qTfy 1.0.0
Getting started

Stavroula Skylaki, PhD

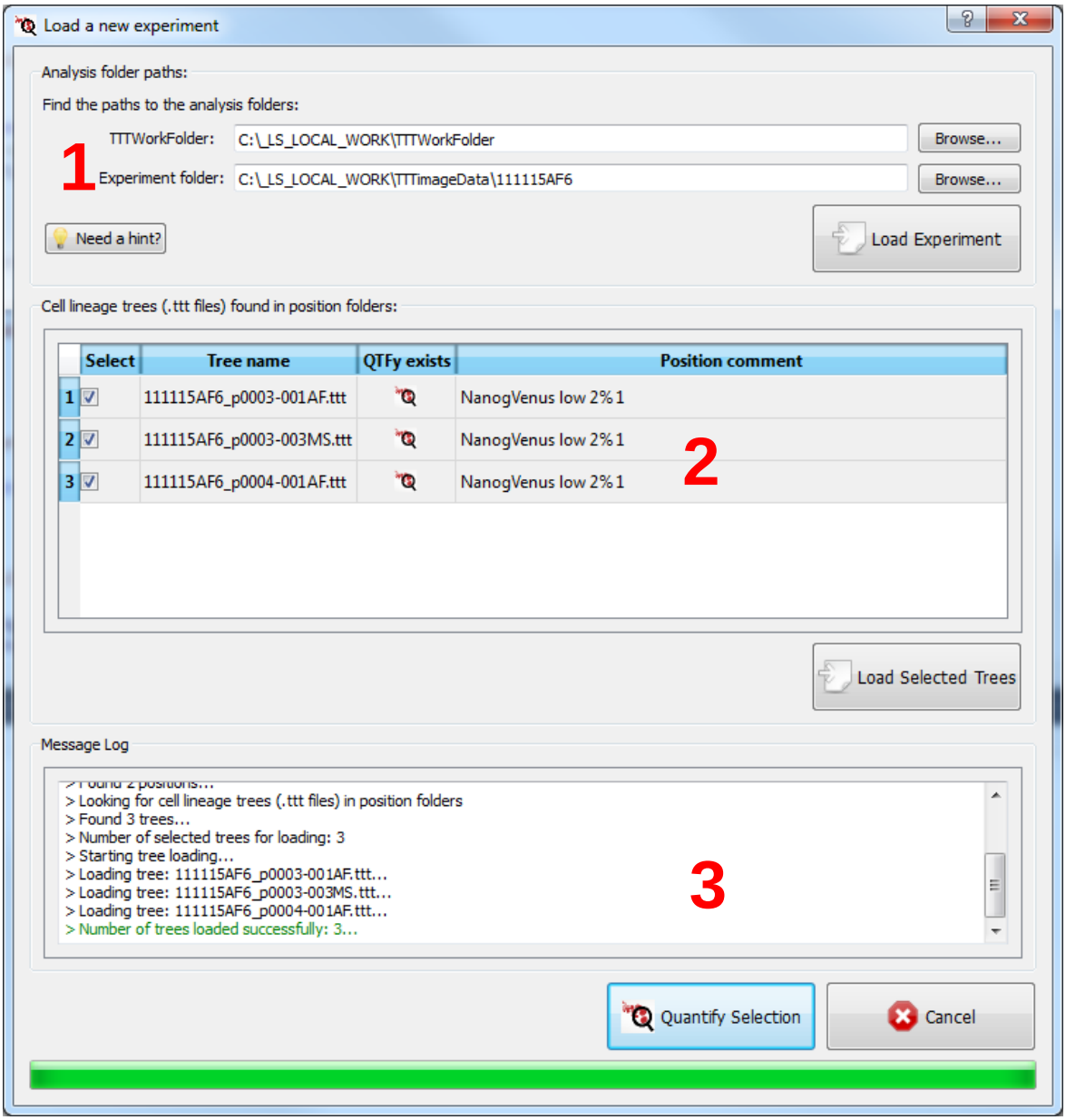
Introduction to qTfy 1.0.0

- A tool for the quantification of the levels of fluorescence intensity in single cells of a lineage tree over time.
- Generation of quantification traces
- Identification of outliers
- Correction of segmentation mistakes
- Updated data and experiment images

Important:
You can use qTfy only after you have generated cell lineage trees for your experiment. Cell lineage trees can be generated by manual tracking using the tTt software. If you have lineage trees generated by other tools (i.e. TrackMate, CellProfiler) you can convert them to .ttt files. Please refer to the tTt manual for the conversion how-to.

The cell lineage trees are stored as .ttt files in the experiment subfolders in the TTTWorkFolder\TTTfiles folder. If no .ttt files can be found qTfy won't be able to proceed with the quantification step as it uses the track point locations to identify the cells.

Data loading

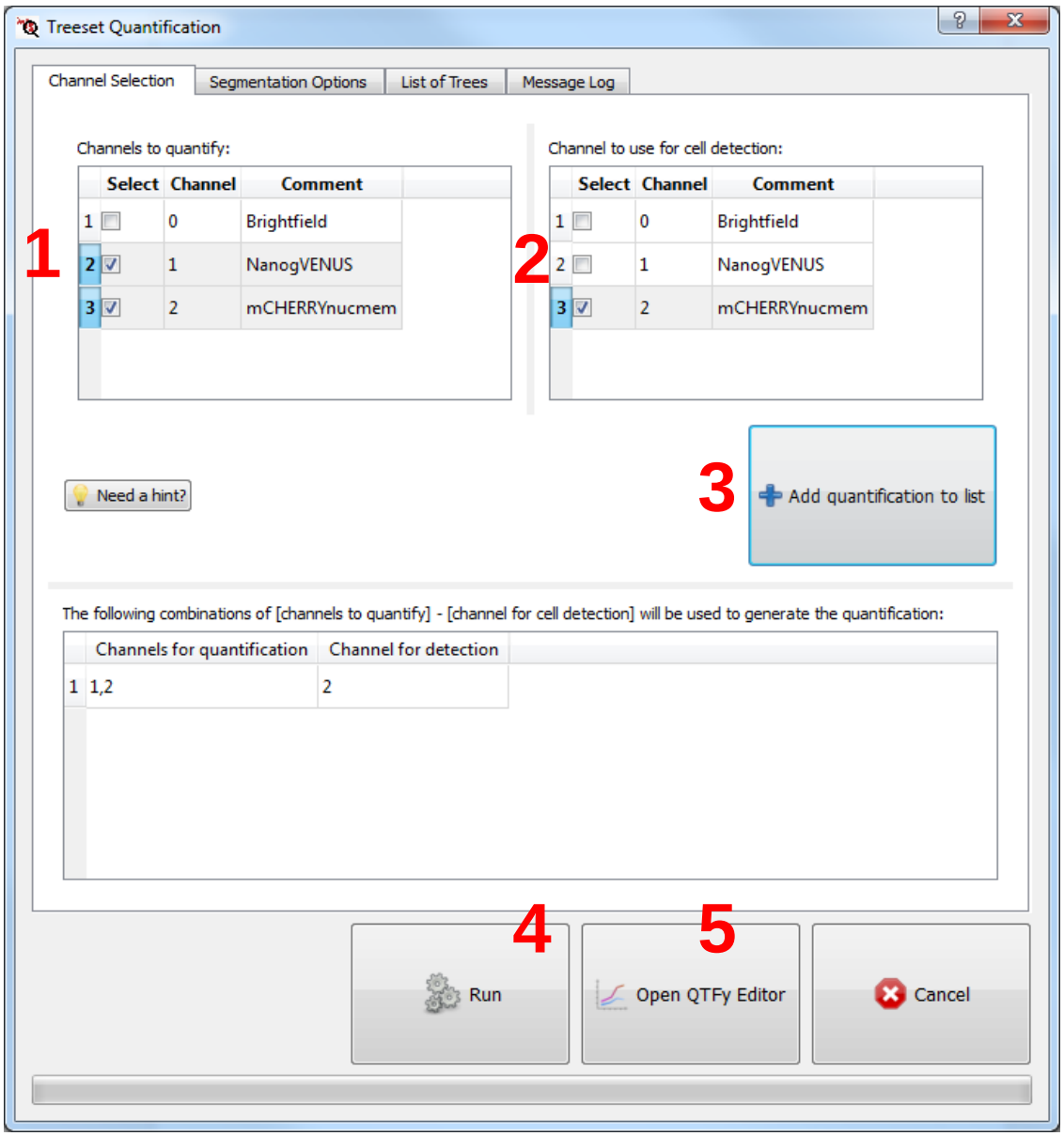


1. Choose the analysis folder paths.
2. Display available ttt trees for the experiment and select trees for the analysis.
3. Message Log: feedback to the user when operations are successful or where things went wrong.

If previous quantification is available in the TTTWorkFolder it will be loaded along with the tree file. Those are .csv files generated by qTfy.

Need a hint?
Help available in key locations of the software based on user feedback.

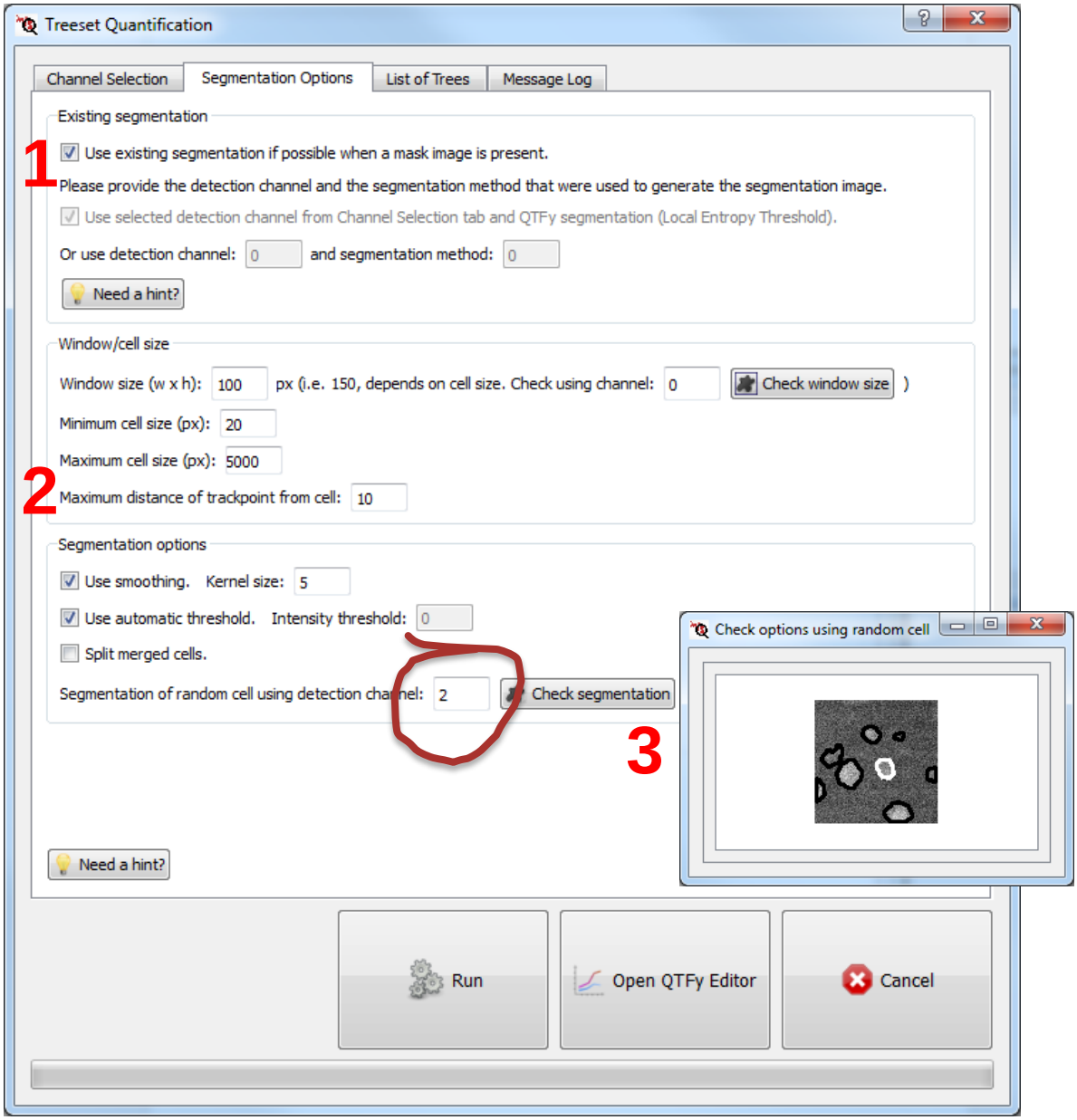
Treeset Quantification: Channel selection



1. Channels to quantify: This channel will be quantified as defined by the contours from the cell detection channel.
2. Channel to use for cell detection: This channel is used to create a segmentation image and identify the cell contours. Use a channel with high singal-to-noise ratio i.e. the mCHERRYnucmem channel.
3. Add quantification to the list
4. Run a quantification or…
5. If at least one quantified tree exist in the selection you can already open the qTfy editor to inspect

** You can also have a look at the tab Segmentation Options (next slide). The default selections should work for the test data.

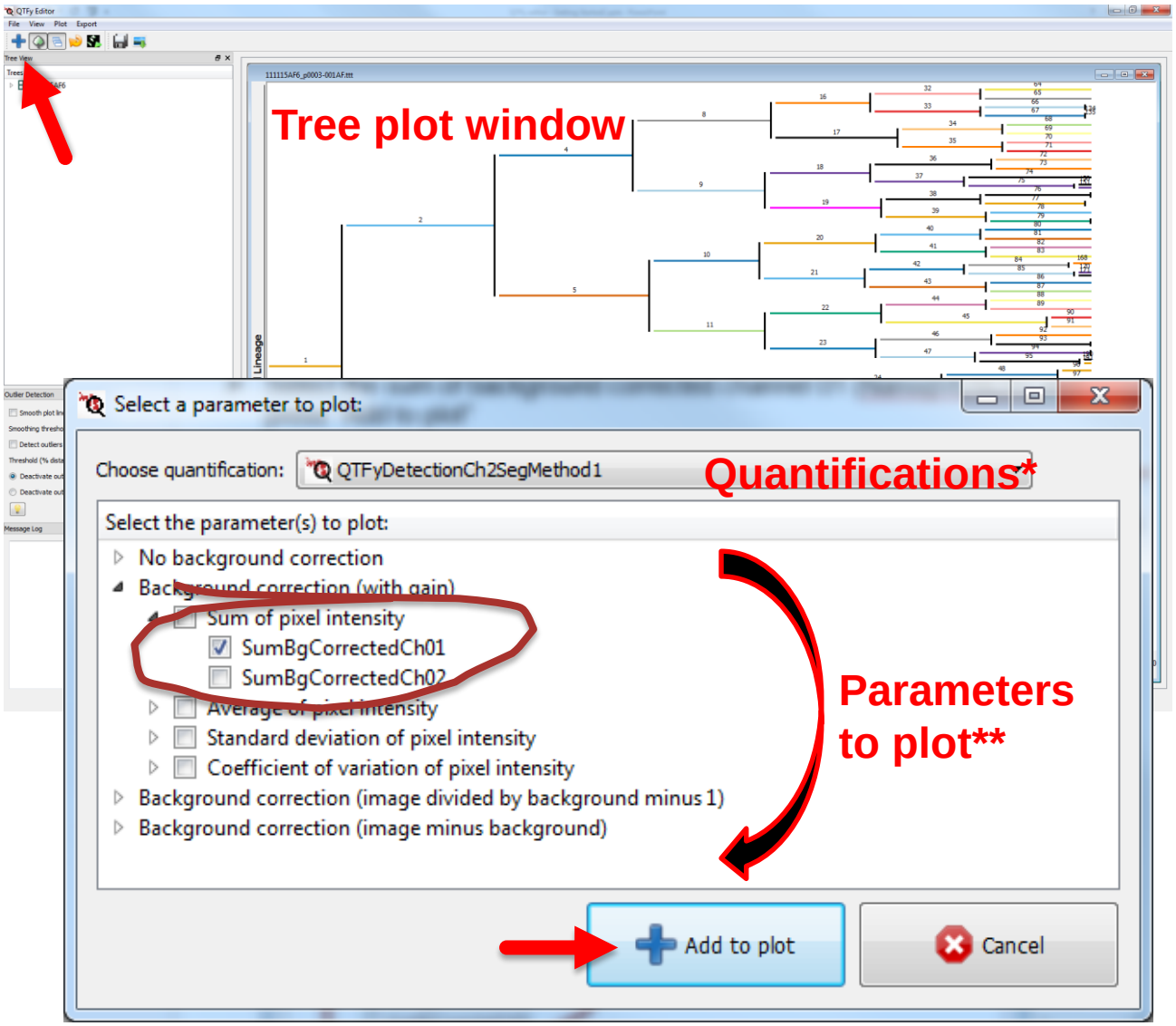
Advanced options: Segmentation options



1. Keep checked to use previously generated segmentation images. For now use default (Entropy Split Thresholding – qTfy method id: 01)
2. What's the allowed distance of the trackpoint from the actual cell? Depends also on the cell size and window size. Otherwise, the trackpoint might not be matched to the right cell!
3. Use the check buttons for a preview. It displays a random cell every time the user clicks the check button. (Use the detection channel i.e. the mCHERRYnucmem channel.)

The qTfy Editor

- The qTfy Editor initially only displays the Tree plot window.
- At first, the “Select a parameter to plot” dialog pops up to start by adding a parameter plot.
- Select the parameter SumBgCorrectedCh01 (sum of background corrected intensities for channel NanogVENUS) and press “Add to plot”

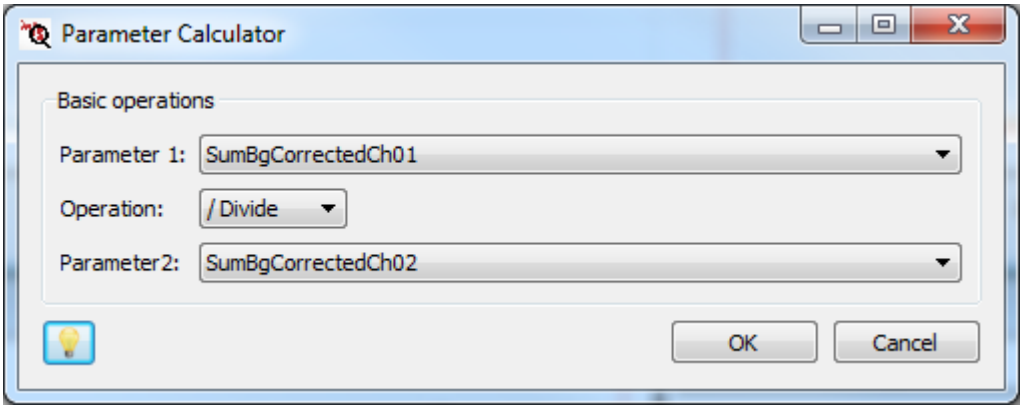


* Quantification: Defined by the Detection Channel and the Segmentation Method used and contains the parameters for all selected Quantification Channels
** Parameters: a statistical property of the pixel intensities. Currently available: Sum, Average, Standard deviation and Coefficient of variation.

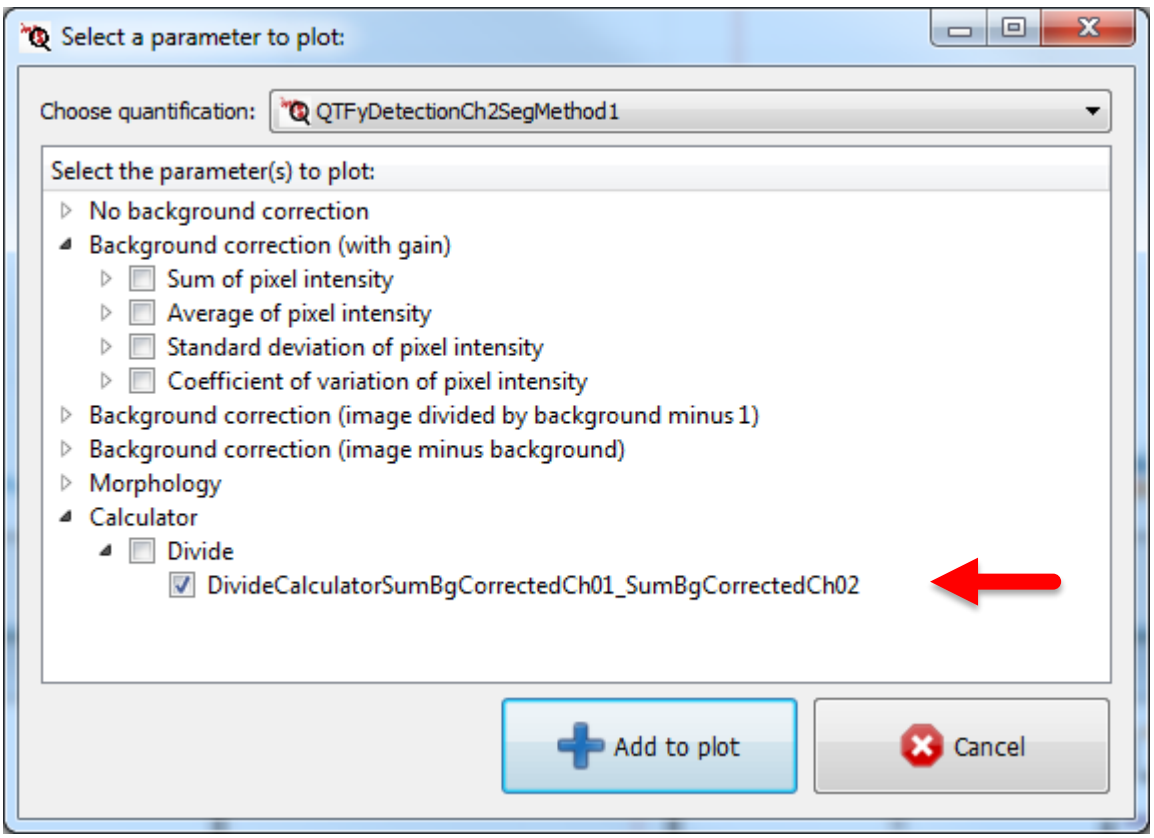
(Four methods of quantification based on how the background has been calculated or not i.e. no correction, with gain calculation, division, subtraction)

The qTfy Editor – Parameter Calculator

- The qTfy Editor allows the creation of derived parameters based on existing quantifications using standard mathematical operations.
- For example, this could be useful for the calculation of nucleus vs cytoplasm ratios.
- From the main menu, select “Process” -> “Parameter Calculator” and choose the configuration of parameters and mathematical operation.



- You can plot the newly created parameter by going to the “Select a parameter to plot” dialog and select it under the category “Calculator”.

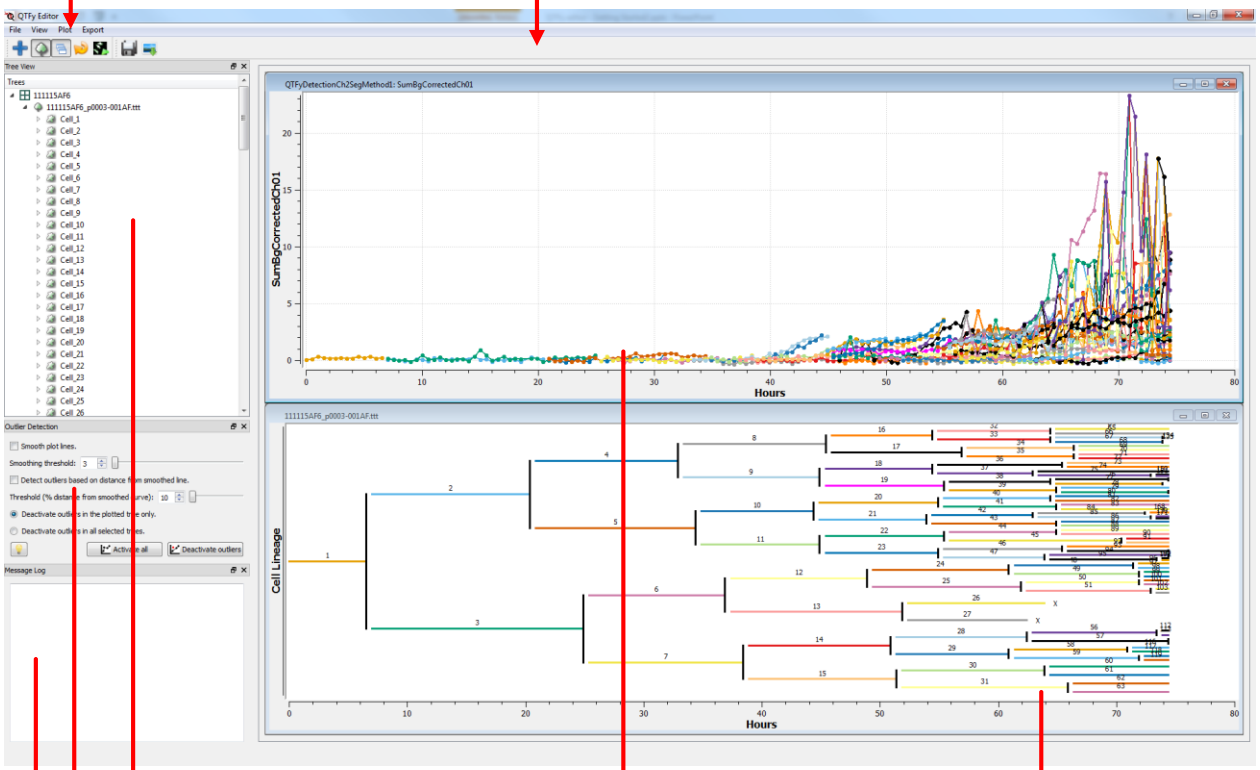


The qTfy Editor

- The interface comprises of dock windows that you can drag and drop as desired

Main toolbar

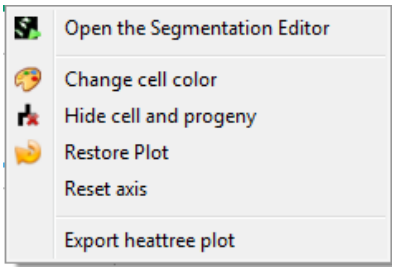
Right click here (Main toolbar) to recover closed dock windows

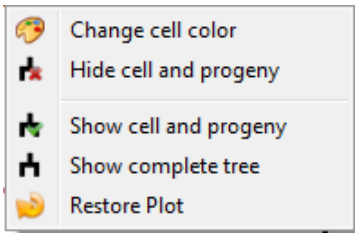


Tree View dock window

Outlier detection dock window

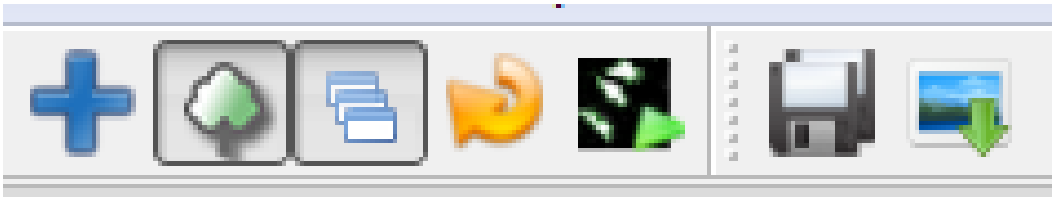
Message Log dock window





Right click on a cell in the Tree plot or the Parameter plot to get additional functions i.e. facilitate visual inspection of complicated trees by hiding one major branch

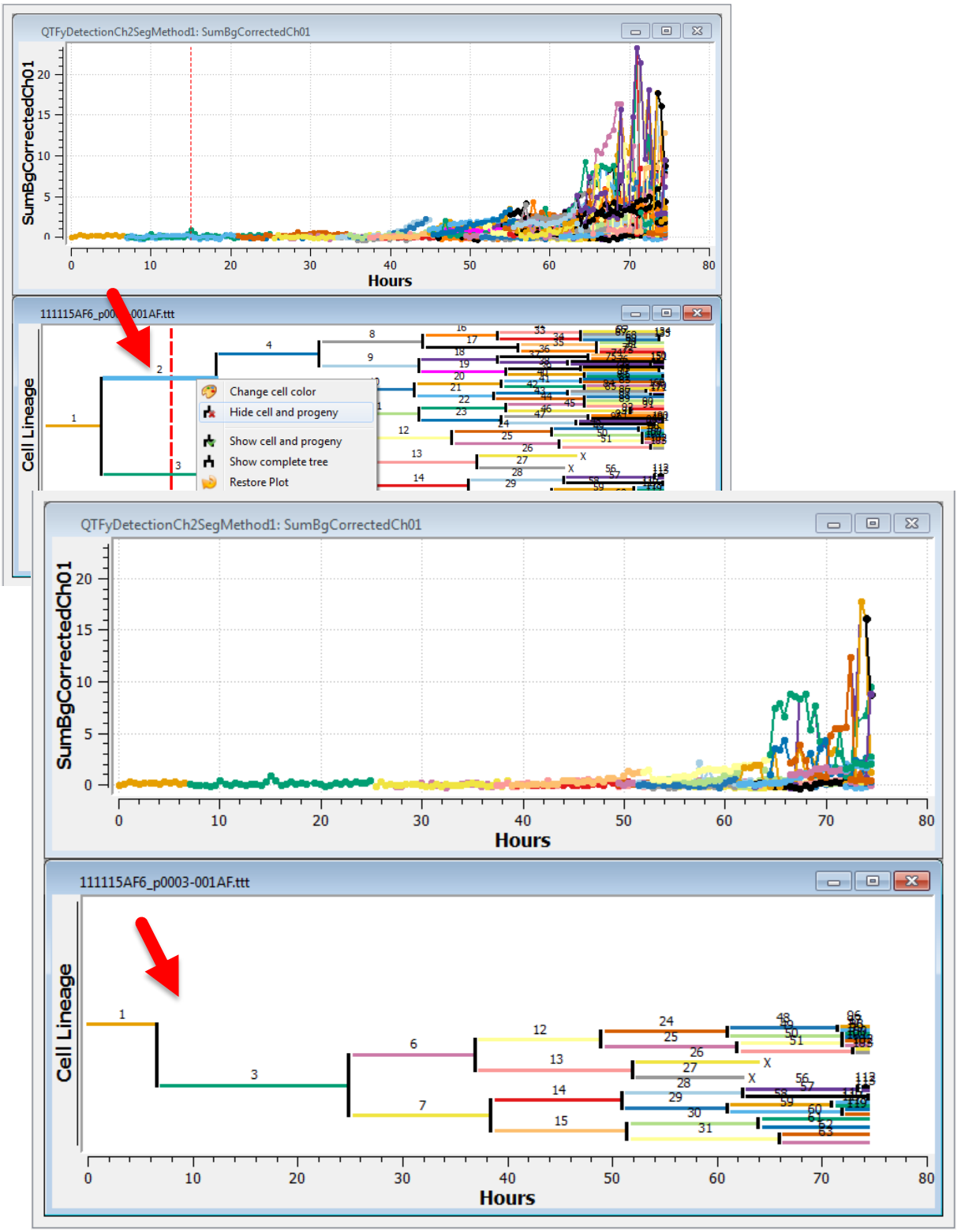
qTfy Editor: Main toolbar



	Add an attribute
	Uncheck to hide the Tree plot window
	Uncheck to disable auto-align. Auto-align tries to optimally use the screen space to align the Tree plot and the Parameter plots every time you move to a new timepoint.
	Restore the plot (show all cells) and the default color scheme.
	Open the segmentation editor
	Save and export data to .csv files (or update existing .csv files)
	Export plot to .png image (either single or combined)

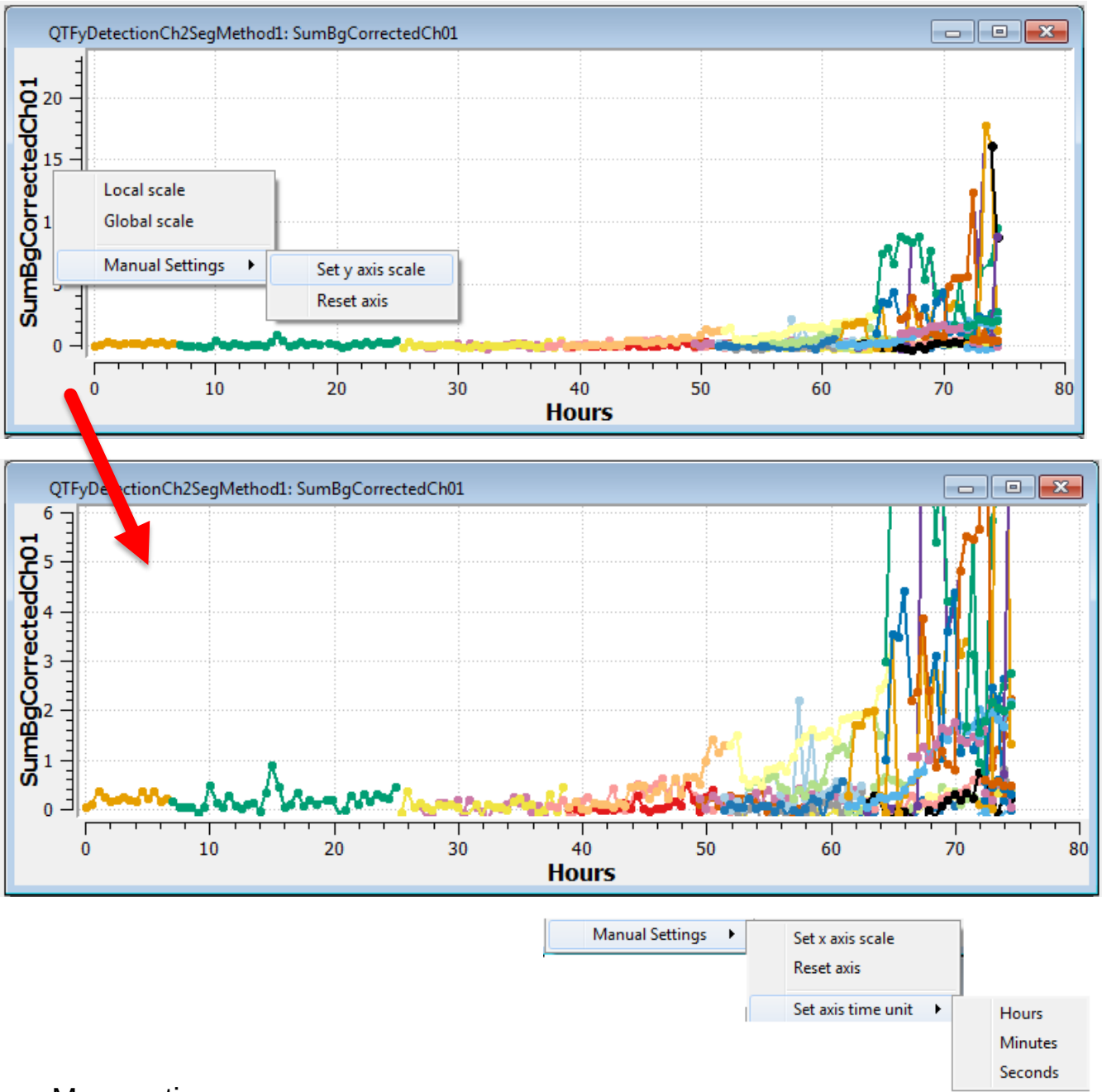
qTfy Editor: Adjust and inspect Parameter plot

Remove daughter cell number 2 to facilitate visualization: Right-click on branch 2 of the Tree plot and select “Hide cell and progeny” from the pop-up menu.



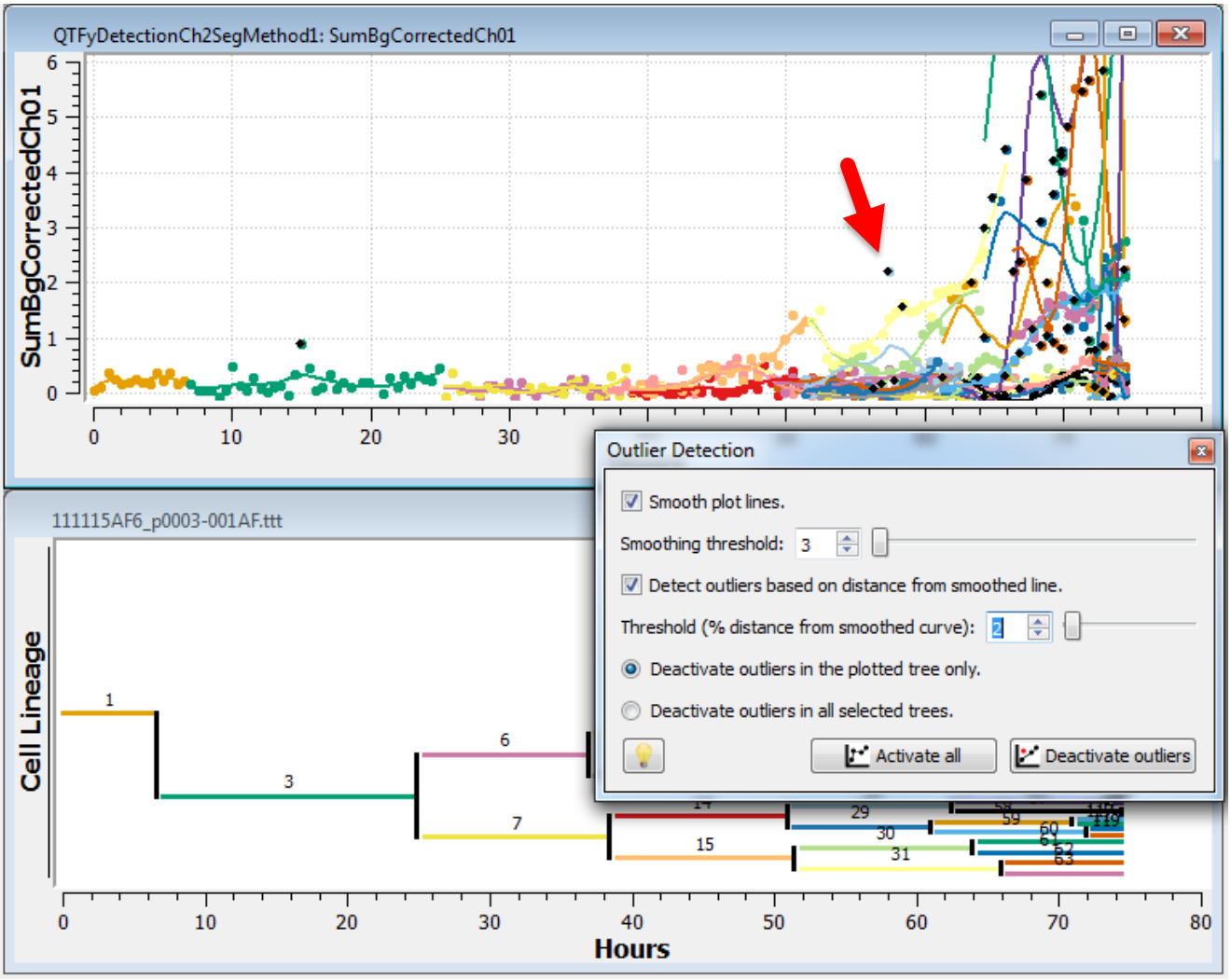
qTfy Editor: Adjust and inspect Parameter plot

Adjust the y-axis to get a detailed view of values. Available options also for x-axis adjustment.



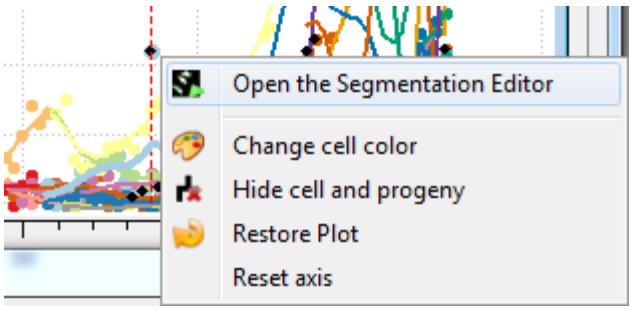
- More options:
- Local scale: the min and max of the y-axis is determined by the min and max values available in the tree for the plotted attribute.
- Global scale: the min and max of the y-axis is determined by the min and max of all trees in the selected treeset for the plotted attribute.

qTfy Editor: Outlier detection

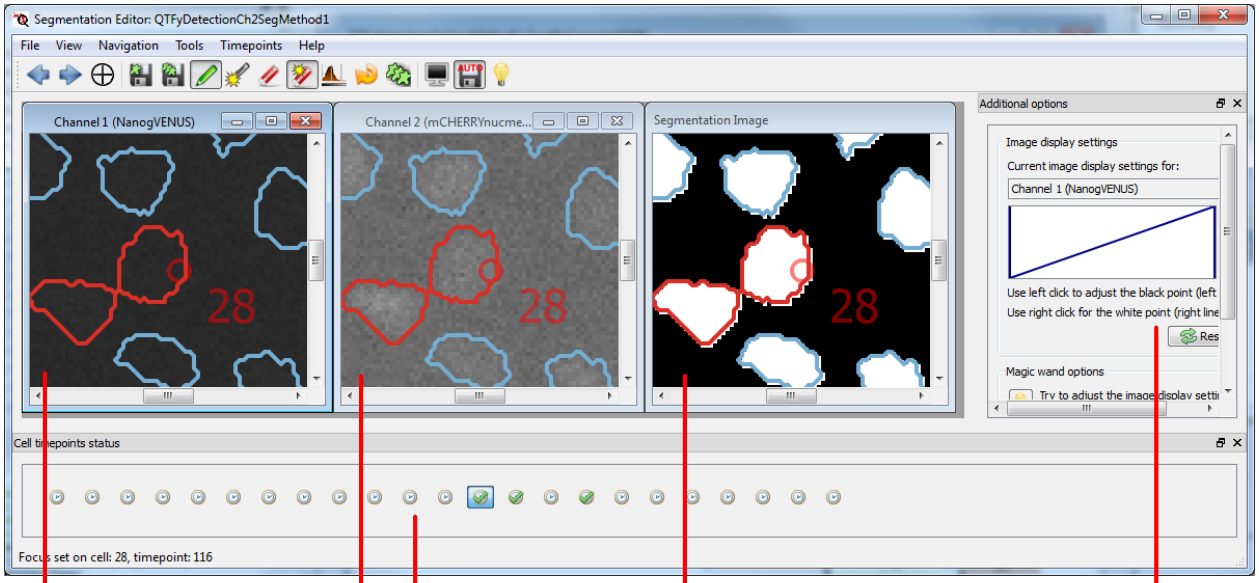


- Check "Smooth plot lines" to generate the smoothed lines for each plot.
- Adjust the amount of smoothing through the smoothing threshold.
- Check "Detect outliers based on distance from smoothed line".
- Predicted outliers indicated with black color (see red arrow)
- Right click on an outlier and Open the Segmentation Editor to correct

i.e. Cell 28, Timepoint 116
in example dataset



The Segmentation Editor

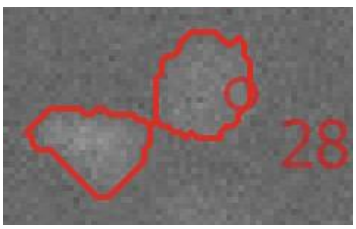


Channel Image View

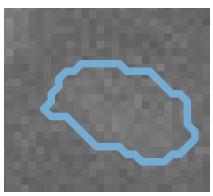
Segmentation Image View (see here what's final correction)

Cell timepoint status dock window

Additional option dock window



With red is the contour matched to the current trackpoint (here the quantification is an outlier where two cells have been merged)



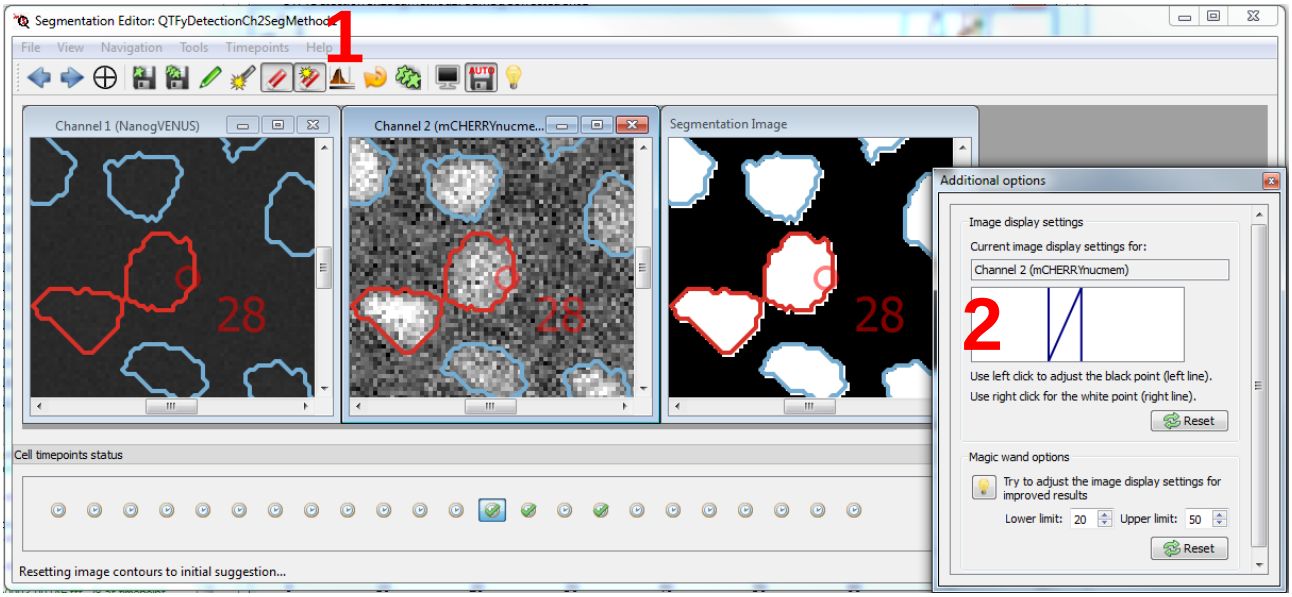
With blue is the contours of other cells in the image

The circle is the actual location of the trackpoint and number the cell ID. If the trackpoint is placed very far away from the cell (more than the specified distance see advanced Segmentation options) then no matched contour will be found.

Segmentation Editor: Main toolbar

	Move to previous timepoint (also “A” or Left arrow)
	Move to next timepoint (also “D” or Right arrow)
	Centre to current trackpoint (also “X”)
	Re-quantify cell that is matched to current trackpoint (also Ctrl+”S”)
	Re-quantify all cells present in the image (slower especially when many cells are present in latest timepoints) (also Ctrl+Shift+”S”)
	Pick the pencil to draw the cell contour by freehand. By default you are holding the pencil
	Pick/drop the magic wand (also “W”)
	Pick/drop the eraser (also “E”)
	Auto-eraser on/off. When auto-eraser is on the cell contour will be automatically replaced with the user-generated new one
	Adjust the image display settings, the intensity histogram of the channel images
	Restore initial contours
	Show all contours on/off. When off it displays only the contour that is matched to the current trackpoint.
	Channels to display
	Auto-save on/off. When on changes are automatically saved when the user moves to the new timepoint
	View the shortcuts help

Segmentation Editor: Adjust image display settings



1. Image display settings
2. Adjust the histogram of intensities of the channel views by using the mouse right and left buttons to drag the two vertical lines that represent the histogram black and white point.

Three available modes (also indicated by the cursor):


Manual mode (Drag and draw)


Magic wand mode (Click at a pixel)


Eraser mode (Drag over contour)

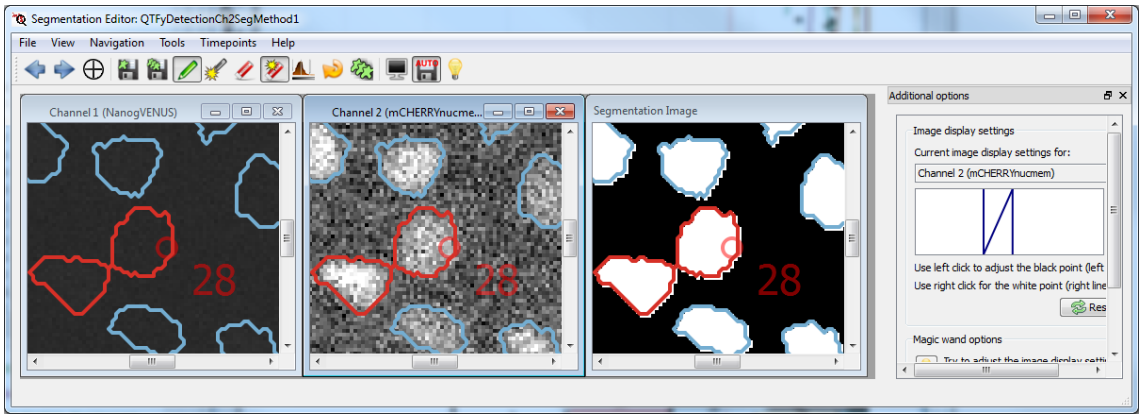
Adjust the contrast until you get a visible cell before you use the magic wand.


Auto-Eraser ON: The contour that has been assigned to the trackpoint is automatically replaced with any new contour that the user creates either by pencil or by magic wand.

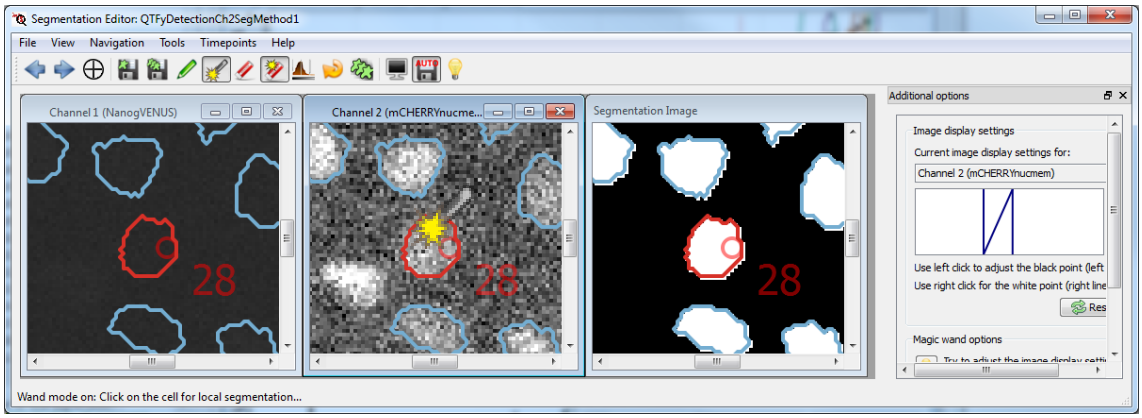

Auto-Eraser OFF: The user has to manually erase any contours by picking the eraser and dragging it over the contour. Auto-eraser OFF is useful for situations: 1. Two cells are really close together, 2. The user wants to correct many cells simultaneously in one image and not only the active cell etc…

Segmentation Editor: Correcting

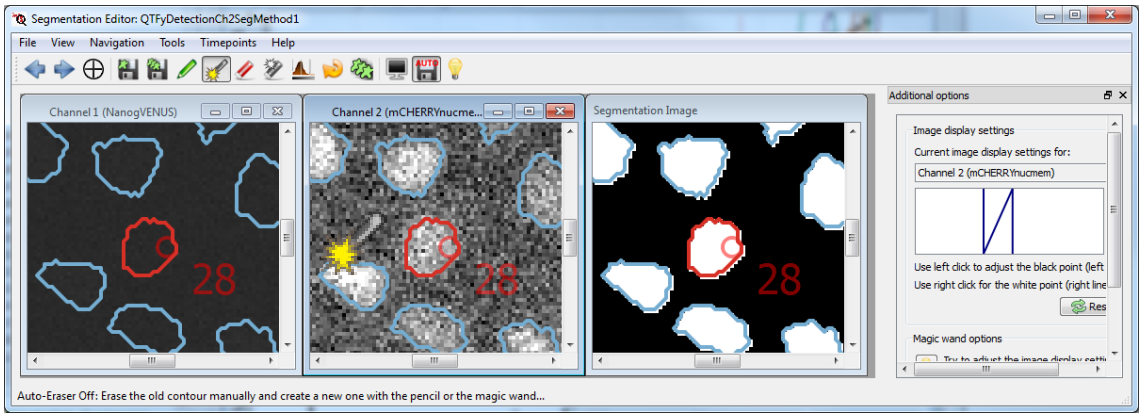
1. Adjust the image display settings of the detection channel



2. Pick the magic wand and click on a pixel at the perimeter of the cell

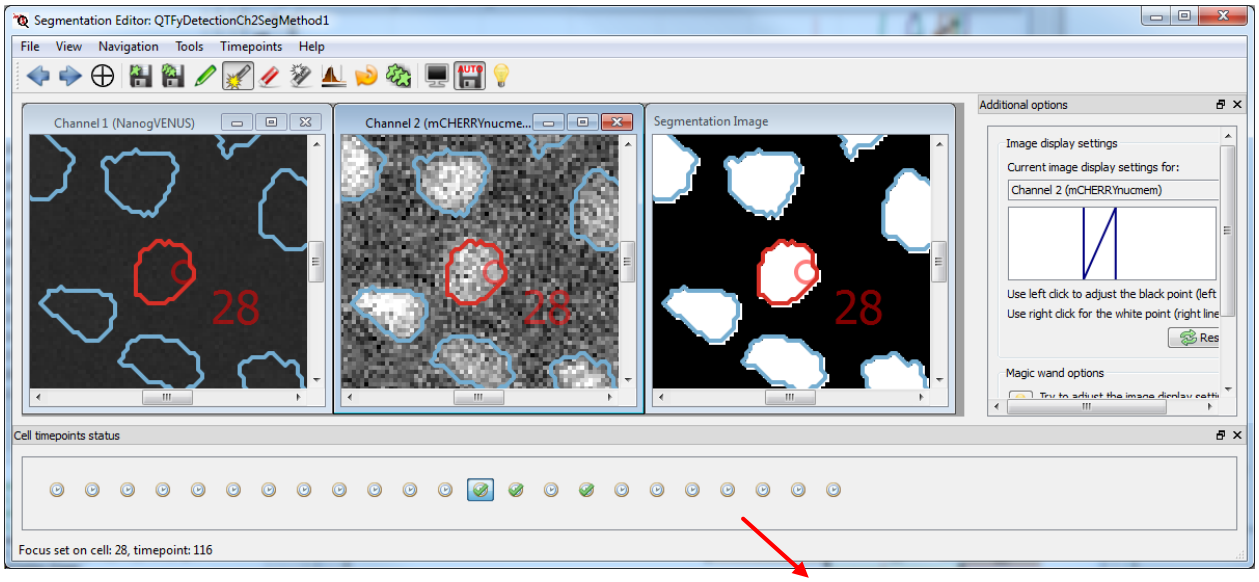


3. Uncheck Auto-eraser and repeat for second merged cell

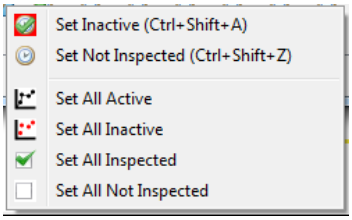


4. Press Ctrl + Shift + S to save the quantification of both cells and see the change in the qTfy Editor Parameter plot.
5. The cells that have been re-quantified are also marked as inspected (see next slide).

The Segmentation Editor: Cell timepoints status



2. Right click on a timepoint to see more options



1. The “Cell timepoints status” is another dock window. Every timepoint is a button. Press it to move to the respective timepoint.
2. Timepoint status:


Active, inspected


Inactive, inspected

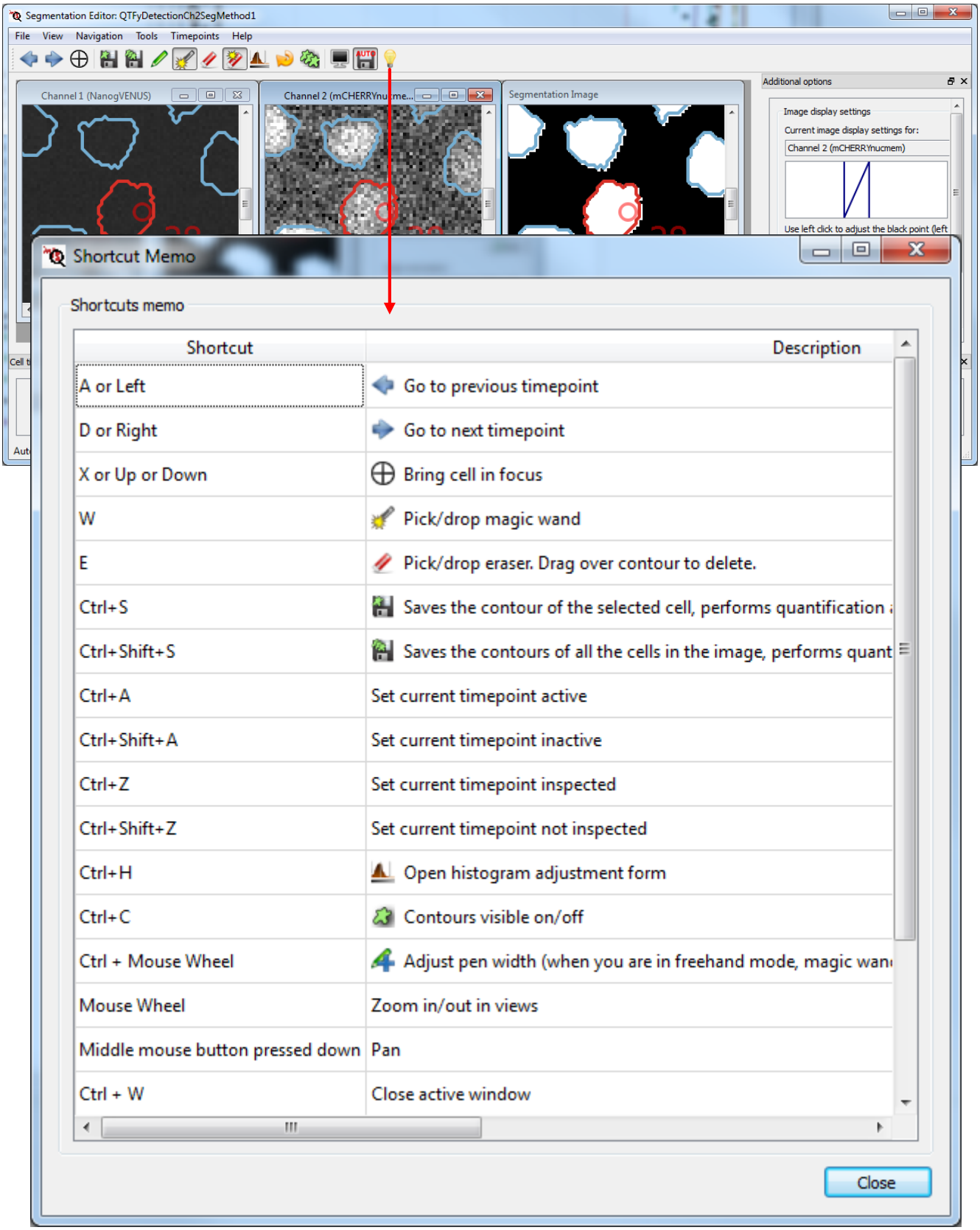

Active, not inspected


Not available (i.e. no contour was identified for the cell at that timepoint)

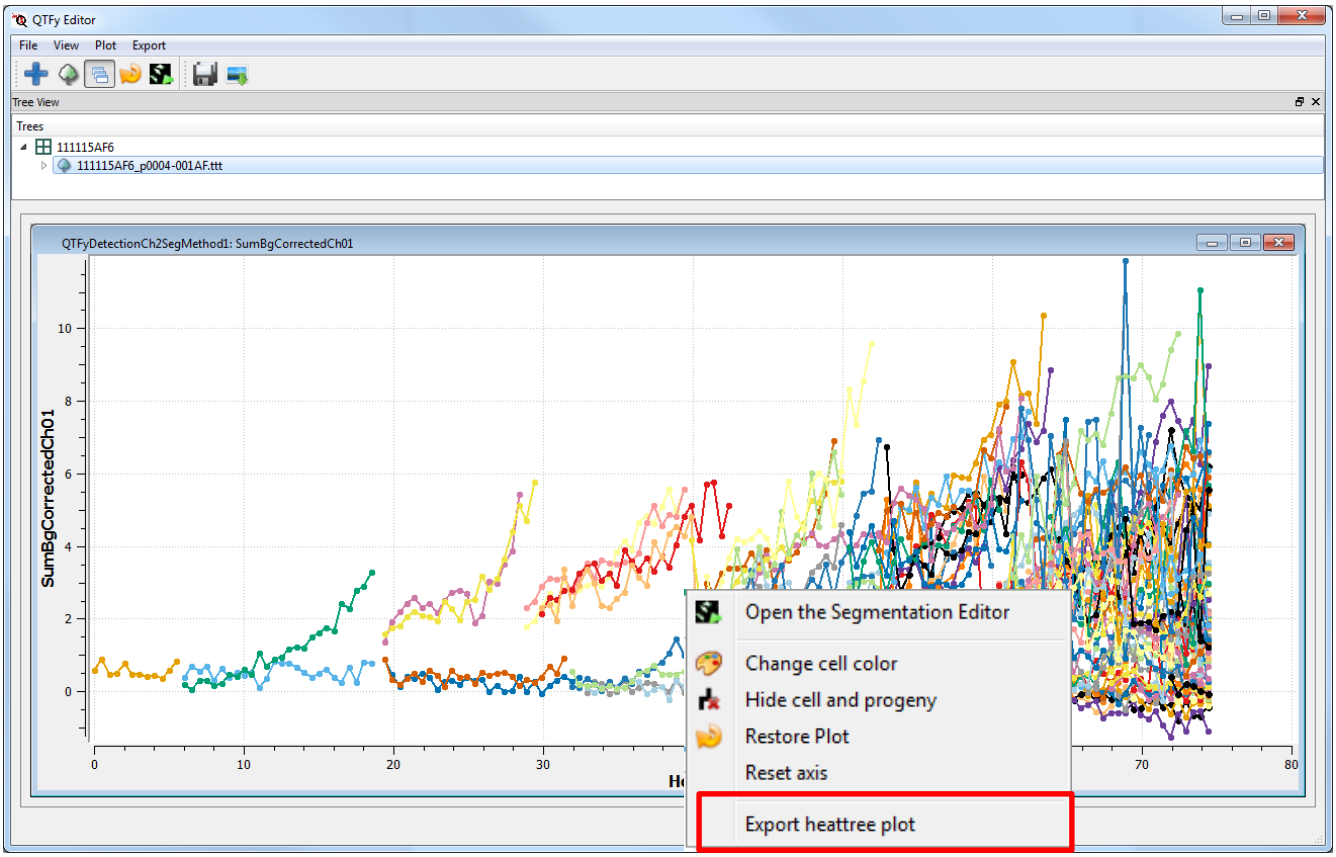
Inactive, not inspected

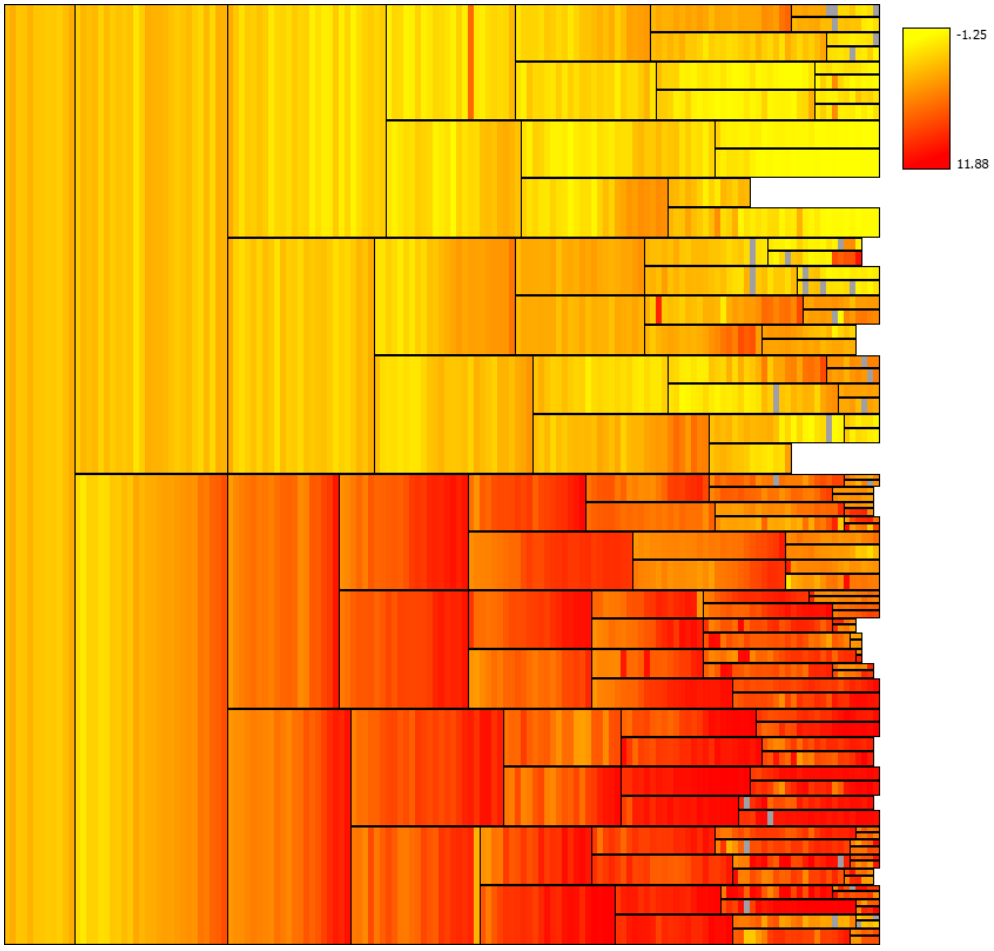
Generally red background means inactive, check mark means inspected

The Segmentation Editor: Advanced handling through shortcuts



Export a Heattree plot





Note: What happens when you run a quantification?

- Look in segmentation folder for mask file that is derived from the specified detection channel using the selected segmentation method
  - qTfy: 01
  - 140630LS5_p0021_t00002_z001_**w00_m01_mask**.png
- If the file is available use existing file (it also supports connected components label images).
- Otherwise, generate a new mask file using the qTfy default method (entropy split based thresholding within a user-specified size window around the trackpoint).
- If there are no identified cells, no mask file will be generated.
- What are possible errors at this stage:
  - When “use existing segmentation” is selected by no mask file can be found, a warning is generated.
  - When no cells have been found near the trackpoint a warning is generated.
  - These warnings will not stop your analysis. It is good however to have a look that everything is as expected.