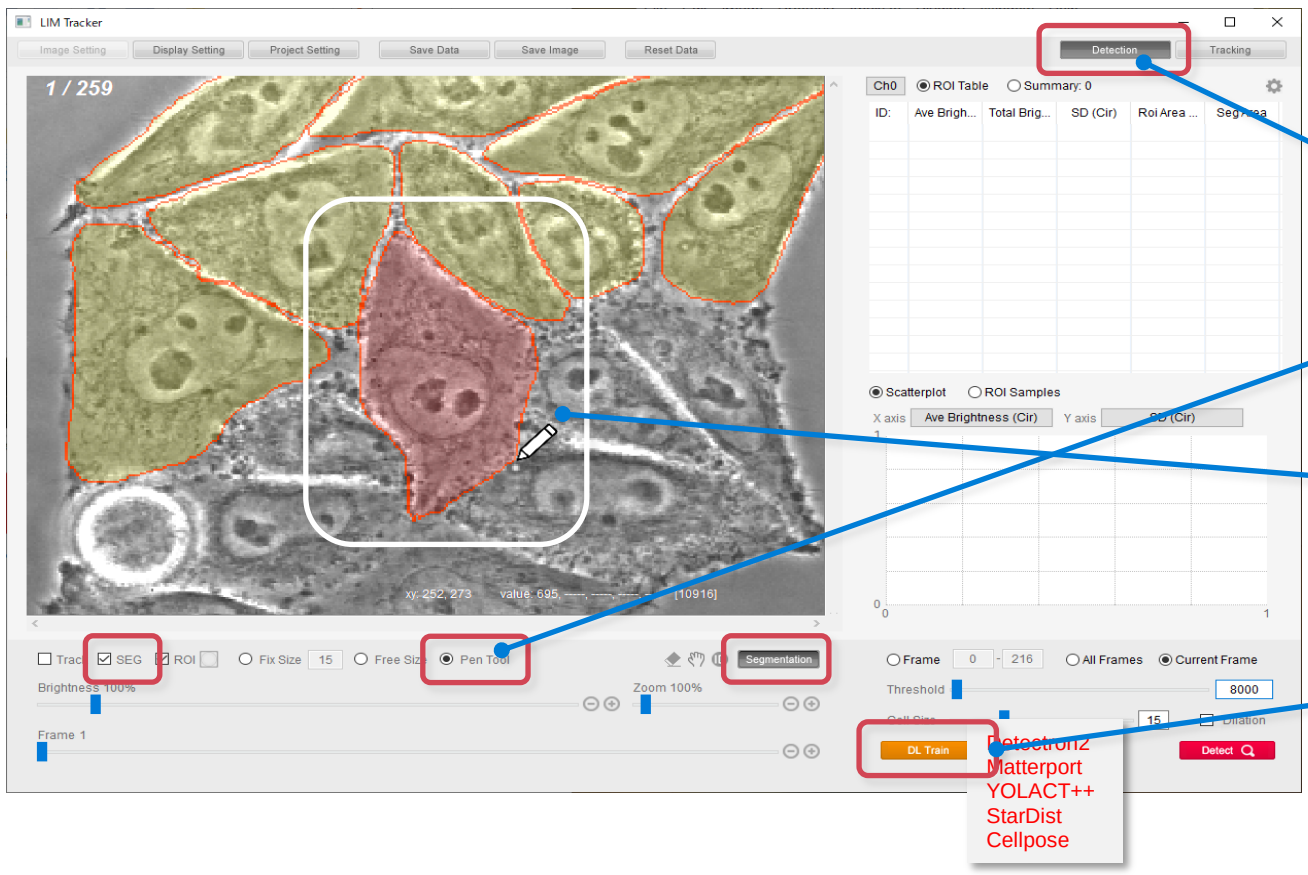
※ You can invoke this dialog from the right-click menu "Save Montage" of the ROI.

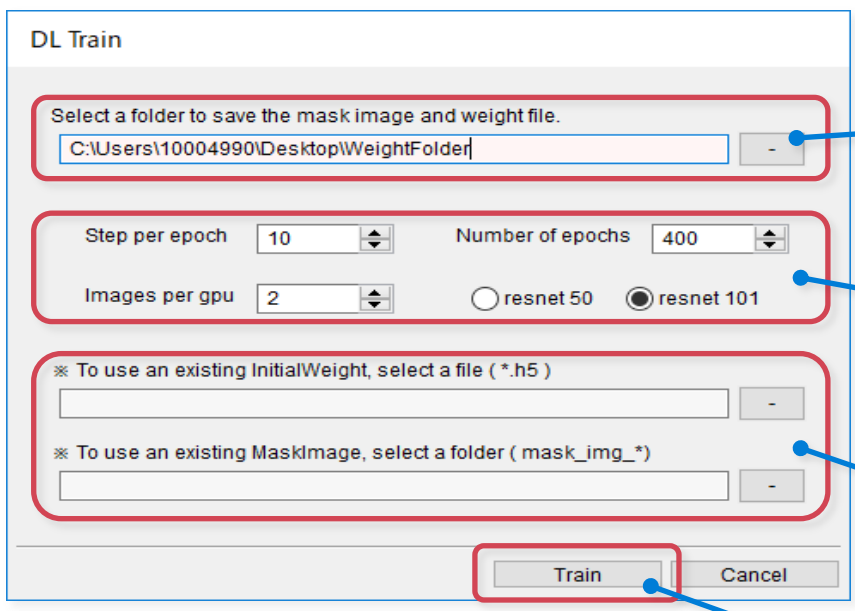
16. Deep learning recognition function

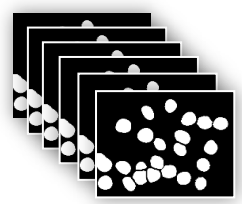
Combining high-precision recognition based on deep learning (DL) with linked tracking capabilities can greatly improve tracking accuracy.
Users can create training data on this software and build the most suitable recognition process for their own dataset.
(The GPU and library programs must be set up correctly. Please refer to the official Github repository for setup details.)

16.1. DL training

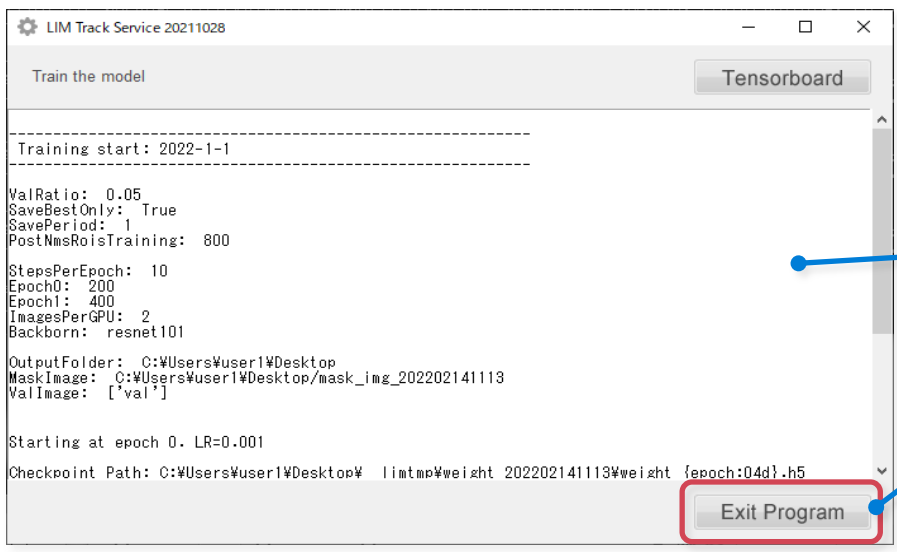
Use the "Pen Tool" to annotate individual cells and create the "training mages". Based on the training images, the DL trains and generates a "trained weight file".







Training images



Trained weight file

1 Load the image files that will be the training images in ImageJ and launch LIM Tracker.

2 Select the Detection mode.

3 Select the "Pen Tool", "SEG", and "Segmentation" buttons.

4 Trace the cell boundaries while holding down the left mouse button. Do this for all images.
(See section 6)

5 Click the "DL Train" button.
＞The DL Train dialog appears.

※ You can select the DL algorithm from the right-click menu.

6 Set the destination to save the "training images" to be trained and the "trained weight files" generated by the training.

7 Set training parameters such as the number of Epochs as needed.

※ The parameters are different for each algorithm. For details, please refer to the description in the official Github repository of each algorithm.

8 It is possible to train with previously saved training images, or to use trained weight files as initial weights. In this case, set the folder path or file path for them.

9 Click the "Train" button.
＞The training images ("mask_img_*") will be saved in the folder you specified in Step 6.

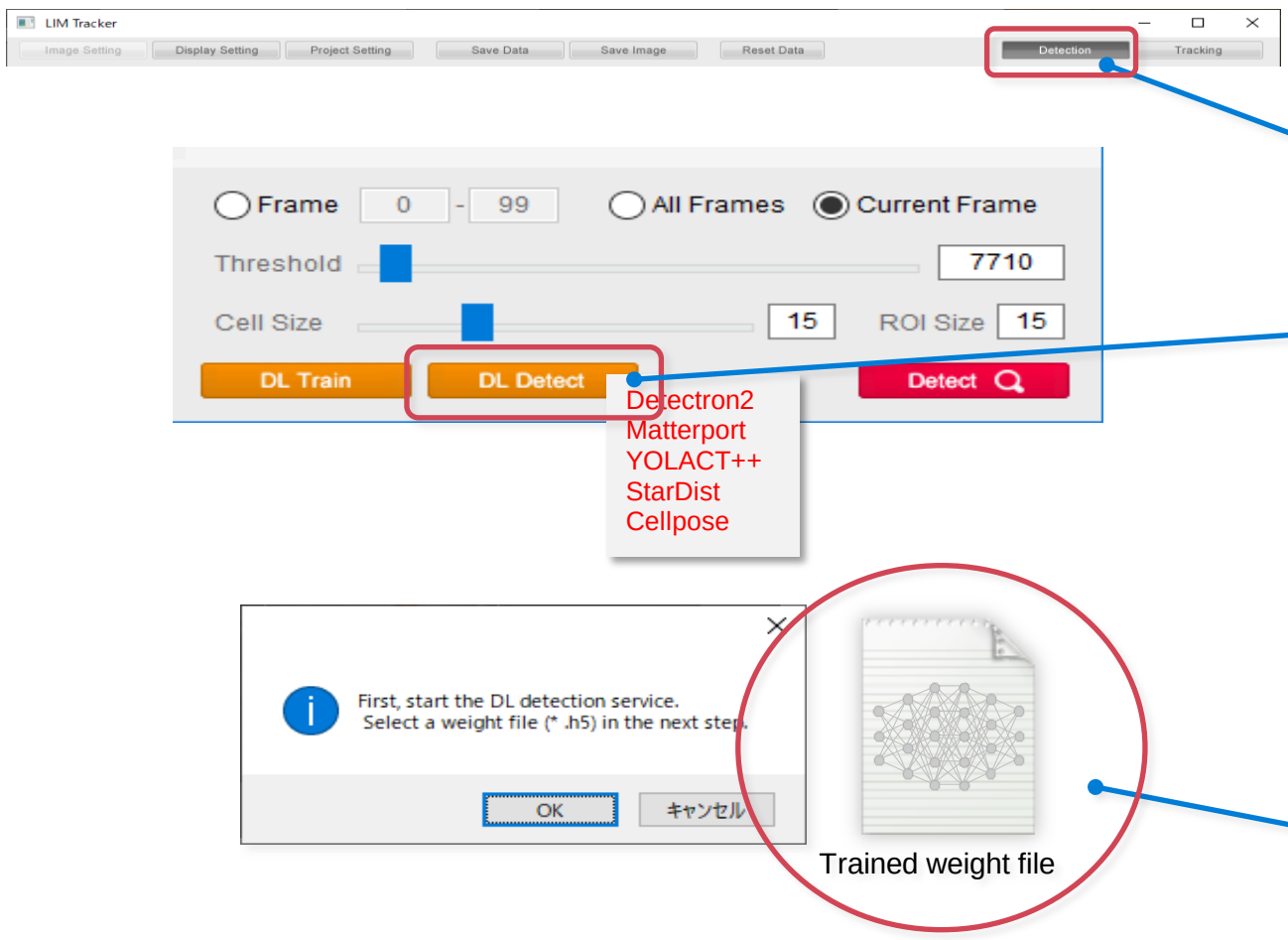
10 The DL Service program will launch automatically and start training.
＞ The progress will be displayed in the dialog.
＞ After a few hours to a few days, when the training process is complete, the message "Training process Completed" will be displayed.
＞ Click on the "Exit Program" button to exit the program.

11 Check the generated trained weight file.

※ The weight file may be saved for each epoch, in which case it will be named "weight_[epoch].h5".
In the recognition process (Section 16.2), the weight file with the highest "epoch number" is basically used.

16.2. DL recognition

Select "Trained weight file" and start the DL recognition service program. The recognition process will be executed in cooperation with the program.



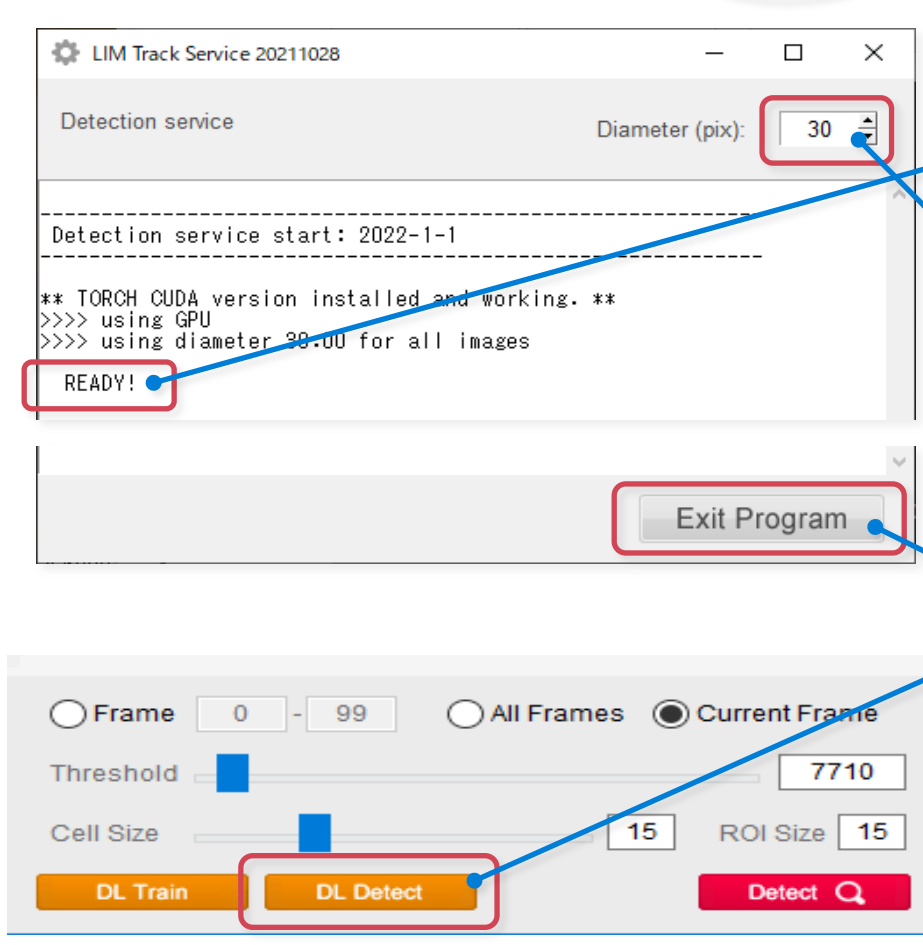
1 Load the image files you want to recognize in ImageJ and launch LIM Tracker.

2 Select the Detection mode.

3 Click the "DL Detect" button.

※ You can select the DL algorithm you want to use from the right-click menu.

4 A message box will appear.
> Select the weight file according to the message.
> After a few moments, the DL service program will be launched.



5 Wait until you see the "READY!" message.

6 "READY!" is displayed, click the "DL Detect" button again
> The recognition process will start.

※ When using Cellpose, set Diameter (pix) in advance.

7 If you want to use a different weight file, click the "Exit Program" button to quit once and repeat Steps 1~6.