

An efficient method for counting DAPI-stained cells using Fiji

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Abstract: *Counting cells is a crucial procedure in evaluating the success of a treatment. A fast yet exact way of doing this for DAPI-stained cells with the ImageJ-derivate 'Fiji' is described in this paper, written as a step-by-step tutorial with screenshots.*

Keywords: *cell counting, quantity, DAPI, Fiji, ImageJ, tutorial*

Counting Process

1. After taking the usual pictures and the DAPI¹-stained pictures at the same places (use at least 2 places per well) and putting them into explicitly labelled directories, you are ready to conduct a cell counting.
2. Get **Fiji**², an enhanced and extended version of ImageJ. The program offers several different ways to count the cells in an open picture. One of the most exact ways is to use the Fiji plugin 'Cell counter' (http://pacific.mpi-cbg.de/wiki/index.php/Cell_Counter).
3. Before you start Fiji, download the plugin from the page mentioned above and put the .jar-file into the 'plugins' directory of the Fiji program folder (this directory is full of .jar-files, all of them are plugins).
4. Now you can start Fiji (usually fiji-win32.exe) and open the DAPI-picture where you want to count cells using the 'File'-menu or just dragging your picture to the program window.

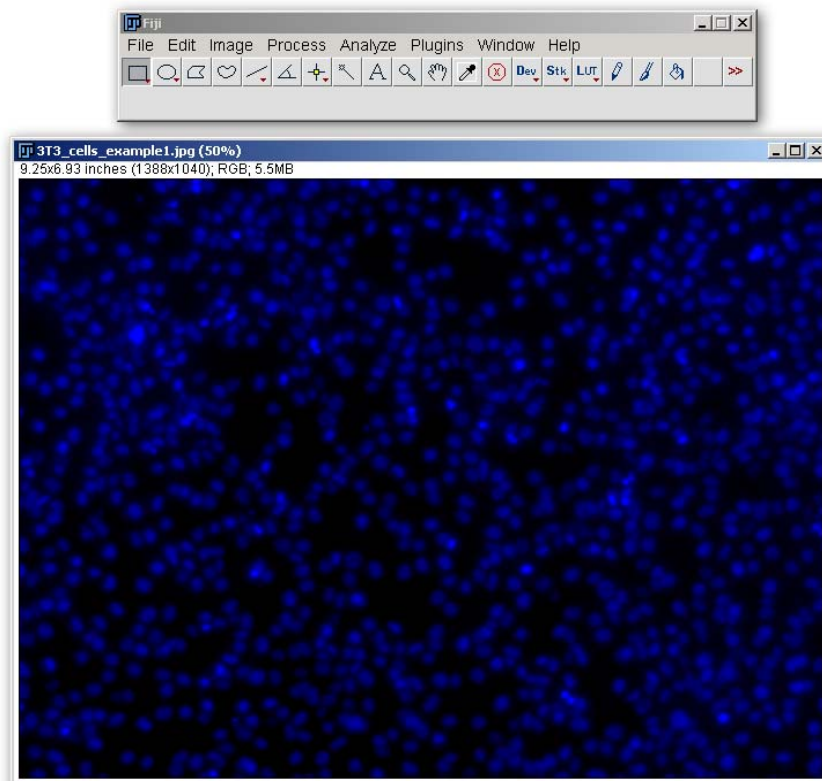


Figure 1: Fiji main window with opened DAPI-picture

¹ DAPI (4',6-Diamidino-2-phenylindole) is a fluorescent stain binding especially on DNA.

² Fiji can be downloaded at <http://pacific.mpi-cbg.de/wiki/index.php>. For this paper, Fiji Heidelberg and Fiji Madison were used.

5. It is better to have the microscopic picture opened alongside the DAPI-picture to recognize **places of accumulated dead cells**. Yellow squares in the figure below mark places with a big amount of dead cells which should not be counted in most cases or counted separately.
During cell counting, however, you work only with your DAPI-picture where you can count the DAPI-stained nuclei of your cells.

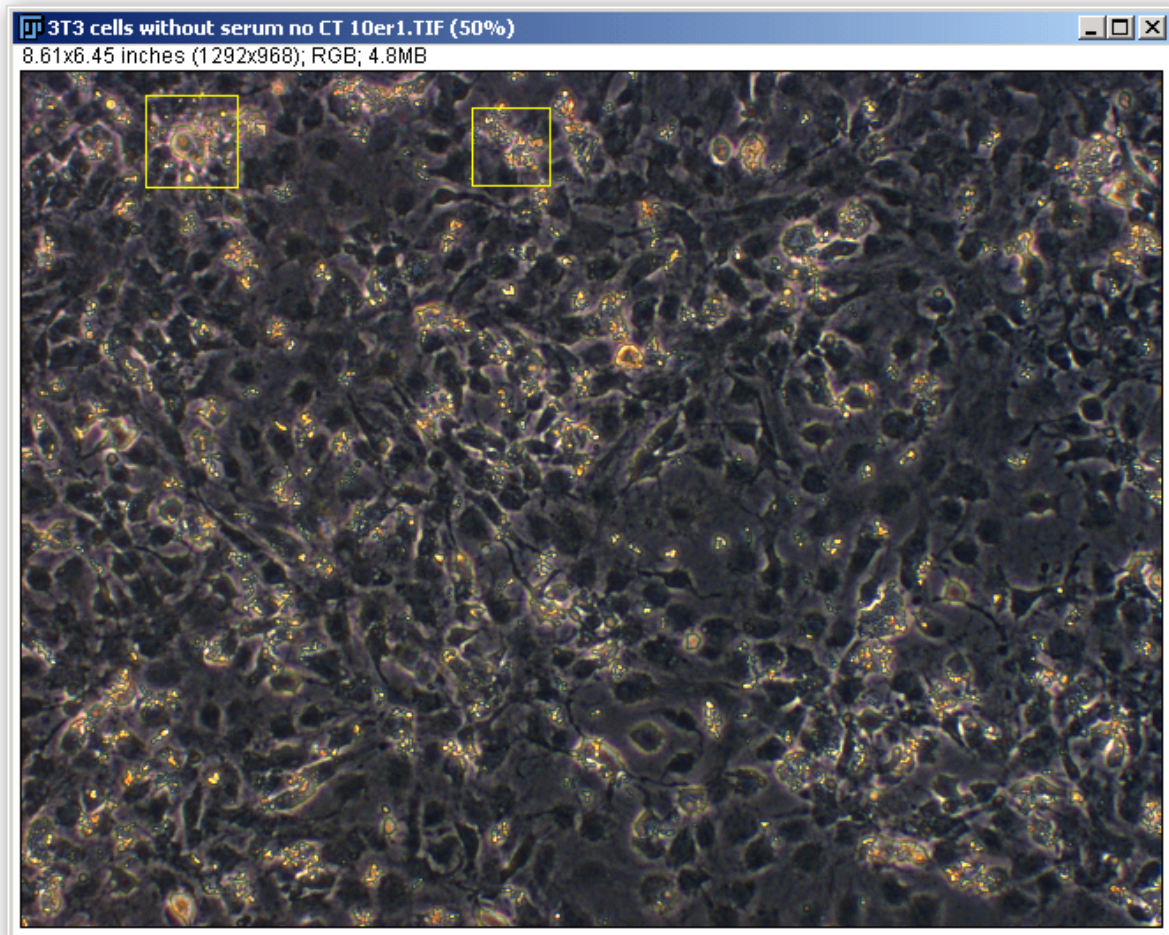


Figure 2: Microscopic picture with yellow squares marking areas of dead cells in high density

6. At first, you need to split the channels of the original DAPI-RGB-image. In the 'Image'-menu, go to the 'Color'-submenu and choose 'Split Channels'.

Image -> Color -> Split Channels

7. This function creates three 8-bit grayscale pictures containing red, green and blue components of the original image. As **DAPI has its emission maximum in the blue area, only the grayscale picture with the blue components will have a signal: gray and white dots showing the nuclei of the cells** (cf. Figure 1). Windows with green and red signals will be empty (black background), hence you can close them.
8. Now you should have a picture like the one in Figure 4 in front of you. This is your workspace.

- If the contrast does not fit, you may adjust the brightness and other values of the picture. In the 'Image'-menu, go to the 'Adjust'-submenu and choose 'Brightness/Contrast'. Play with the slides until the values fit.

Image -> Adjust -> Brightness/Contrast

Figure 3: Brightness and Contrast window

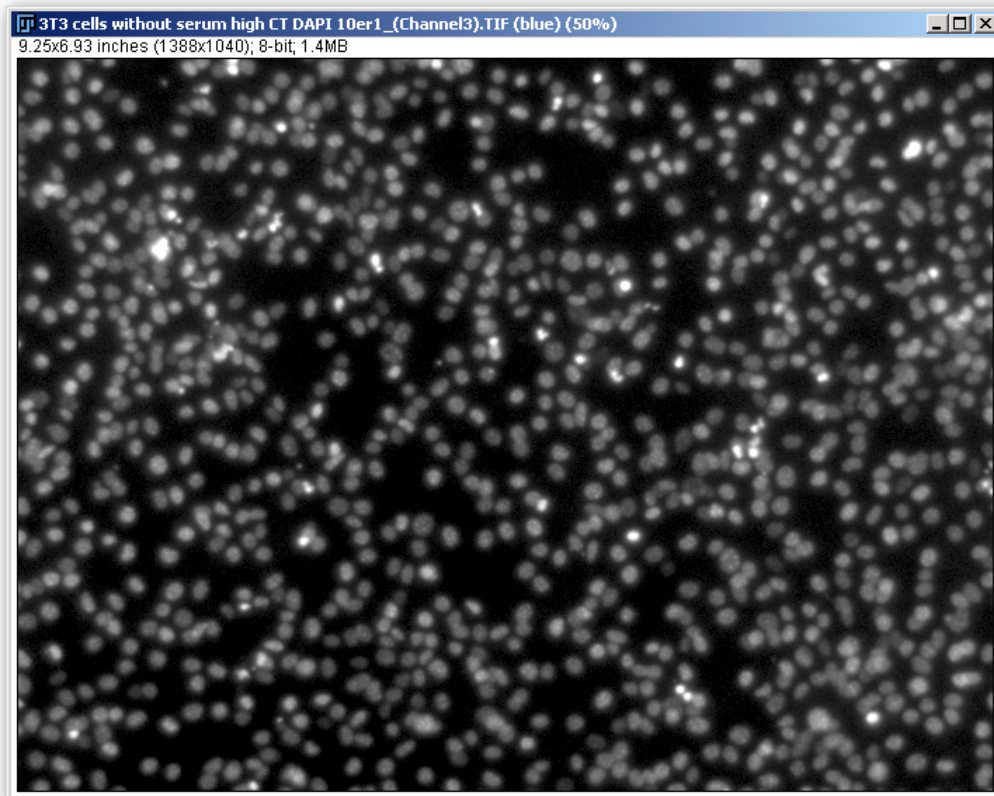
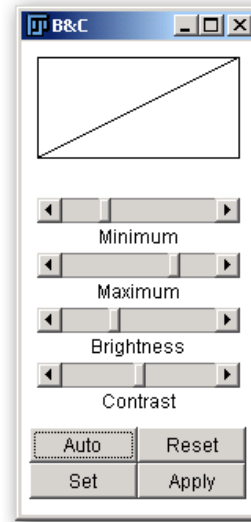
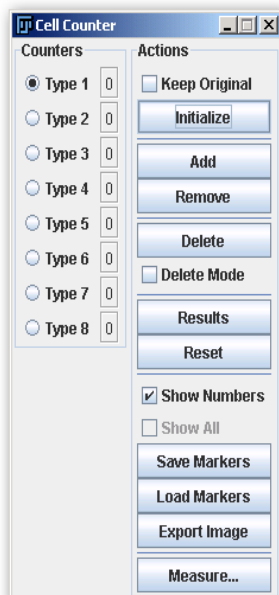


Figure 4: Picture after channel split with only blue signal. This is the workspace for counting the cells.



Now use the 'Cell Counter'-plugin. Go to the 'Plugins'-menu and choose 'Cell Counter'. A window like the one in *Figure 5* emerges. Click on 'Initialize' and choose the 'Type 1' counter.

Plugins -> Cell Counter -> 'Initialize' button -> 'Type 1' control box

Figure 5: Window of the cell counter with all controls. Different types represent different colours. The result is shown right to the respective type radio button.

- Count the cells by clicking on them. As you see there are

eight types of counters. Each of them has a different colour. If the blue colour of the counter type 1 is not optimal for you, chose 'Type 2' or something else. Distinguish between the types or colours, respectively, to count different types of cells (e. g. stuck cells and dead cells).

This is one of the most effective ways to count the cells since it is fast yet exact. Eventually, you can use the described techniques to count also cells not stained with DAPI.

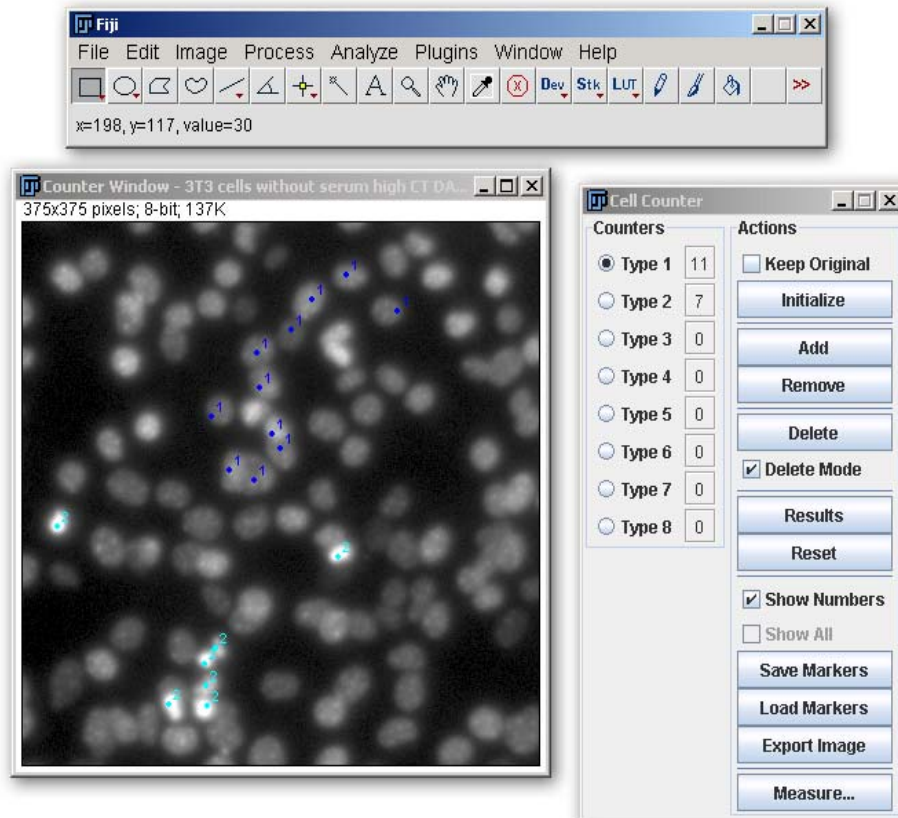


Figure 5: Counting different types of cells

Results

- You may check your results automatically. To do this, just go to

Analyze -> Analyze Particles

Adjust the necessary values. Usually the images have to be thresholded before. Use

Image -> Adjust -> Color Threshold

for this.

- To document your results, use any spreadsheet program you want and log the number of 'cells alive', 'cells dead' and the total number. Now you can calculate the percentages which show a perfect overview of the status of your cells after a certain treatment.

Useful suggestions

- It is very useful to display the number of the colour you are counting (e. g. '1' for type 1 etc.)
- If a mistake occurred, i. e. a miscalculation, use the 'Delete Mode' (*activated in Figure 5*) to revert it. Just click on the cell which was counted by mistake and it is subtracted from the total number.

References

- [1] Ferreira, T., and Rasband, W.: The ImageJ User Guide. February 2011.
(<http://imagej.nih.gov/ij/docs/user-guide.pdf>, 22/02/2011 15:28).

Protocol on how to count (DAPI-stained) cells using Fiji

This is a short laboratory version (protocol). For a more comprehensive overview, see the whole paper.

1. Get **Fiji** (<http://pacific.mpi-cbg.de/wiki/index.php>), an enhanced and extended version of ImageJ, and use the Fiji plugin '**Cell counter**' (http://pacific.mpi-cbg.de/wiki/index.php/Cell_Counter).
2. Before you start Fiji, download the plugin from the page mentioned above and put the .jar-file into the 'plugins' directory of the Fiji program folder (this directory is full of .jar-files, all of them are plugins).
3. Now you can start Fiji (usually fiji-win32.exe) and open the DAPI-picture where you want to count cells using the 'File'-menu or just dragging your picture to the program window.
4. It is better to have the microscopic picture opened alongside the DAPI-picture to recognize **places of accumulated dead cells**.
5. At first, you need to split the channels of the original DAPI-**RGB**-image.

Image -> Color -> Split Channels

6. Three 8-bit grayscale pictures containing red, green and blue components of the original image are created. **As DAPI has its emission maximum in the blue area, only the grayscale picture with the blue components will have a signal.** Windows with green and red signals will be empty (black background), hence you can close them.
7. If the contrast does not fit, you may adjust the brightness and other values of the picture.

Image -> Adjust -> Brightness/Contrast

Play with the slides until the values fit.

8. Now use the 'Cell Counter'-plugin. Go to the 'Plugins'-menu and choose 'Cell Counter'. Click on 'Initialize' and choose the 'Type 1' counter or any other type. Each type has a different colour.

Plugins -> Cell Counter -> 'Initialize' button -> 'Type 1' control box or any other type

9. Count the cells by clicking on them. If the blue colour of the counter type 1 is not optimal for you, chose 'Type 2' or something else. Distinguish between the types or colours, respectively, to count different types of cells (e. g. stuck cells and dead cells). **This is one of the most effective ways to count the cells since it is fast yet exact. Eventually, you can use the described techniques to count also cells not stained with DAPI.**

Useful suggestions

- It is very useful to display the number of the colour you are counting (e. g. '1' for type 1 etc.)
- If a mistake occurred, i. e. a miscalculation, use the 'Delete Mode' to revert it. Just click on the cell which was counted by mistake and it is subtracted from the total number.