CellStar tutorial

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What is CellStar?

CellStar basic function is to segment bright-field microscopy images of budding yeast (i.e. find in images which pixels correspond to each cell) and to track these cells in time lapse (i.e. recognize a cell in different frames). If you also have fluorescence images, CellStar can also extract the single cell fluorescence trajectories. For time lapse, make sure you have your movie saved as separate images (and not a video file). Most common image formats are supported.

CellStar can work in "Batch Mode" (no intervention besides setup) or in "Interactive Mode" thanks to a Matlab embedded user interface (UI). The interactive mode allows you not only to easily setup a segmentation/tracking task, but also to perform a variety of related task like corrections and automatic tuning of its own parameters, making it a very versatile and easy to use tool.

In this tutorial, we will guide you through the basic operations needed to extract single cell fluorescence data from a time lapse set of microscopy images using the User Interface (UI).

Setup and Installation

Copy the Cellstar folder to a directory of your choosing and add the folder and all subfolders to your Matlab Path. That's it! NB: Cellstar requires the image analysis toolbox to function and optionally the global optimization toolbox for auto-tuning of parameters.

To launch CellStar, simply run "InitUI":
e.g. >>InitUI();

This should open a special figure window with an extra tab called CellStar.
In the console, you should see a list of keys and associated actions.
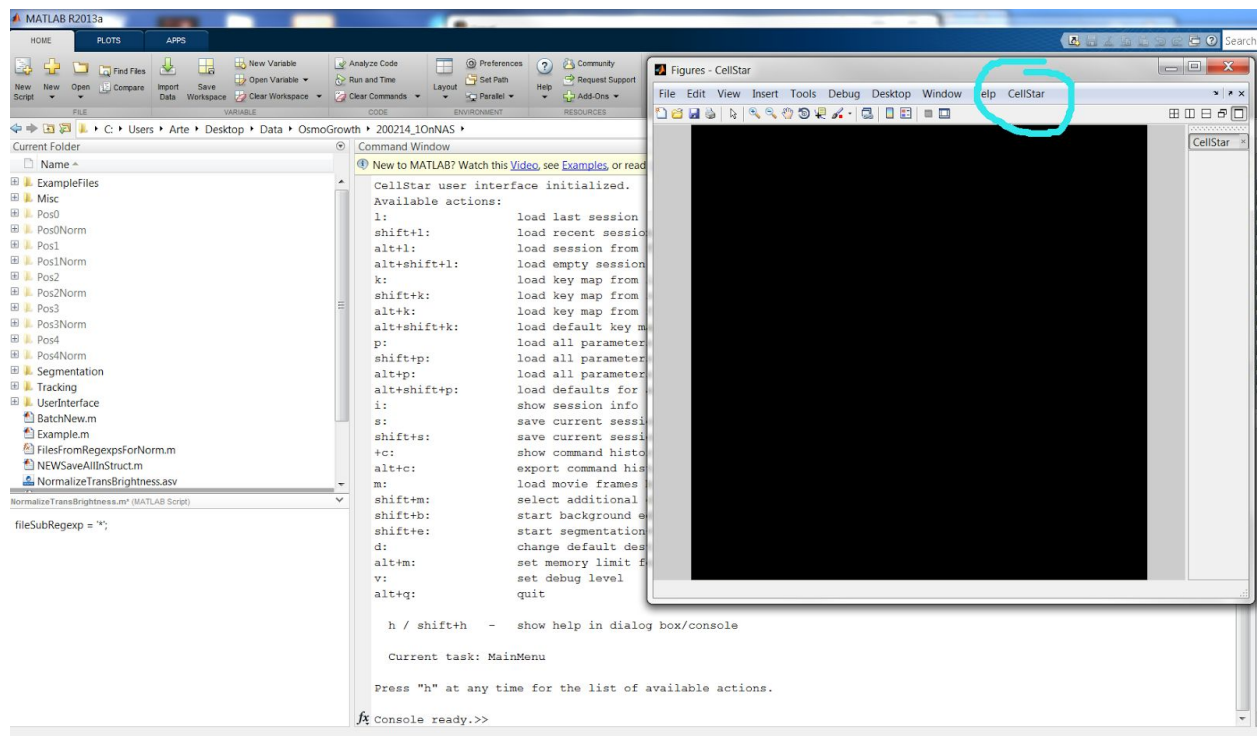
General note on the use of cellstar :

In order to make action in CS you can either use the keys (you can display the list of available actions pressing **'H'** (help) at any moment) or the special tab in the figure window. Last, you can use the console (advanced use).

Note that when using keys for actions, the figure window of CellStar must have the focus.

Loading a movie

Click on the CS figure window which contains the Cellstar UI and press **'M'** to load your bright field frames or use the option from the CellStar Tab "load movie frames by file selection".
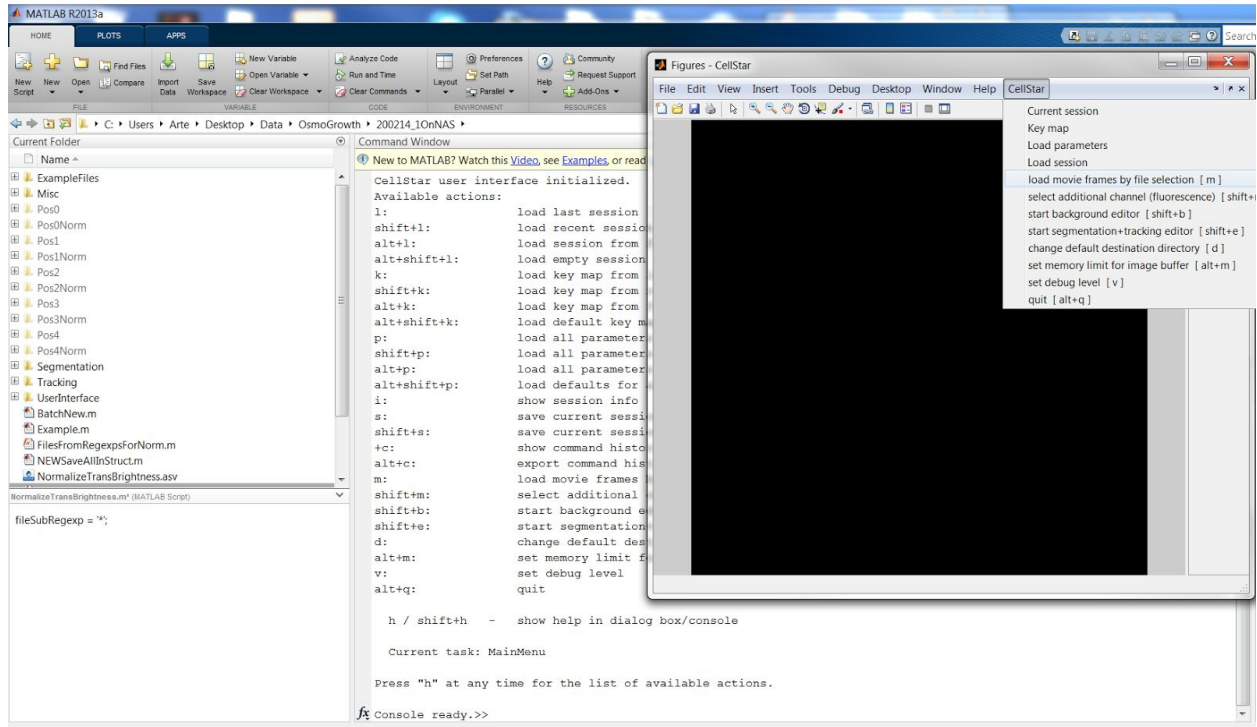

You can now select all bright-field images you wish to load. If you do not have a movie at hand but you want to test CellStar user interface, you can select the example pictures that are in the folder ExampleFiles.
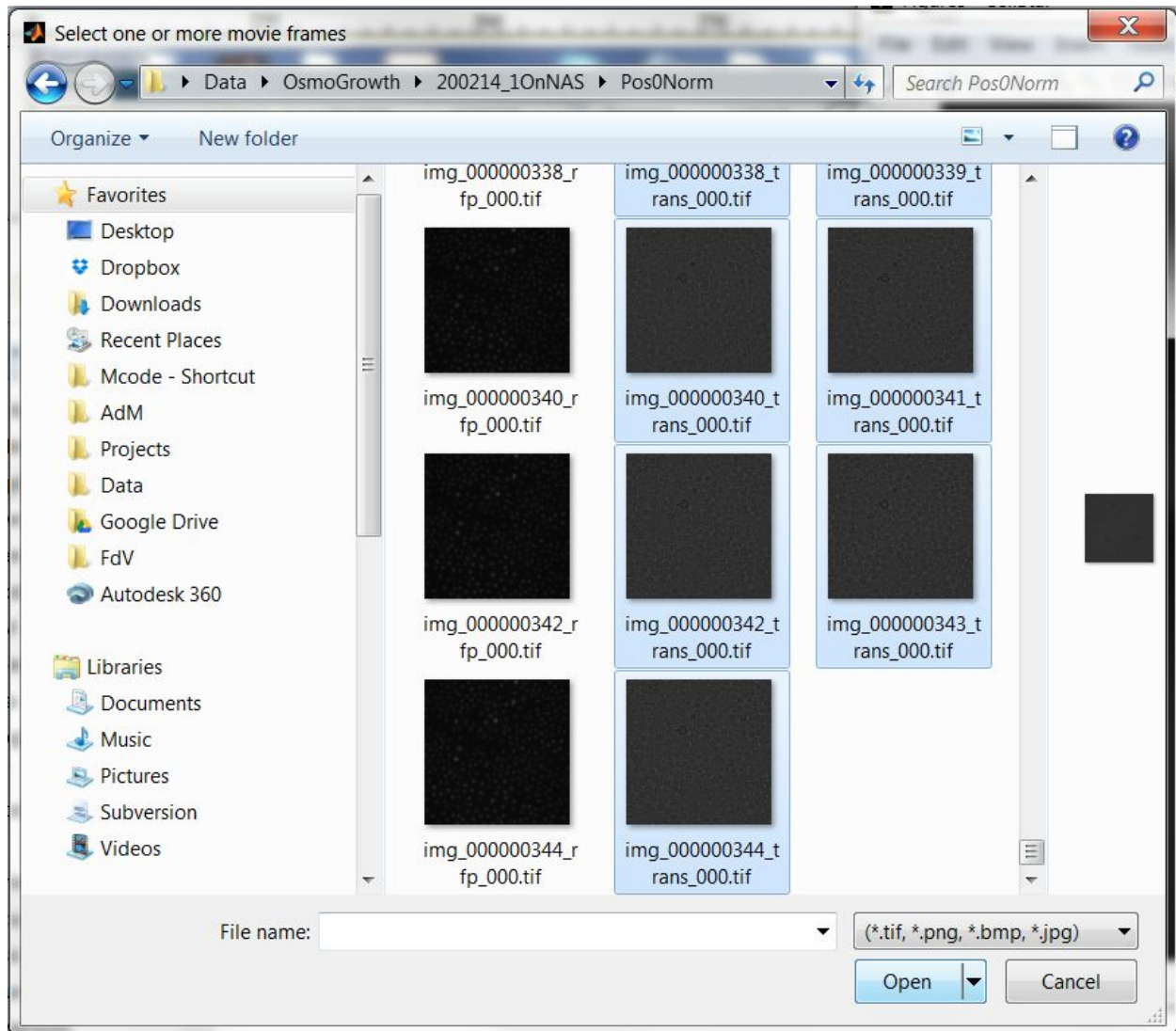
Next you can press **'SHIFT' + 'M'** to load fluorescence images that correspond to your movie or use the "select additional channel (fluorescence)" option in CellStar Tab. To match your needs, there are three ways to import fluorescence data and map it to bright field images.

- Import by "Time": A dialog asks for all the fluorescent images and will map bright field to fluorescence files with the closer creation time.
- Use a "Regular Expression". This is useful if your files have similar names, like in the current example where we have "trans" files and "rfp" files every two frames. You will be prompted to select a regular expression that is replaced for the fluorescence (here "trans" and "rfp".
- Use a "Map": after you have imported all fluorescence files, you can map indexes of bright field to fluorescence with regular matlab expression like 1:2:200.

Lastly, you will also be asked if you wish to compute fluorescence as a mean or maximum.
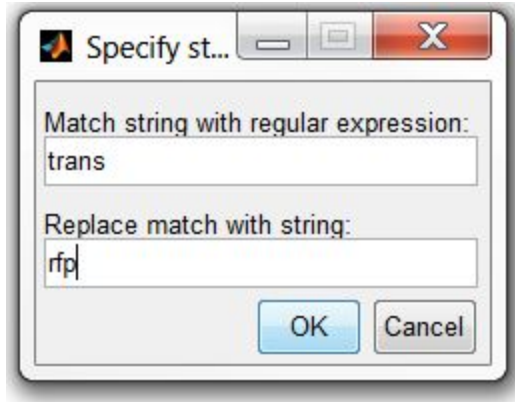
Background Editor

Load the background editor by pressing **'SHIFT' + 'B'** (CellStar Tab -> "start background editor"): this will change the list of available actions under the CellStar Tab. You will be prompted to save your session file if you have not done so yet. You will then be prompted to select your average cell diameter in pixels, or you can let Cellstar to attempt automatic detection of the average cell diameter. This is not advised without human supervision: if you use autodetection, check Matlab console for the detected size and if you find that the value is considerably off, fix it manually by pressing **'C'** (CellStar Tab -> "set average (adult) cell diameter").

You will then need to select one or more frames to compute the background. You can press **'A'** to manually select frames to use for the background, **'SHIFT' + 'A'** to set the background as the average of ALL frames, or **'ALT' + 'SHIFT' + 'A'** to use the first frame only (CellStar Tab -> "Set background as..."). We recommend pressing **'A'** and selecting the first 5-10 frames average of the movie as a background. You should then see an image similar to the one below.
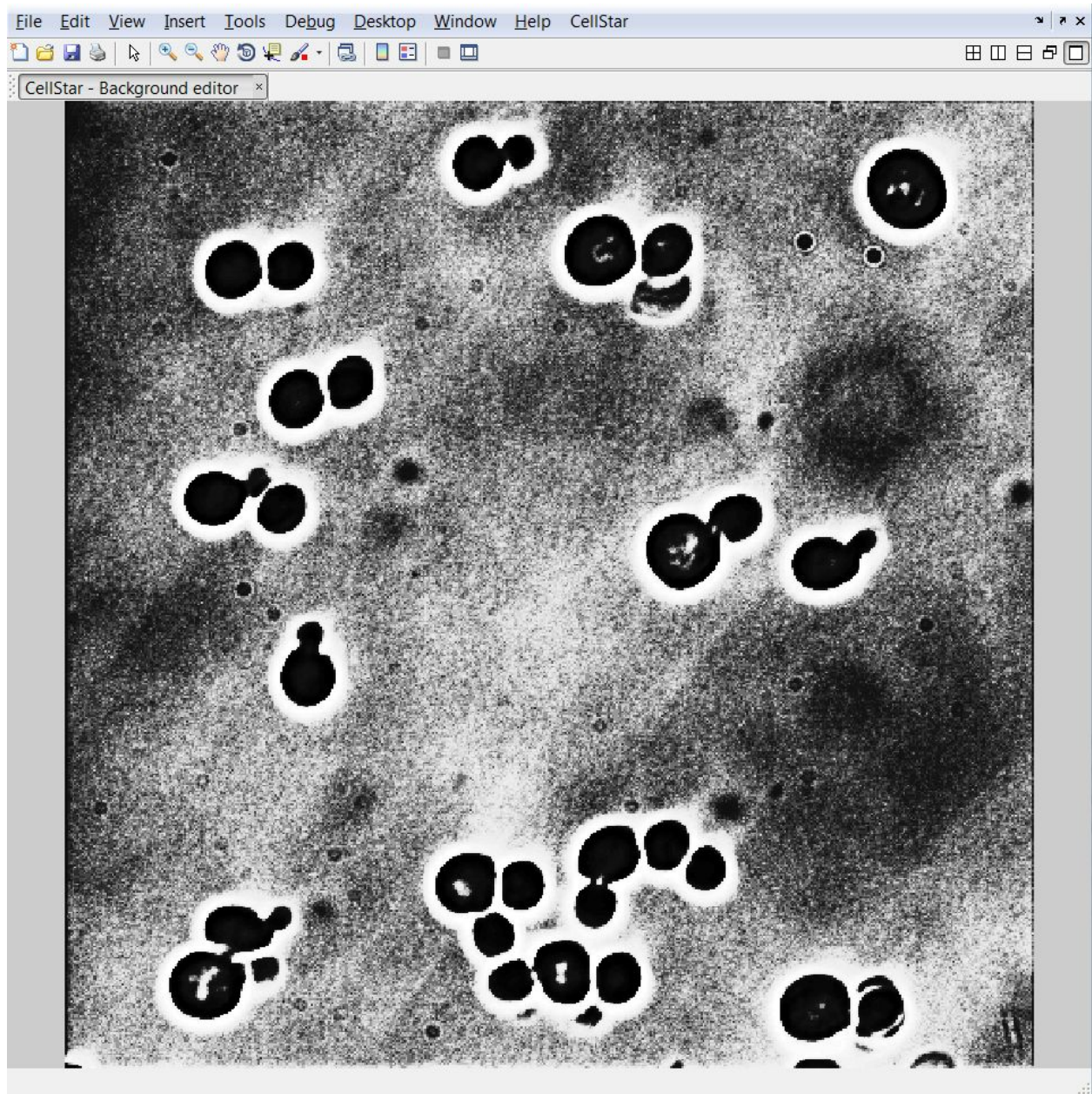
Auto-detect the background mask by pressing **'SHIFT' + 'M'** (CellStar Tab -> "Edit background mask"). You can manually adjust the background mask anywhere on the image with mouse **Left Click** (add a disk to the mask) or **Right Click** (remove a disk from the mask). You can increase or decrease the size of the disk that is added to or removed from the mask by pressing **'1'** or **'2'** (CellStar Tab -> "Circle size").

You can also invert the mask by repeatedly pressing **'I'.**
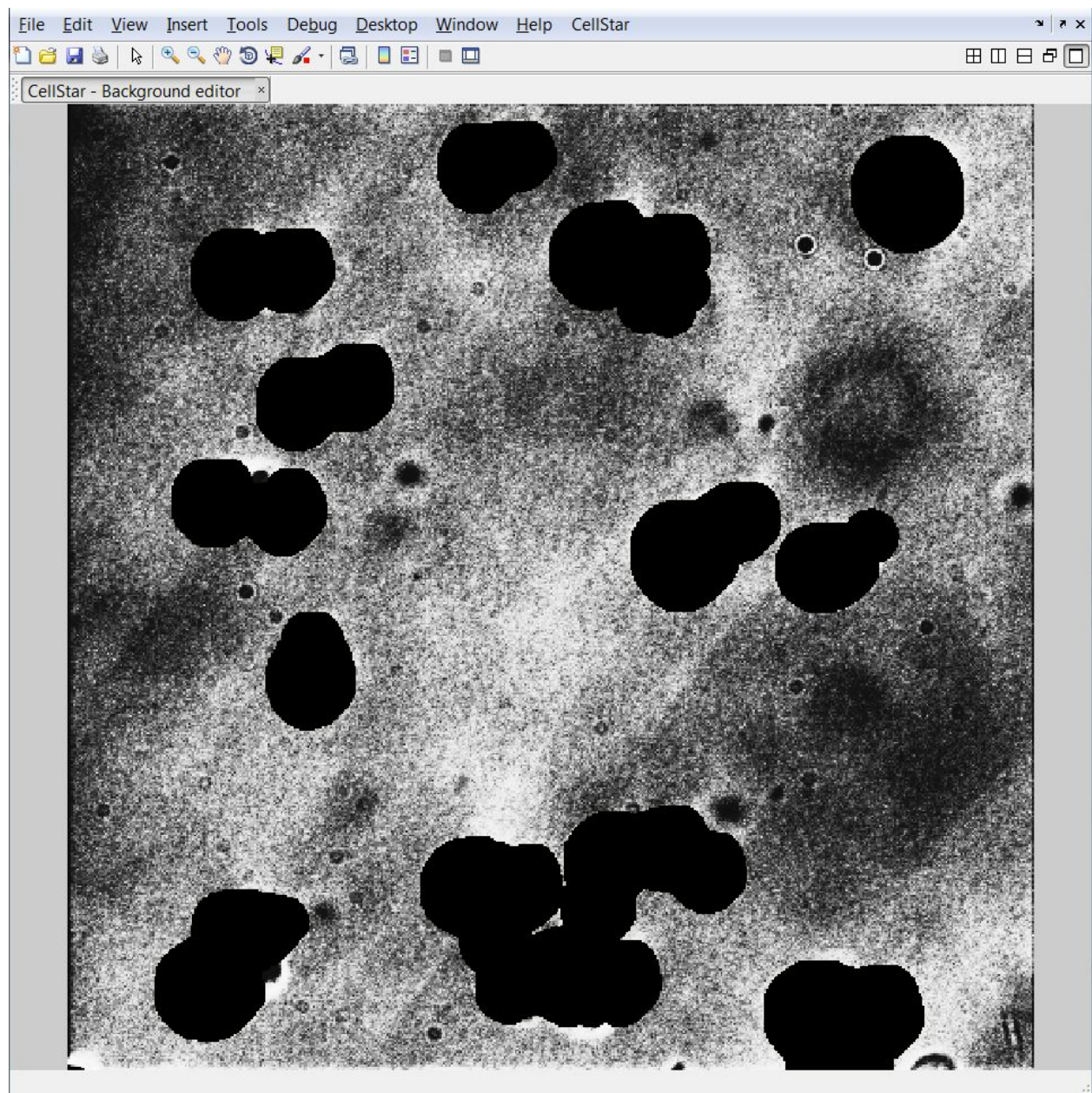
Once all your cells are covered by the background mask you can fill in the mask by selecting **'SHIFT' + 'B'** (CellStar Tab -> "Blur background") to blur by neighbor pixels. You should then see something like this:
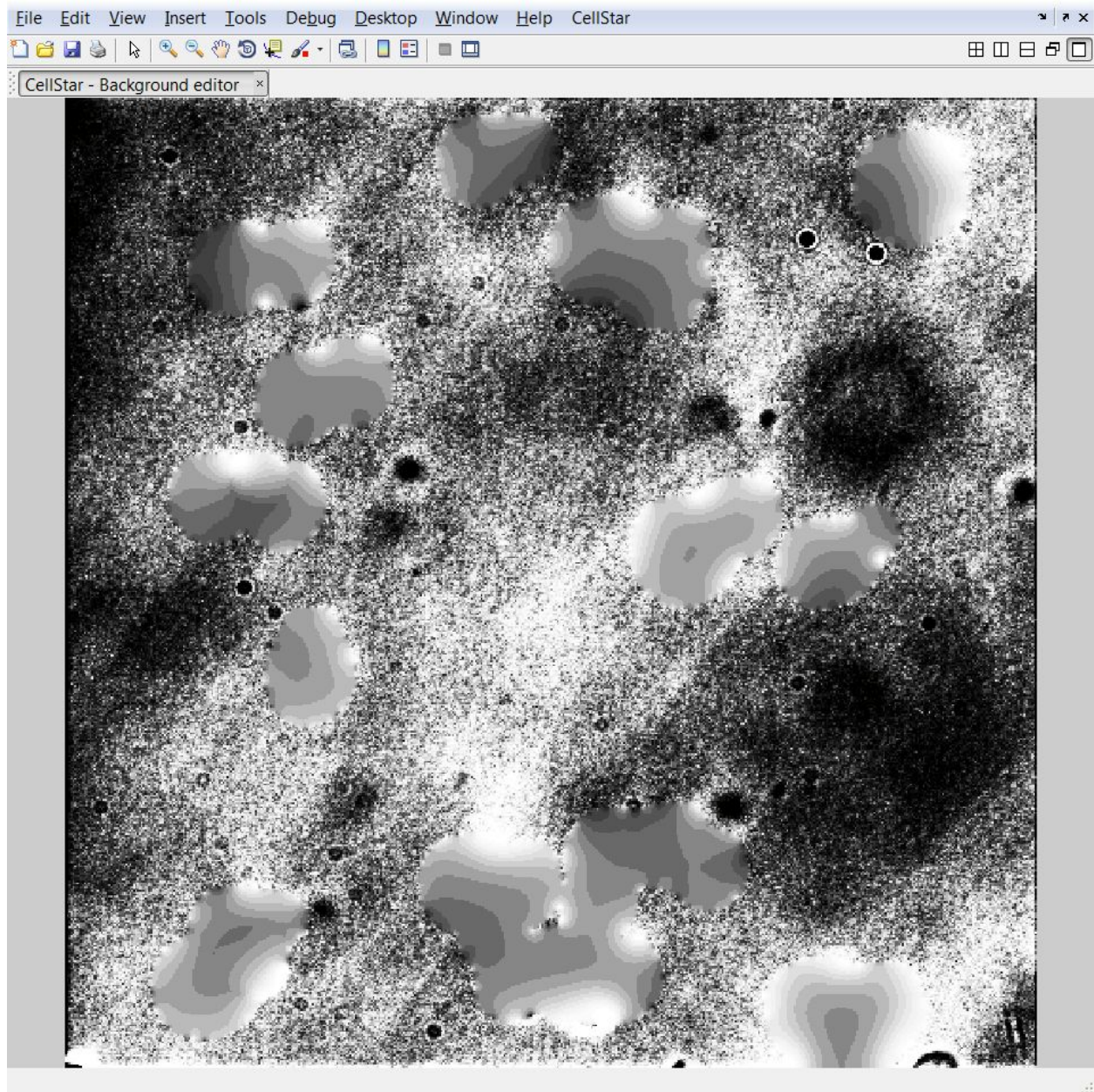
This will prevent Cellstar from counting cells as part of the background image. You are now ready to start the segmentation and tracking editor!

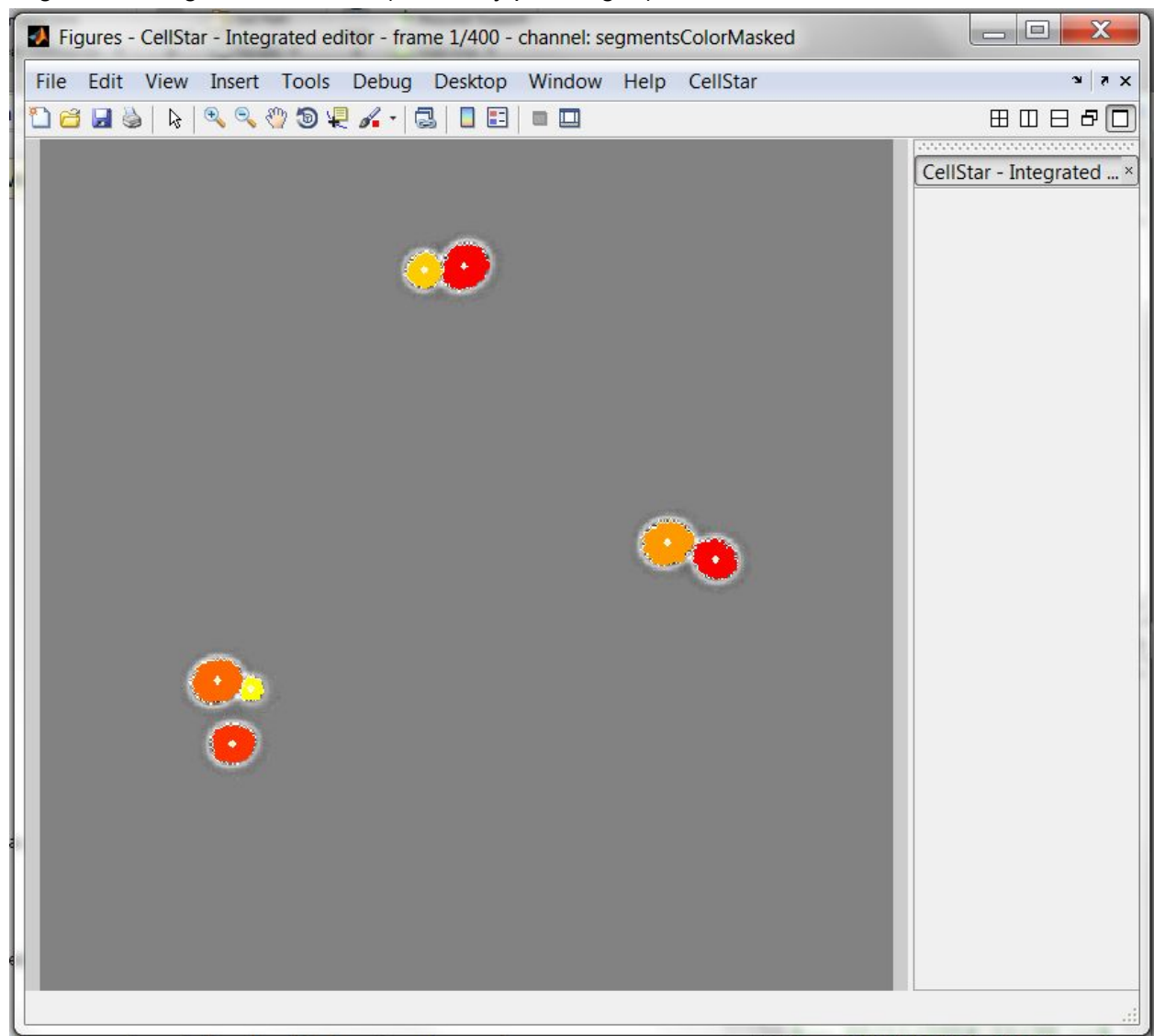## Segmentation and Tracking

To load the "Segmentation and Tracking Editor", press **'Shift' + 'E'** (CellStar Tab -> "start segmentation-tracking editor"). This will change again the list of available actions in CellStar tab, and it will also start you on the first frame of your video. You can navigate frames by rolling the **Mouse Wheel** or pressing the **'Left Arrow'** and **'Right Arrow'** keys.

To begin automatic segmentation and tracking for ALL frames, press **'SHIFT' + 'ALT' + 'Z'** (CellStar Tab -> "(re)Do segmentation-tracking"). This can be very time consuming and computer resources intensive, so for less powerful computers it is advisable to close all other programs before beginning. It is recommended to perform segmentation and tracking separately which can be done by pressing **'SHIFT' + 'ALT' + 'S'** to segment all frames and **'SHIFT' + 'ALT' + 'T'** to track all frames. This can allow you to correct errors in the segmentation before proceeding with tracking (see below). Additionally, you can segment or track individual frames by pressing **'SHIFT' + 'S'** or **'SHIFT' + 'T'** respectively or select a set of frames to segment or track by pressing **'ALT' + 'S'** or **'ALT' + T'** respectively.
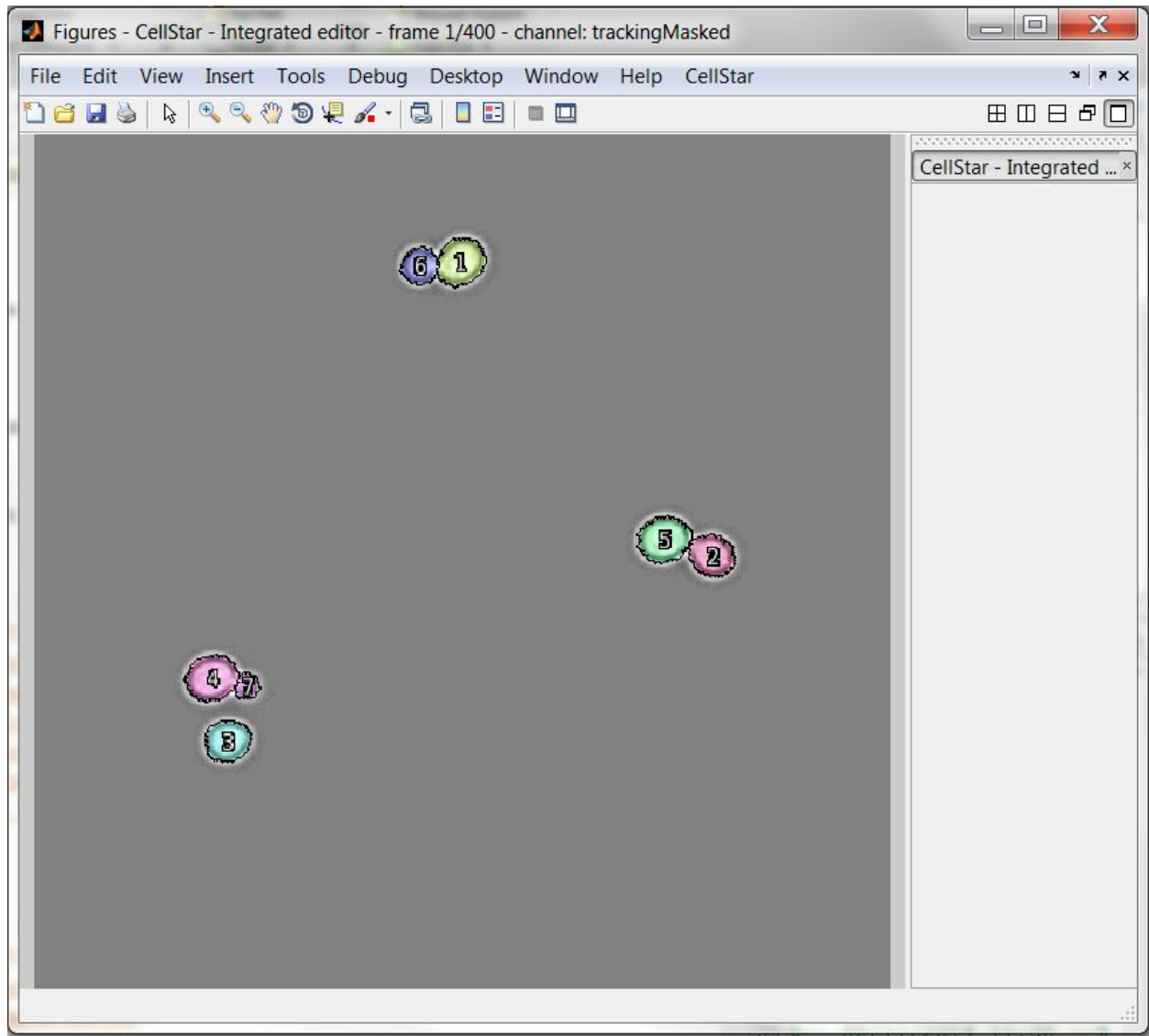
In the Segmentation and tracking editor, you can browse in between channels: bright light, fluorescence(s), segmentation result, tracking result along with several intermediate images that are computed for segmentation which are useful for troubleshooting (to see the list of all the available channels you can see all the options in the CellStar tab -> "Toggle channel-info")
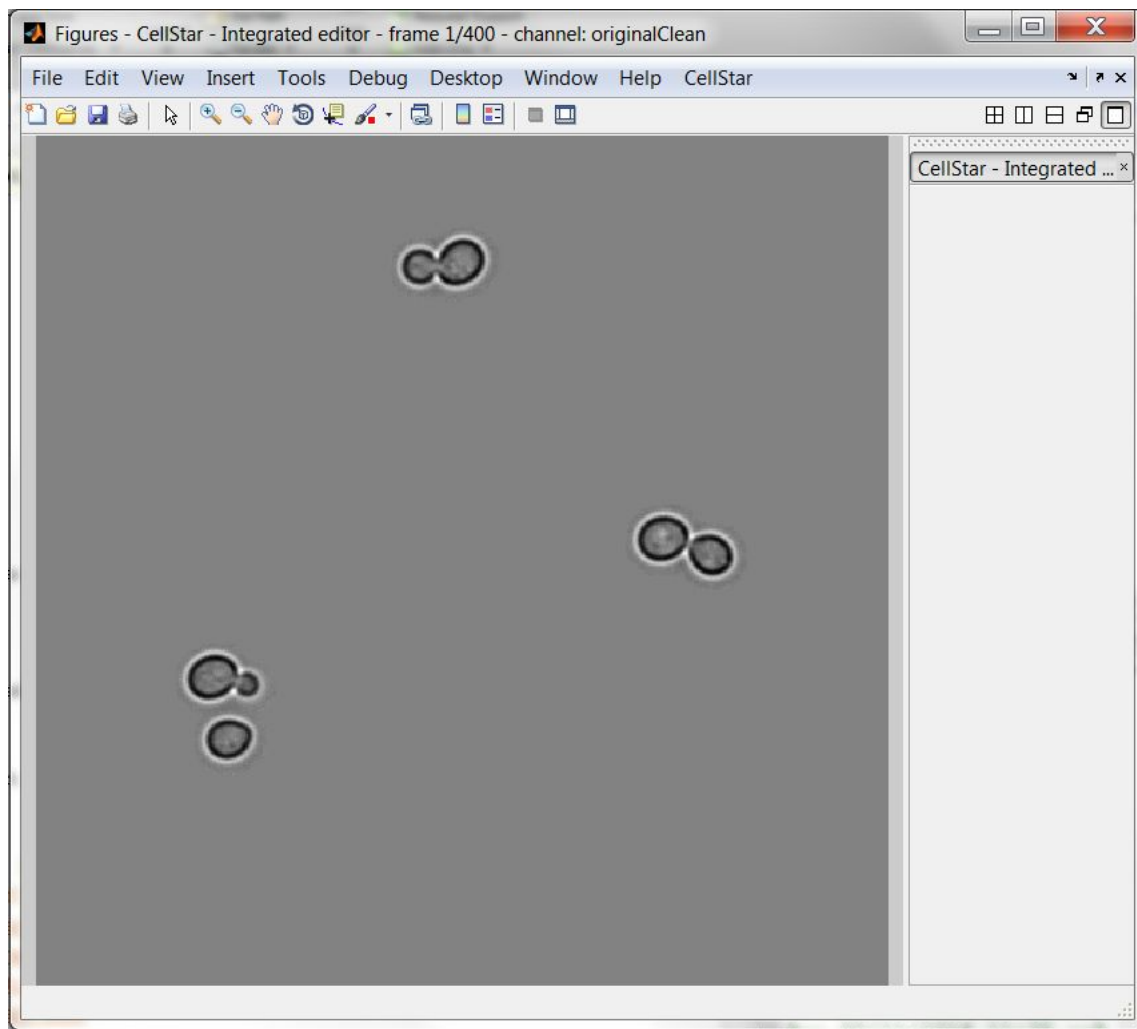
Bright field image in S&T editor (shown by pressing **'I'**)

Segmentation result in the Segmentation and Tracking Editor (shown by pressing **'S'** after completing segmentation)

Tracking result in Segmentation and Tracking editor (by pressing **'T'**, after completing tracking)

You can quickly switch between the last two shown channels by pressing repeatedly the key corresponding to the last channel you switched to.

Corrections

Although you may want to achieve the best possible result automatically, some errors in the segmentation and/or tracking are often present. Another great feature of CellStar is that it allows you to easily fix these errors by hand.

In the following we will guide you through fixing the most common errors but more features are available.

To bring up the segmentation data, press **'S'**. This will show you the areas of the image that Cellstar thinks are Cells, called also traces of segments. You can select a cell with a **Left-Click.**
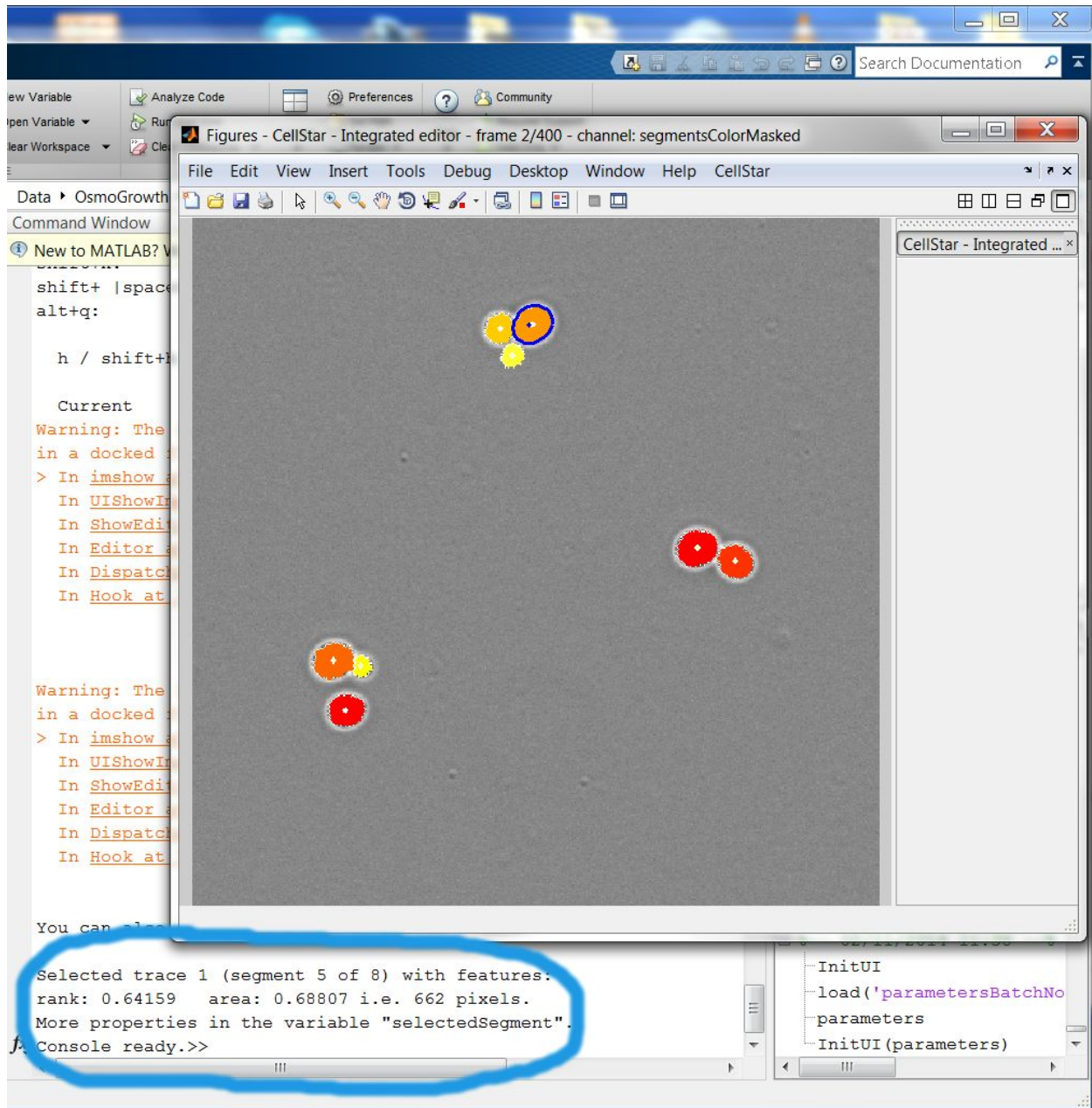
Selecting a cell shows information on that cell in the console and creates a variable with additional information.

False positive error

Like in the previous pictire, you may find false positives (i.e. part of the image segmented as cell while not real cells). To correct these, simply select the cell with a **Left Click** and press **'D'** (CellStar tab -> "Edit segmentation"). NB: you can also directly delete a cell with a **Right Click** if that cell is not already selected. Otherwise, a **Right Click** on a selected cell modifies its contour and adds it to the ground truth (see below).
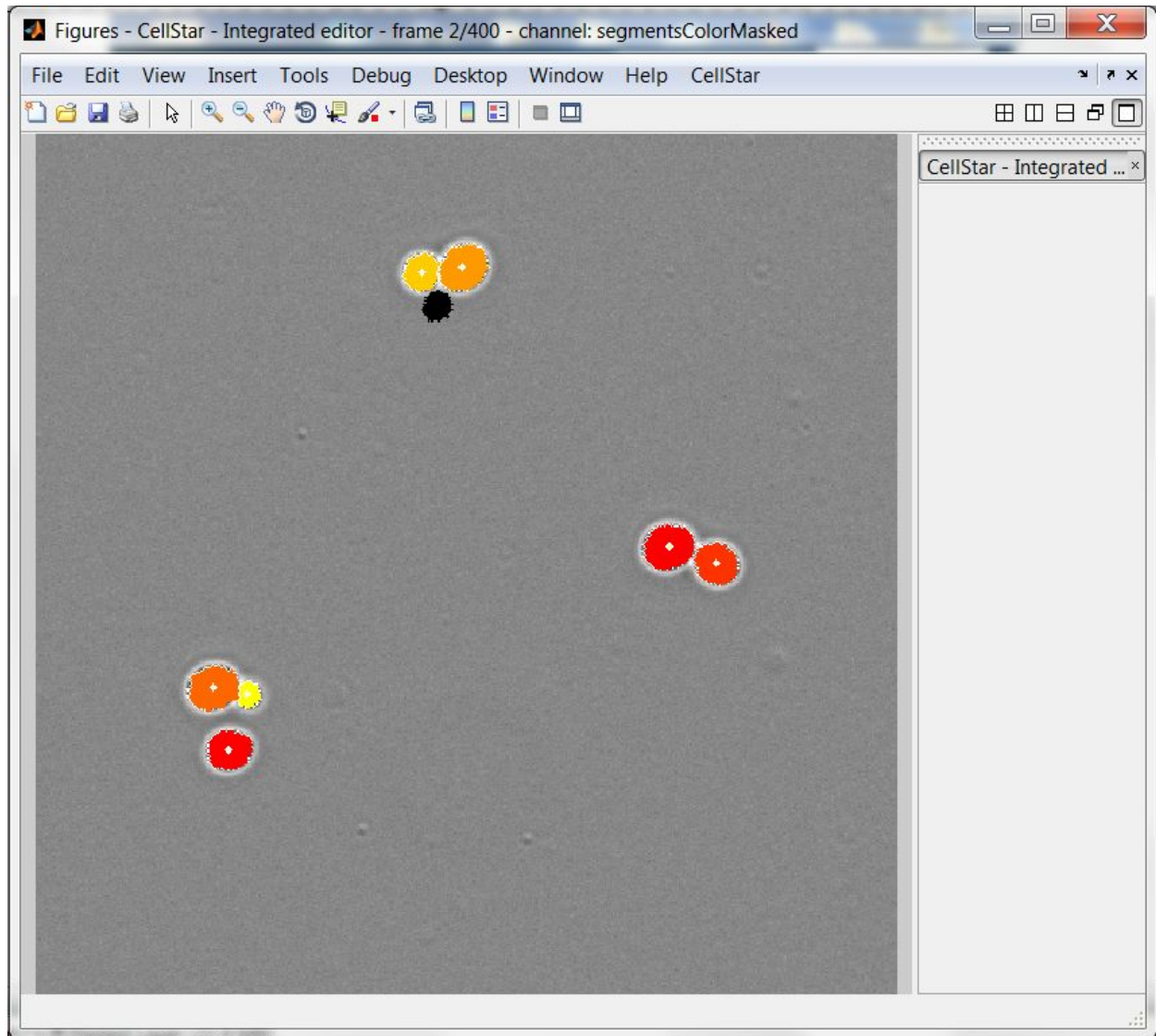
Result after having deleted a false positive. The black area shows where the cell was. This implies the change has still not been saved (in case you deleted it by mistake, so you can add it back by right-clicking it again or by "undeleting" the last deleted segment by pressing **'U'**).

It is possible to delete (or undelete) more cells at once, as well as to apply several further operations available in the CellStar tab -> "Edit segmentation".


Validating a change

In a general manner, when you made changes you need at some point to validate the changes by pressing **'SHIFT' + 'Y'** (CellStar tab -> "Apply changes") and wait for CellStar to save them in files and apply consequent corrections (re-numbering of cells, recomputing tracking for concerned frames, etc.)

False negative error

For cells that Cellstar fails to properly detect, **Left Click** where the cell should be and a green outline should appear around the cell. If this green outline does not properly enclose the cell, you can **Right Click** to move the border of the green outline until it is in the proper place.
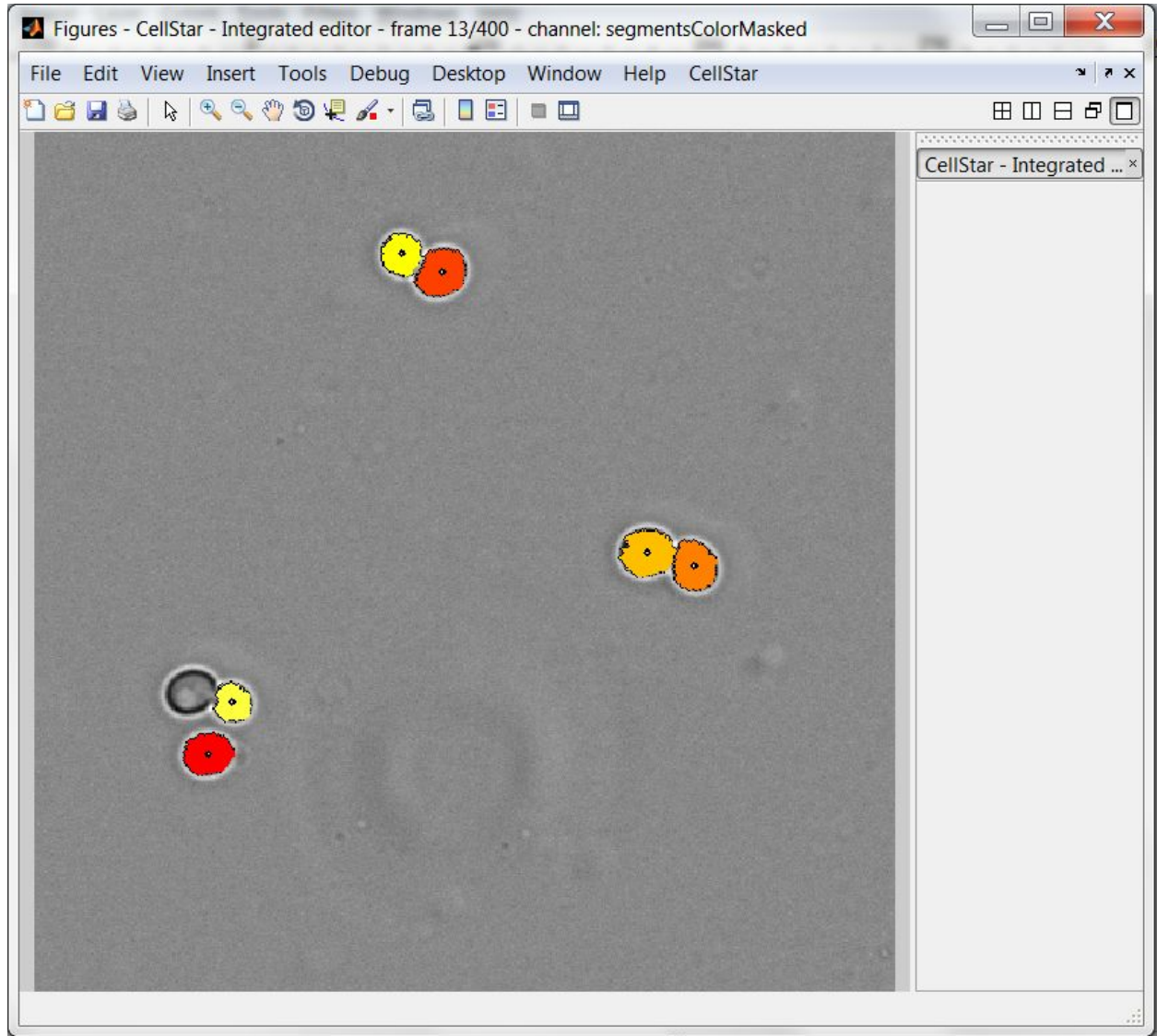

Here a cell is missing
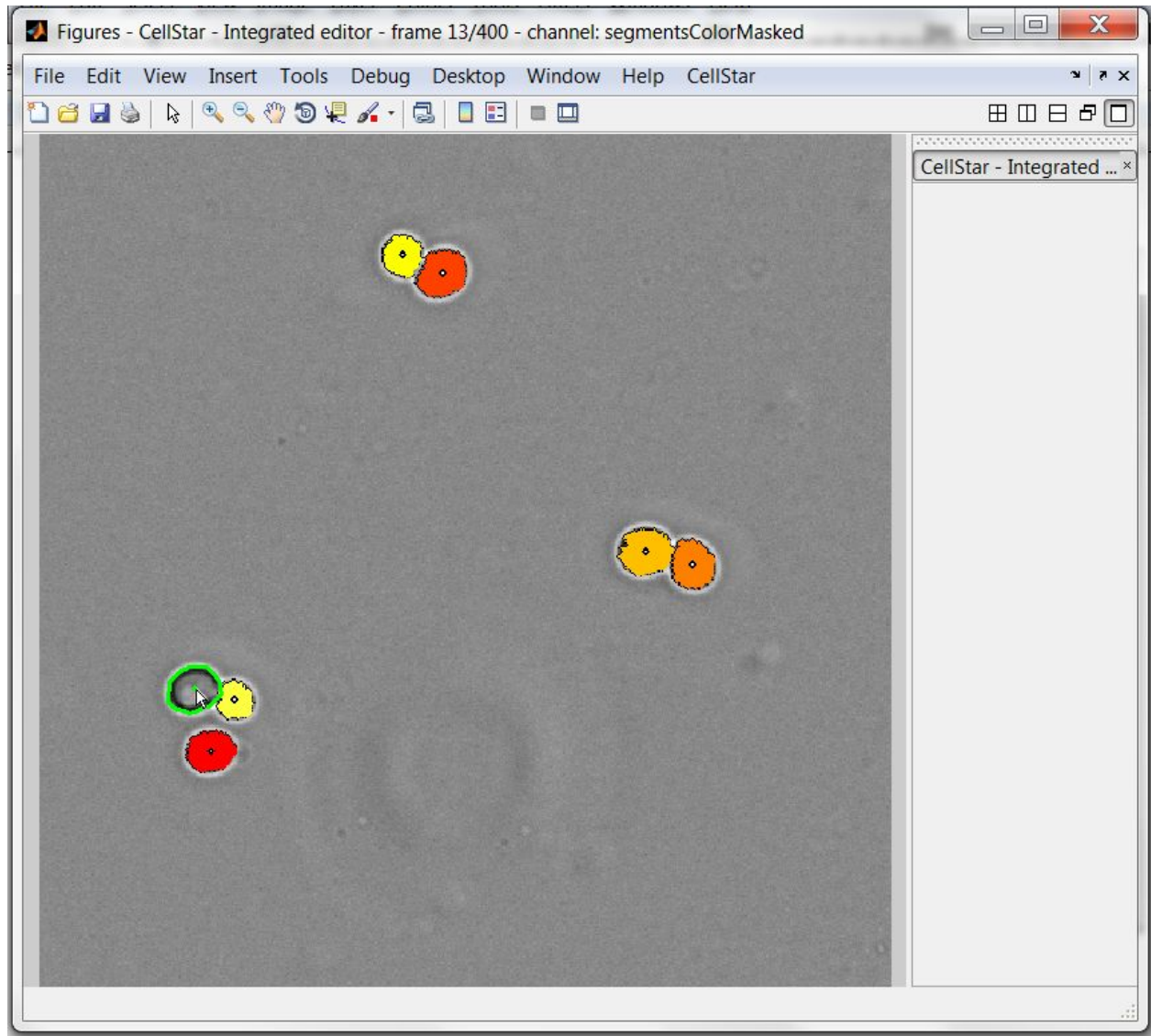
We add the cell back with a left-click.

Ground truth cells

When you add a missing cell and apply changes with **'SHIFT' + 'Y'** you will see that the cell turns green. This mean this cell is part of the Ground Truth. Ground Truth is a category of cells CellStar knows are right since you edited them. These are permanent cells (unless you manually delete them), used also for automatic tuning of parameters (explained later). It may therefore be useful at some point to add other cells to the ground truth, even some of those that you did not edit by hand, so as to tell CellStar what is the perfect segmentation for its parameter learning.
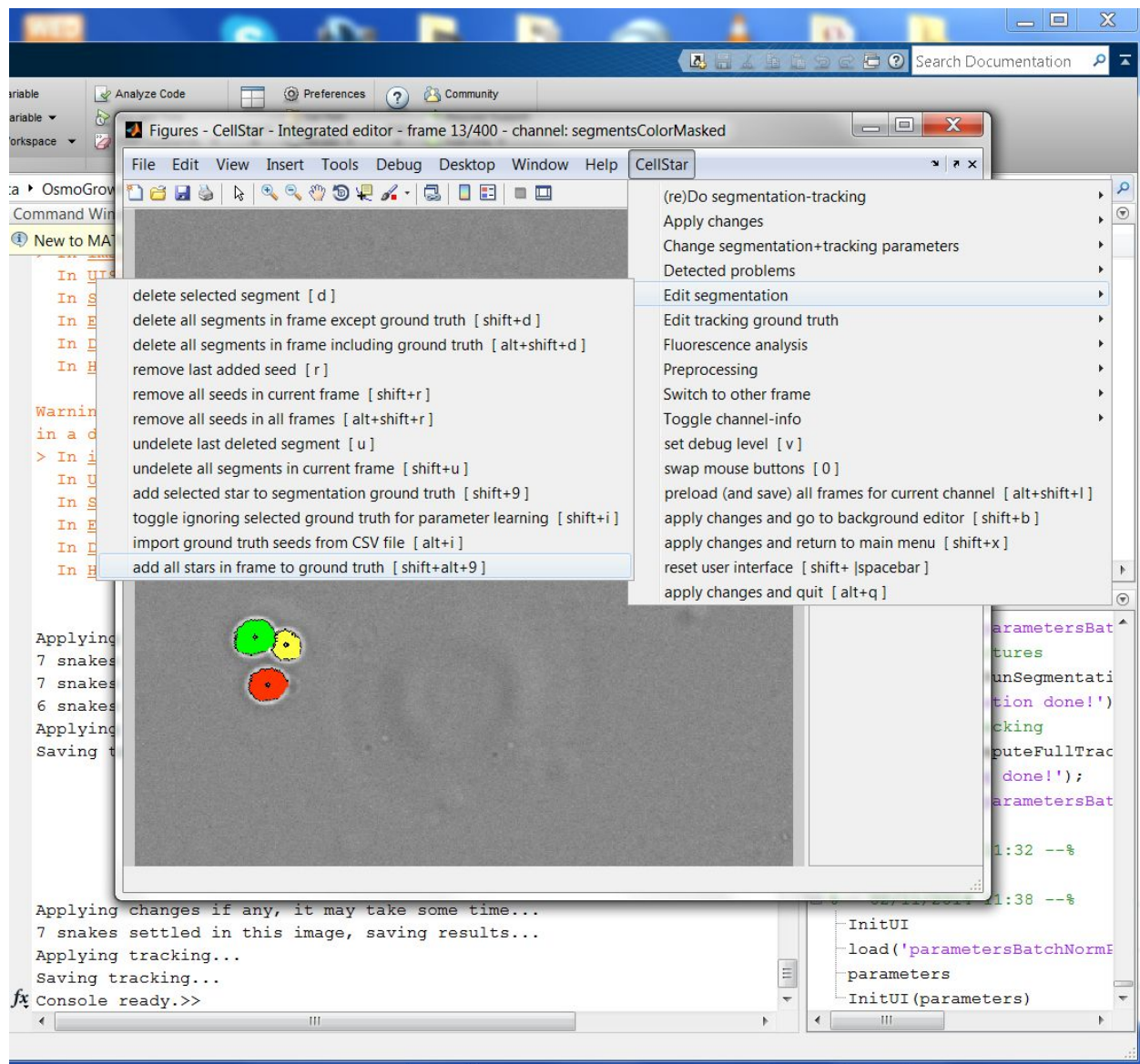
Adding all cells to ground truth
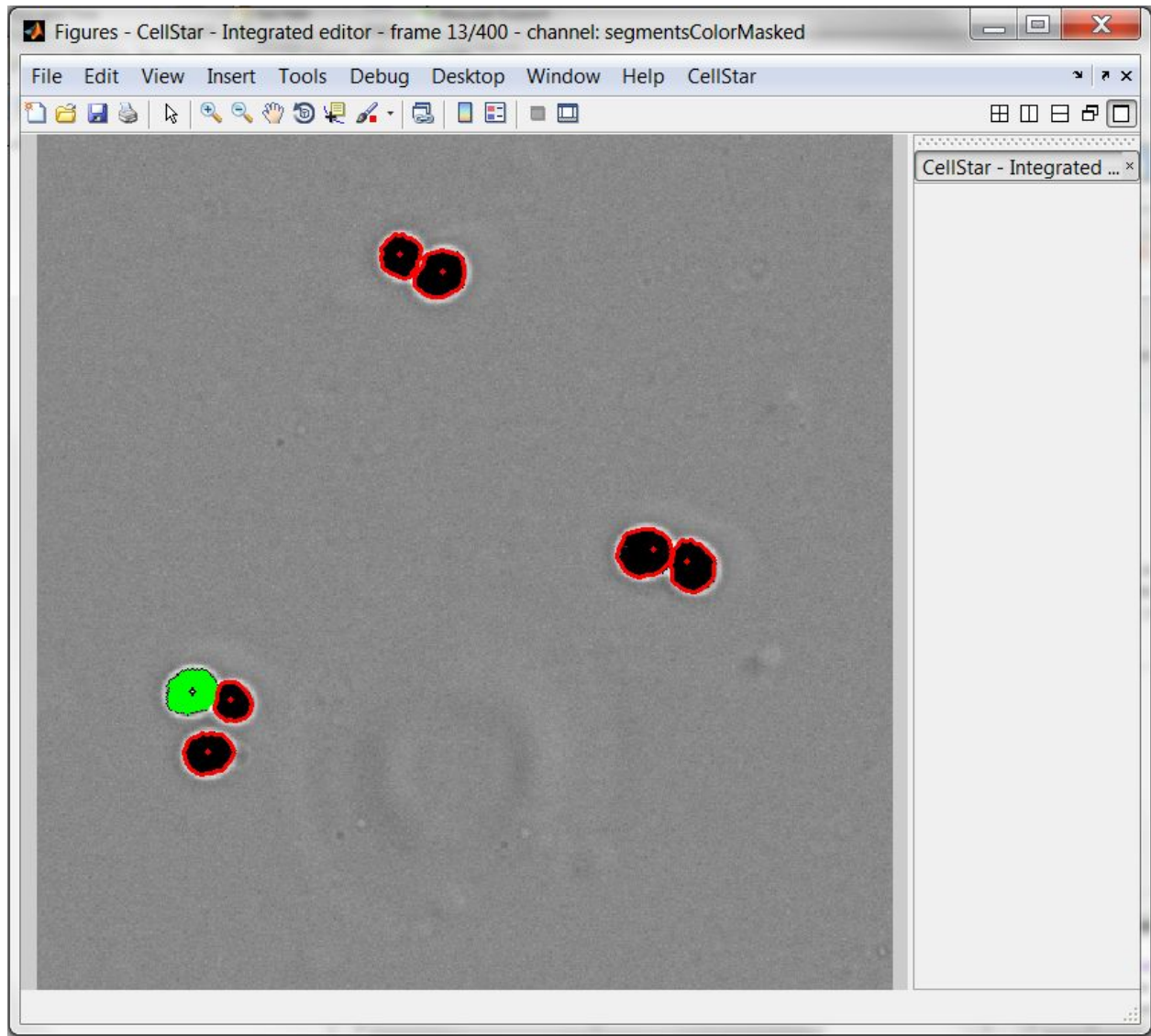
Like any other modification, you need to validate using **'SHIFT' + 'Y'**
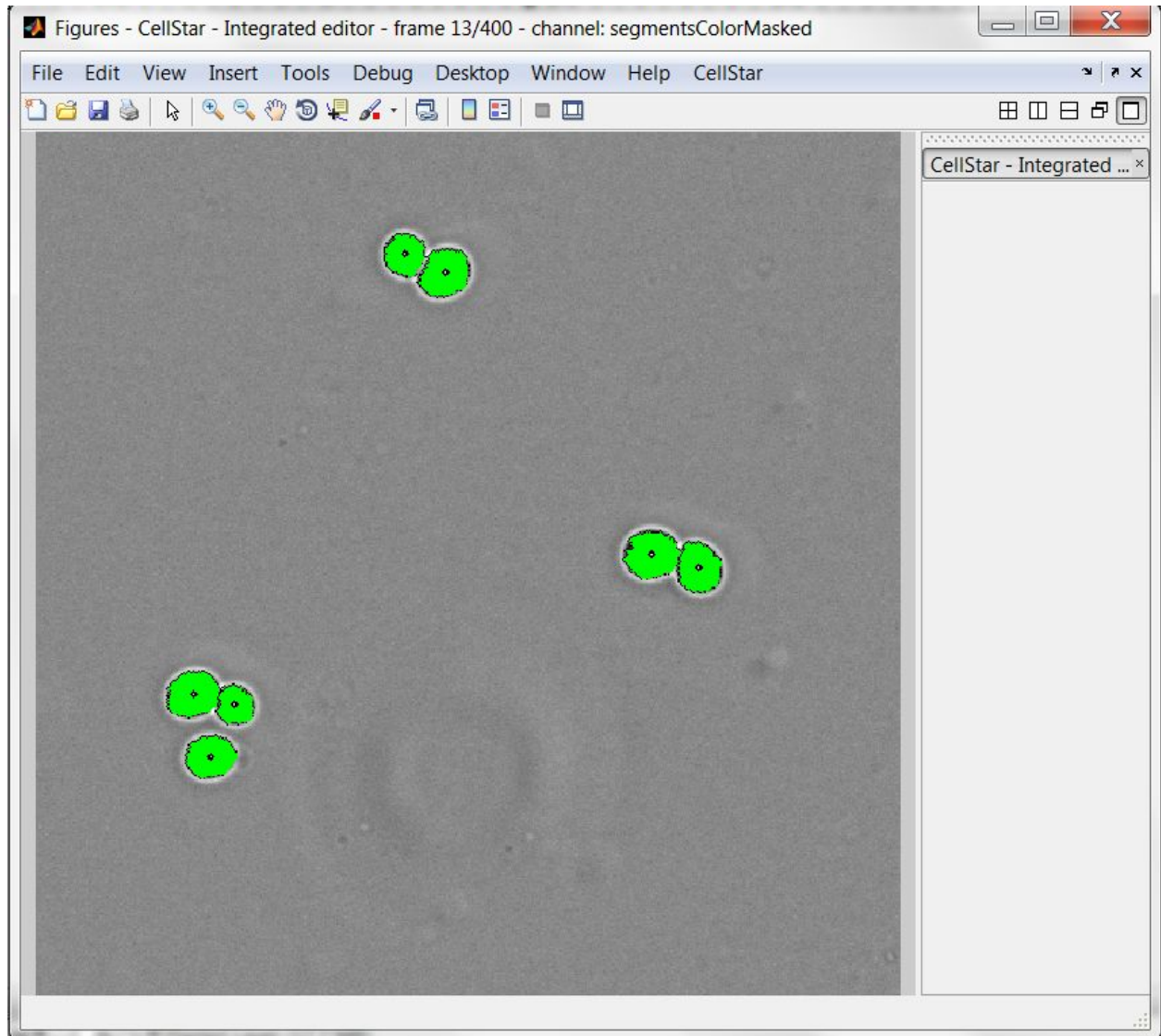
After having applied changes, all cells are green, indicating they are in the segmentation ground truth.

Other segmentation errors (shape)

Similarly for cells that have been segmented but with improper borders, select the cell and **Right Click** to move the border to the correct location. When all segmentation errors for a frame have been corrected, press **'SHIFT' + 'Y'** to apply all changes properly.
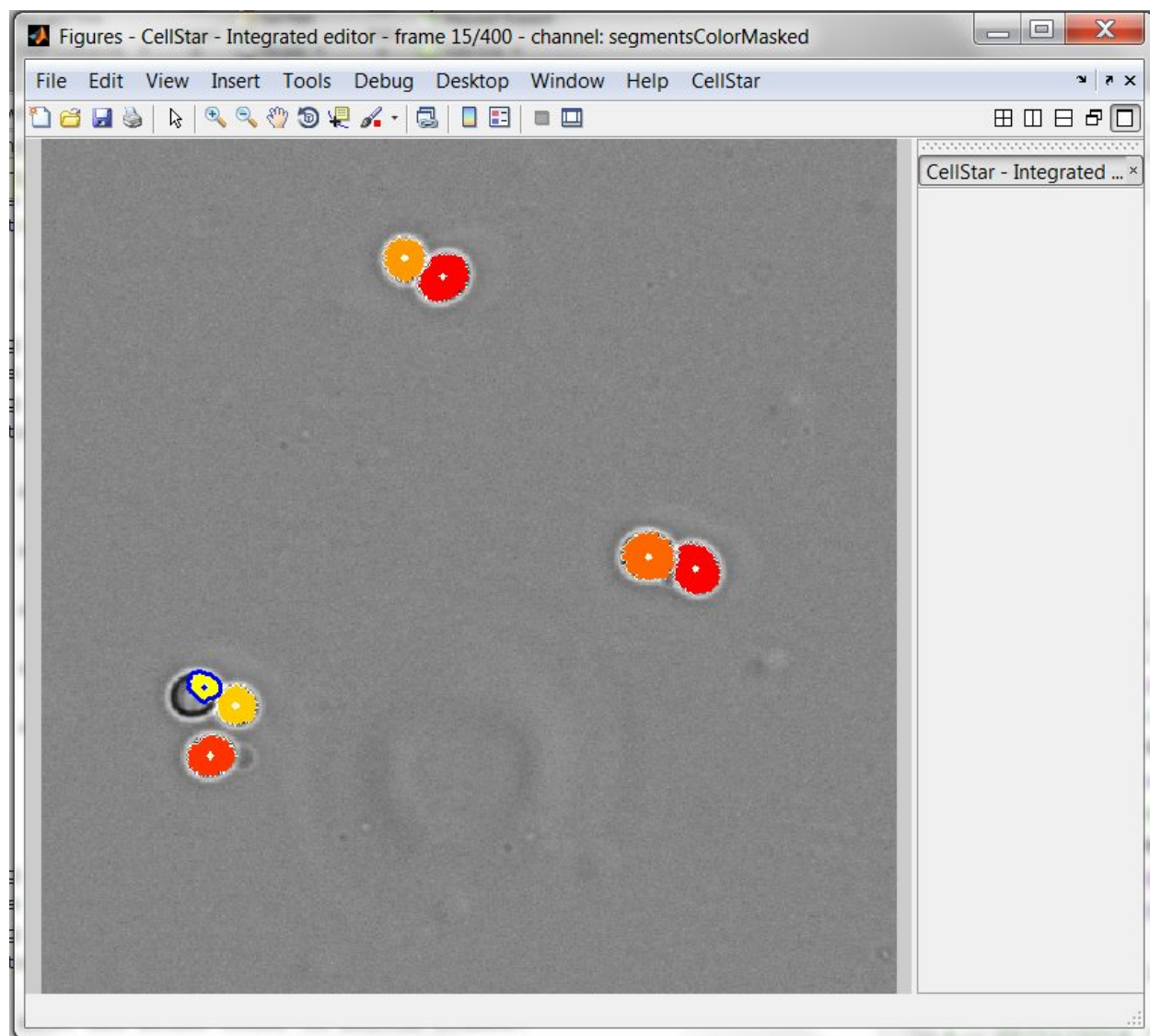
Select the mis-segmented cell
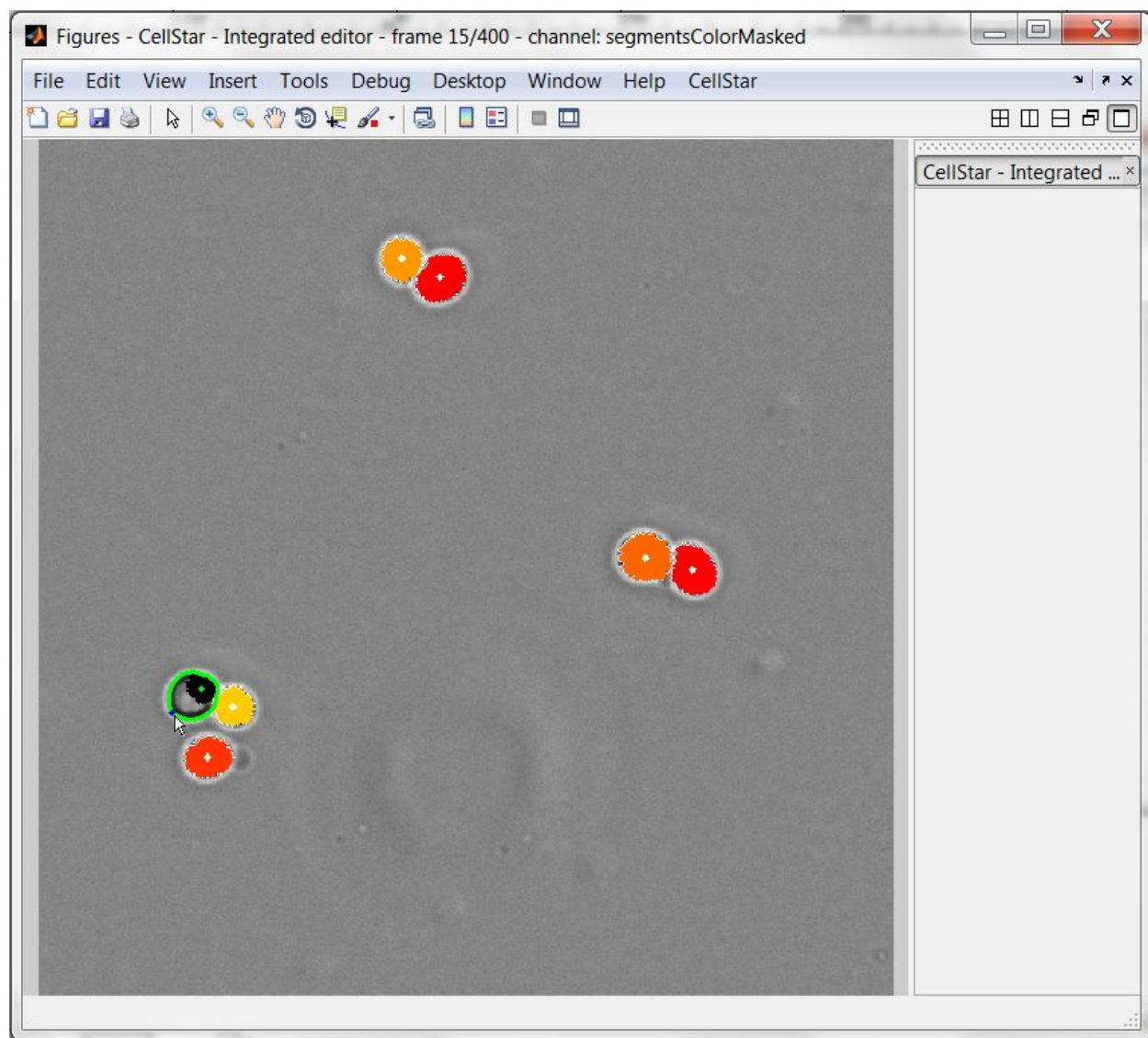
Correct the border adding points with "right-click"

To finish editing cells border, simply "left-click" somewhere else, the border turns red, indicating you need to validate with **'SHIFT' + 'Y'** for it to be taken into account (you can do several modifications before validating).

Tracking errors

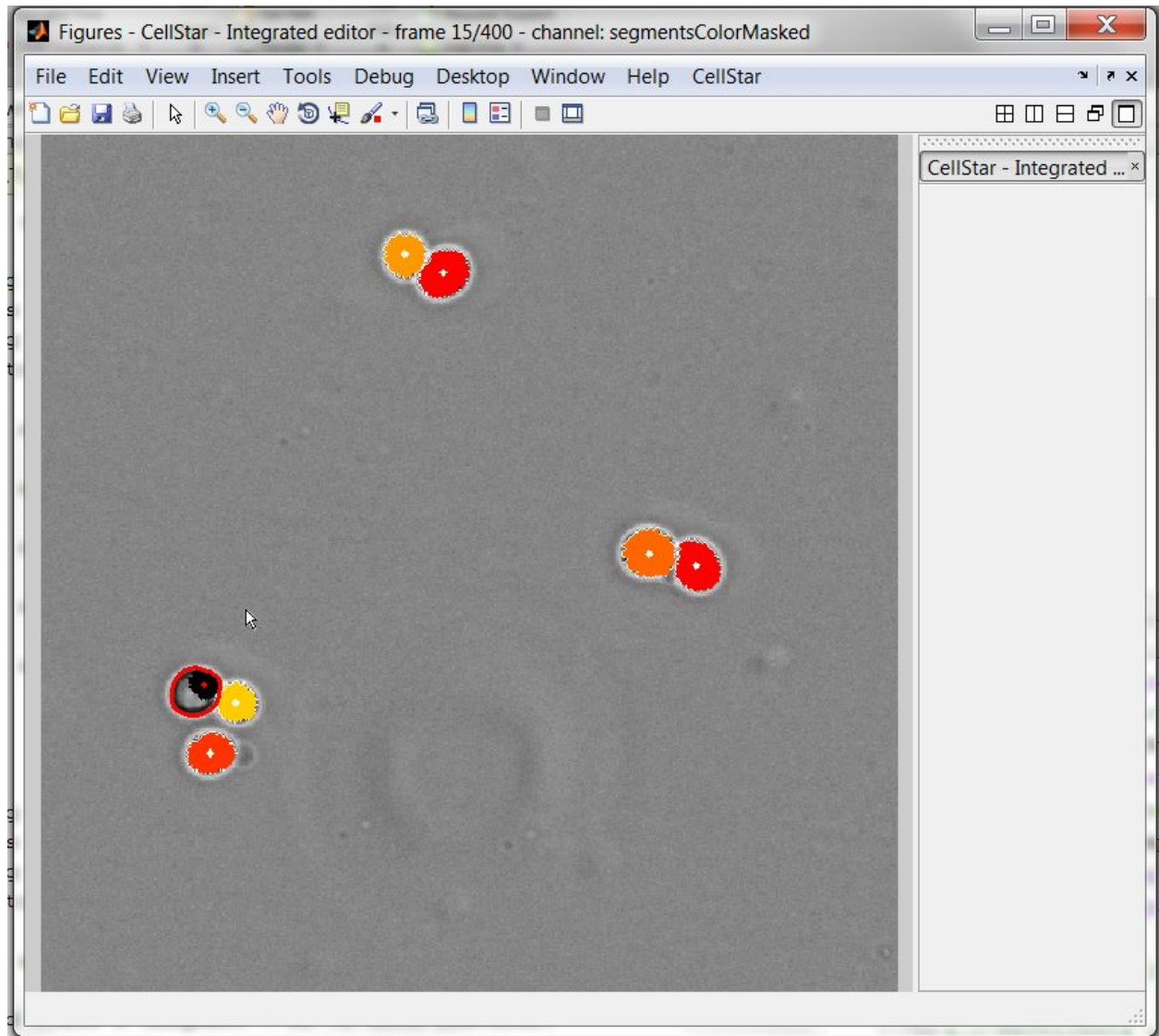If Cellstar fails to properly track a cell, you can manually link two segmented cells by selecting the cell in the first frame and pressing **'-'**. **Left Click** on the cell you want to link to on the next frame. A red line should appear between the location of both cells on each image. You can also link to previous frames by pressing **'SHIFT' + '-'** (CellStar tab -> "Edit tracking ground truth").You can now press **'SHIFT' + 'Y'** to apply all changes properly. See an example below:
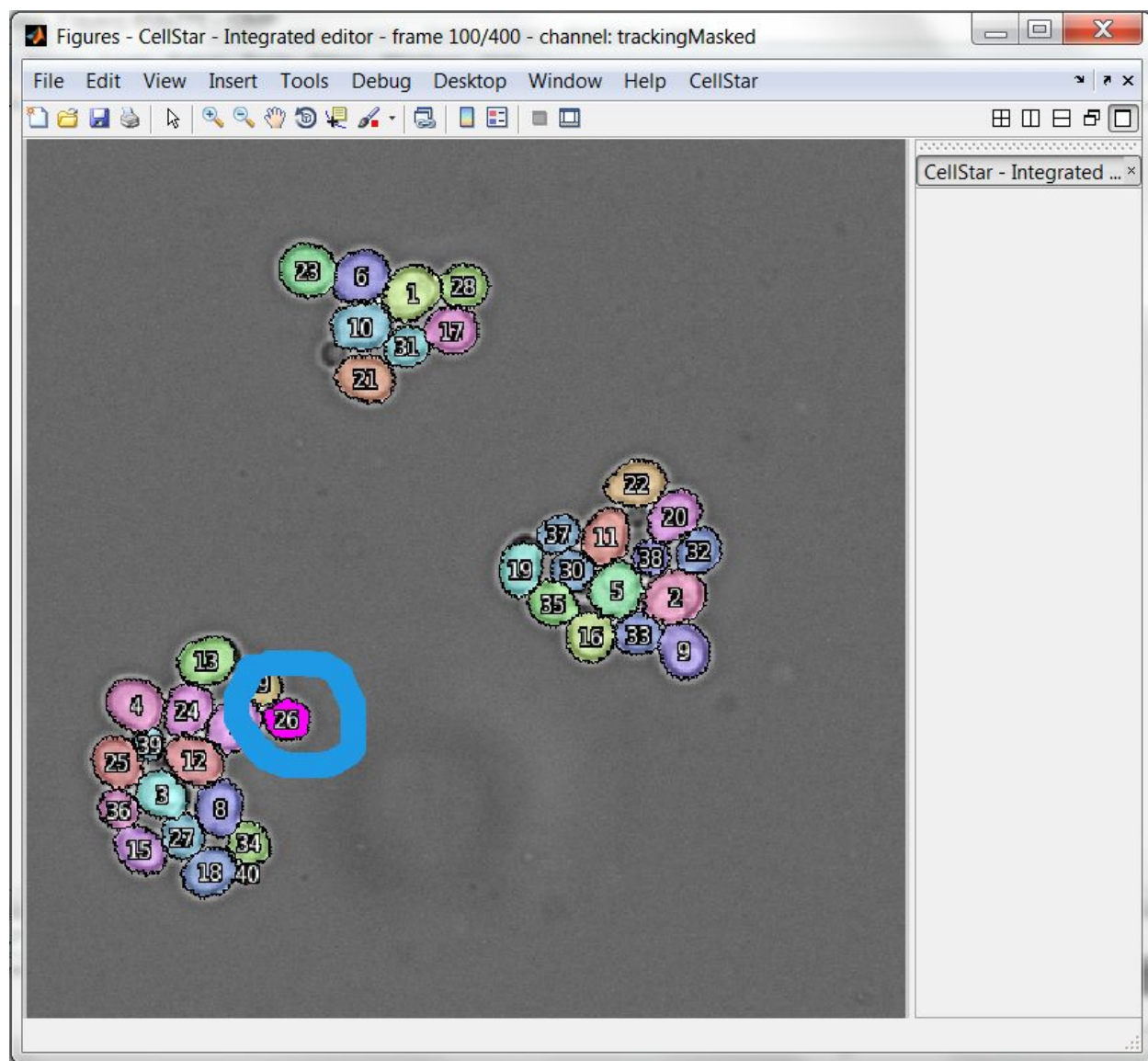
In frame 100 we see cell 26 is pink, indicating it will be lost in the next frame
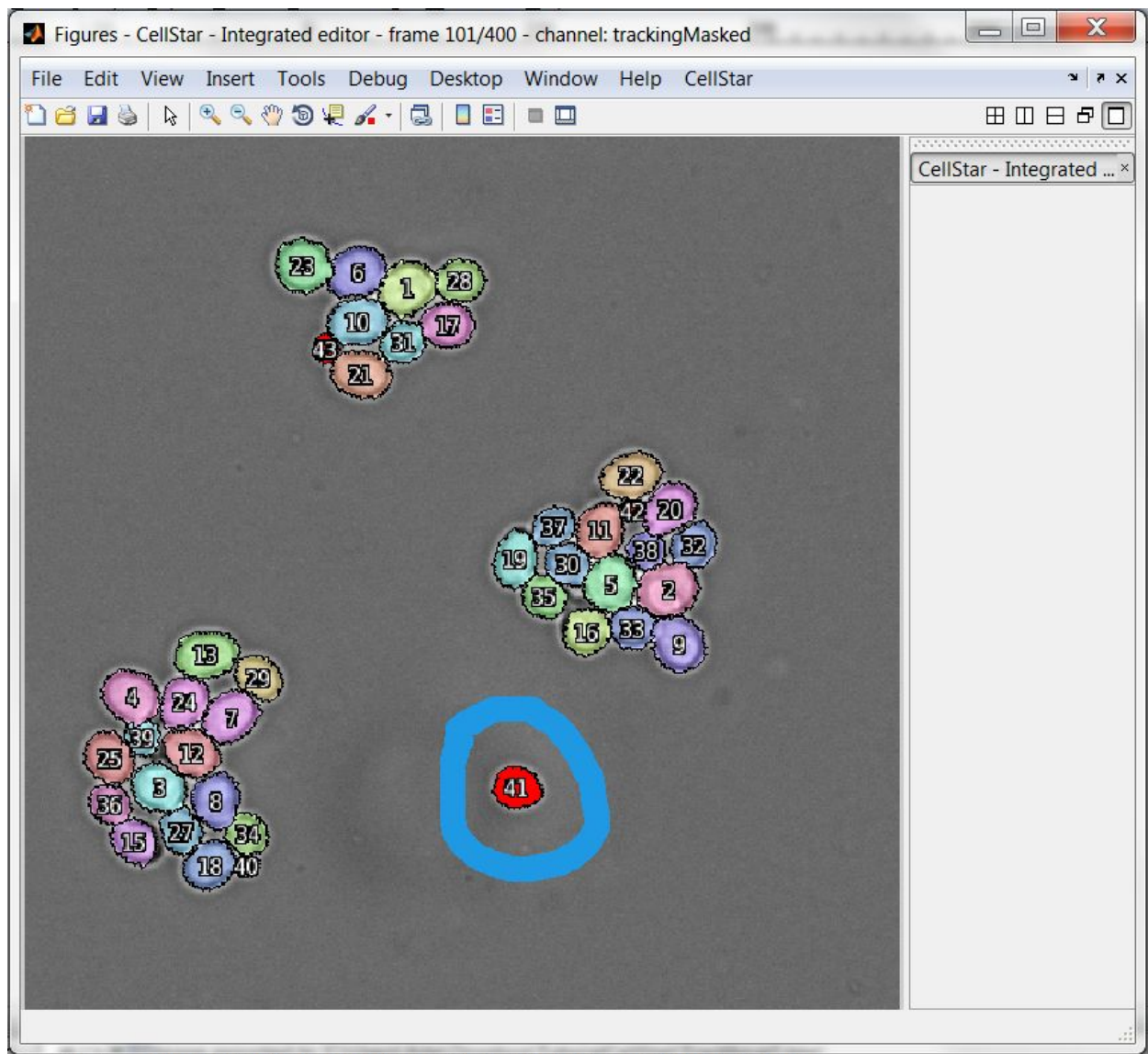
In frame 101, we see the cell detached brutally and therefore was tracked as another cell, number 41 (in red since it appears at this frame)
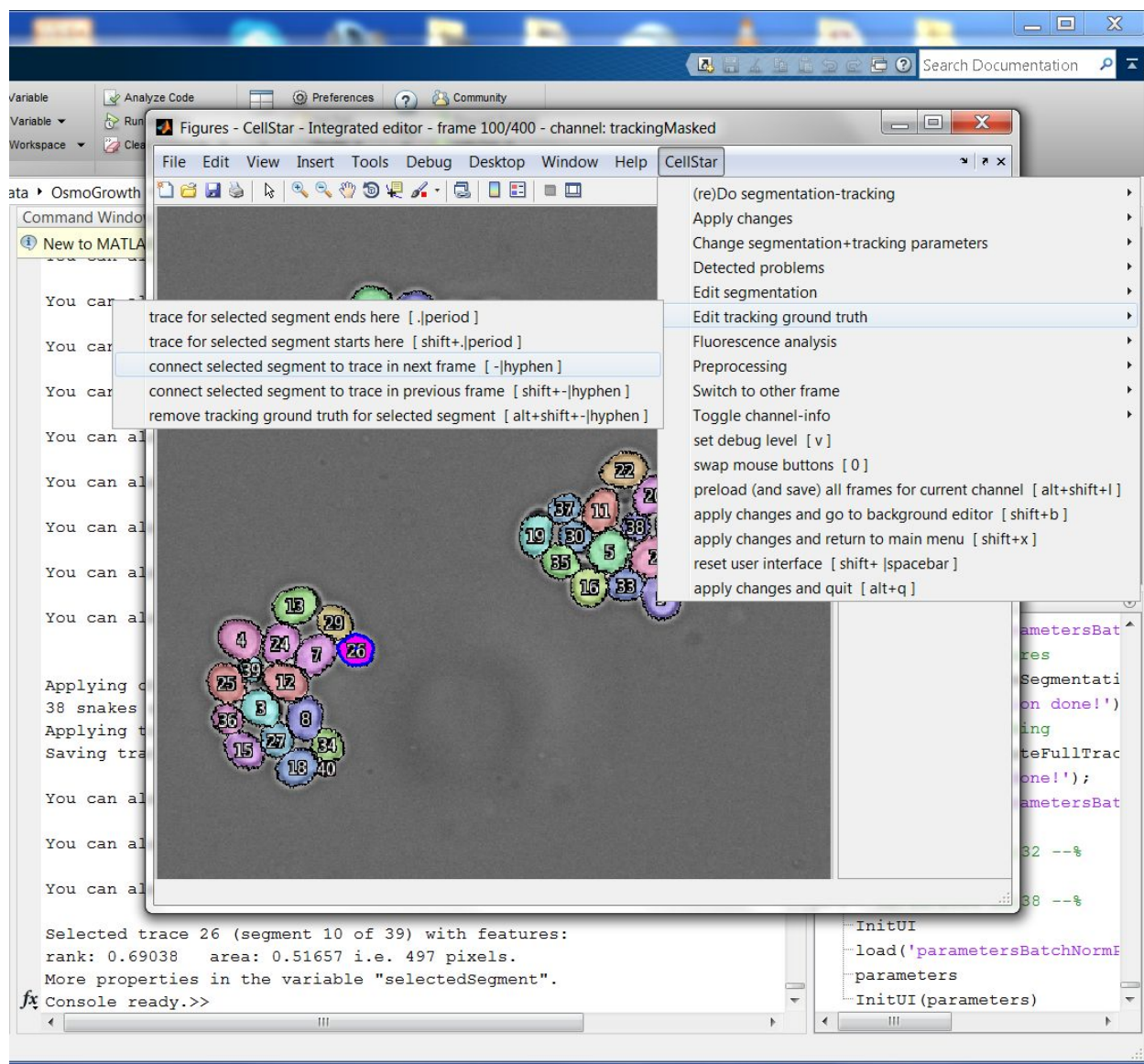
To correct it, on frame 100, select the cell that will be lost and press '**-**' or select the corresponding entry in the menu. This automatically switches to the next frame.
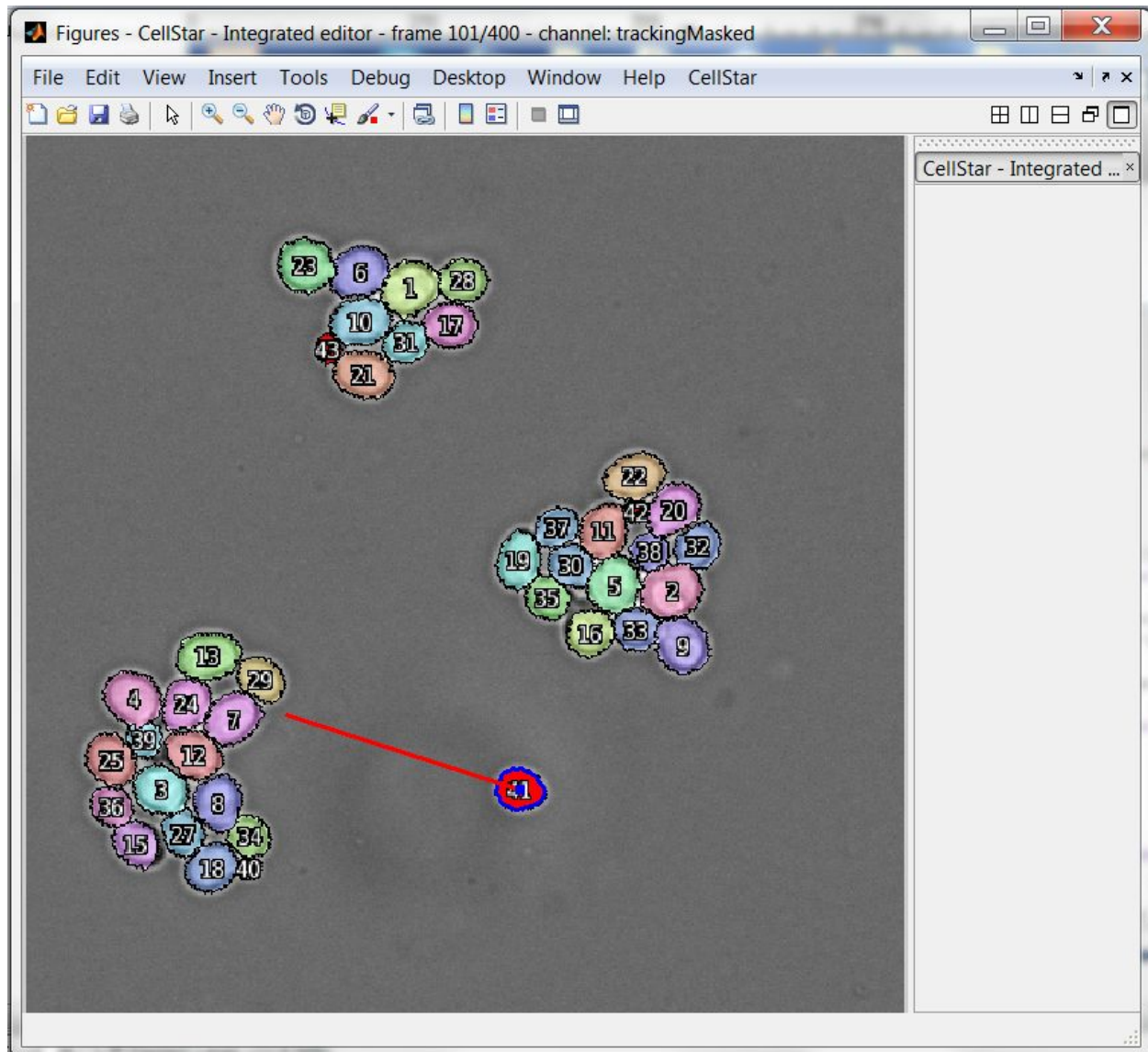
Just select the cell that should be tracked as the cell number 26. A line shows the move from the previous frame. Then simply validate.

Fluorescence data

With an image properly segmented and tracked, the fluorescence data for individual cells can be retrieved by pressing **'CONTROL' + 'F' (**CellStar tab -> "Fluorescence analysis"). This will generate a graph showing the fluorescence level for each cell for all the loaded images. This data can exported as a table in variable form by pressing **'SHIFT' + 'ALT' + 'F'** or exported to CSV by pressing **'CTRL' + 'SHIFT' + 'F'**.

Note that you can also plot the fluorescence data only for a selected cell.

Automatic learning of CellStar parameters

Performance on segmentation and tracking using the default parameters of CellStar may vary with different experimental setups. In order to improve segmentation quality, tuning the parameters to your specific setup is advised. Nevertheless, CellStar relies on many parameters and their manual tuning requires a detailed knowledge of the algorithm. In order to simplify the use of CellStar, an experimental routine for the automatic learning of the most relevant parameters is available.

Parameter learning relies on segmentation ground truth (see section "Ground truth cells" of this tutorial and the entry "Edit segmentation" in CellStar tab), that is cells that are know to be correct. More specifically, segmentation ground truth is composed of two kinds of cells:

- dark green segments, that are the default ground truth segments and are <u>ignored</u> by the parameter learning routines: they represent manual fixes of the segmentation that should not be taken into account because they are not representative of the cells that must be detected by CellStar;
- light green segments, that are the only part of ground truth taken into account for parameter learning: these cells "look like" the cells that CellStar is expected to detect.

It is possible to add cells to ground truth or to switch from dark green to light green ground truth by

- pressing **'8'** (light green = ground truth for parameter learning) or **'9'** (dark green = default ground truth) when a segment is selected;
- **'SHIFT' + '8'** or **'SHIFT' + '9'** to switch all the segments in the current frame,
- **'ALT' + 'SHIFT' + '8'** or **'ALT' + 'SHIFT' + '9'** to switch all the segments in all the frames.

These actions are available under CellStar tab -> "Edit segmentation".

The correct choice of the segments to be added to the ground truth for parameter learning is of fundamental importance for a successful outcome. There is no general recipe, however here are some guidelines from experience:

- it is possible to get good parameters by learning even from few cells (even less than 5), but having at least 10-15 cells is strongly recommended, with 30-40 being a good compromise;
- there is no maximal number of cells to add to ground truth for parameter learning, but the higher the number of cells, the slower the learning procedure although possibly more accurate;
- the cells added to ground truth for parameter learning should "look" as much as possible like those that have to be segmented, in the sense that they should be statistically representative of the population for their dimension, shape, brightness, etc.

Once the cells are chosen, it is possible to start the parameter learning routines that are able to optimize parameters in two successive steps:

- contour parameters: these parameters determine how close the contour of a detected segment is to the real contour of the underlying cell; the "goodness" of these parameters can be easily checked by trying to manually segment a cell in CellStar UI, and check whether the green contour that is created at first by clicking inside the cell matches "sufficiently well" the real contour of the cell; if this is not the case, by pressing **'SHIFT' + 'E'** the learning routine for this subset of parameters will be started;
- ranking parameters: these parameters determine which segment will be chosen when two or more overlapping segments are generated; to check how good these parameters are, one has to segment at least one full image; if the result is not satisfying, ranking parameters can be optimized by pressing **'SHIFT' + 'G'**.

Please note that:
- you may need to run parameter learning (both for contour and ranking) more than once, until you are satisfied with the result;
- contour parameters should be optimized *before* ranking parameters, so you should start working on ranking parameters after being fully satisfied of contour parameters;
- if parameter learning "gets stuck" in some bad parameter set, you may need to reset parameters to their initial values (**'ALT' + 'E'** for contour parameters and **'ALT' + 'G'** for ranking parameters) and start the optimization again;
- several *degrees* of search are available, depending on how long one is willing to wait and how "far" in the parameter space the learning procedure should be allowed to go:
  - quick search, **'E'** for contour parameters and **'G'** for ranking parameters;
  - normal search, as previously described;
  - extensive search, with respective keyboard shortcuts **'ALT' + 'SHIFT' + 'E'** and **'ALT' + 'SHIFT' + 'G'**;

  notice that an extensive search is not always better than a normal (or quick search), because these optimization routines do not work on all segmentation parameters at once, and learning is applied to a little portion of data only;
- at the end of the learning procedure, the best values found are automatically assigned to parameters;
- if you want to interrupt the learning routine (which can last quite long), you can just press **'CTRL' + 'C'**: Matlab may complain about this with an error, but the best parameter set found so far is automatically saved nevertheless and you may continue using CellStar as before.

The above actions for parameter learning are available under CellStar tab -> "Change segmentation+tracking parameters".

Advanced use

If you are an expert Matlab user, you may want deeper access to CellStar data. Actually, all the data used by CellStar is stored in a global variable called **csui**. To make it accessible from command line, you can just type

```
global csui
```

at Matlab console. By browsing this structure you will be able to directly access all the information stored and produced by CellStar.