

## Count-On-It Instructions



Count-on-it is a colony counting suite that is made for quantification of colony-forming units (CFUs) which are spot plated from a 96-well plate in a 8x12 grid onto a rectangular agar plate. It is the first automated CFU counting software which segments the object into a uniform grid and batches the results into a simple spreadsheet, setting the standard for future development of high-throughput CFU counting systems. It is in the form of an imageJ plug-in making it broadly compatible and free to all users. The macros are written in the IJ1 Macro scripting language which is easily modified using the built-in editor in FIJI.

The software suite consists of three Plug-ins; *Count-On-It Gridiron* will batch process a folder of JPEG images, divide each image with a uniform grid, count the colonies in each cell, and output the results into a simple spreadsheet. *Croptacular* is a specialized cropping tool which allows the user to fine-tune the placement and boundaries of the counting grid for each image. *Count-On-It Circus* is included for convenience, this plug-in will batch process the photos of round plates, count the colonies, and output a .csv file.

Download the latest version of FIJI: <https://imagej.net/software/fiji/downloads>

Download the Count-On-It files (Included with supplementary materials)

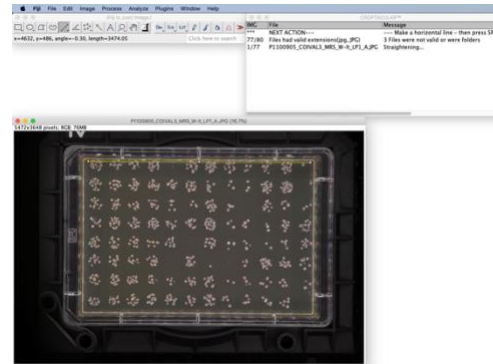
- 1) Install the Count-On-It plug ins to FIJI; Navigate to Applications>FIJI then right-click on the application icon>Show package contents, open the plugins folder (which contains the files Circus\_.ijm, Croptacular\_.ijm, and Gridiron\_.ijm). Drag and drop the Count-On-It folder into the plugins folder. Restart FIJI.
- 2) Organize the images you wish to quantify into a folder. The filenames will become column titles for each set of counts, so name them so you can distinguish the plates. Within this folder make a subfolder named "cropped" and another named "receipts".

- 3) First launch *Croptacular*  
The default settings are usually sufficient, but depending on the resolution of your image, the lighting, the spot size, etcetera, you may find it helpful to adjust the base parameters. Click OK to start cropping. The grid dimensions can be customized. The default filename is the original filename\_crop.jpg. You can change that. Start

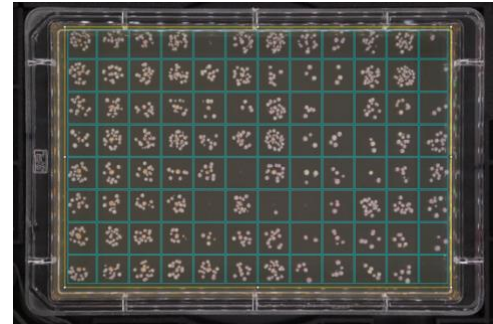


batching a new folder with the “Batch process a folder” option selected. Choose “Start from current image” if the plug-in crashes.

- 4) If the image is already straight, simply press space. Otherwise, straighten the image by drawing a line along an edge that should be horizontal. You may redraw the line as many times as you want if the image still doesn't appear straightened. If you draw the line from right to left it will rotate the image 180°. If you fail to draw a line at all the plug in may crash.  
**Relaunch and choose “start from current image option”**. Press space when you are done.

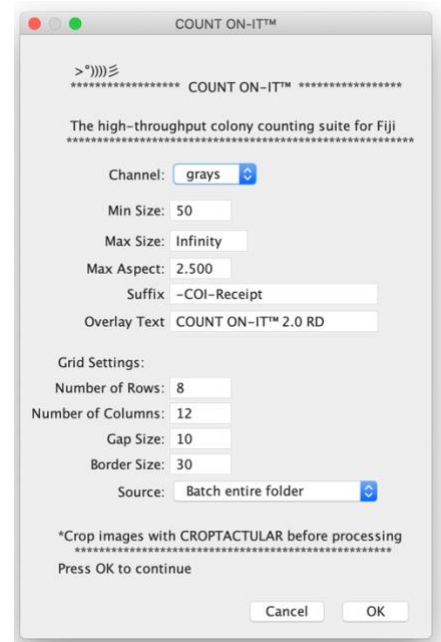


- 5) Next draw a boundary box of the area you wish to count. The first image in a batch, the horizontal line will not disappear. That's ok. draw a rectangle around the agar surface. You can adjust the size and proportion until all the spots are within their cells. You must drag the cursor outside the boundary box to refresh the grid. When the grid looks good, press space.

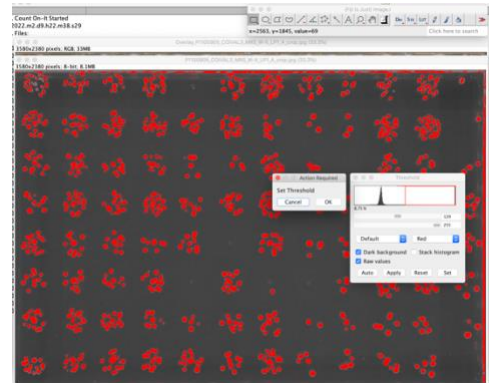


- 6) The next image will automatically rotate to the same angle as the first one, the plugin assumes all the photos are aligned the same. If this is accurate, press space to continue. Otherwise, you can straighten the image as before. The grid also recalls the same position as the previous image, adjust if necessary then press space.

- 7) Once you are done cropping images, launch Count-On-It>*Gridiron*. These default settings are usually good. Depending on the image, it may improve thresholding to use only one of the RGB channels. When using the green filter/blue light settings, it is advantageous to select the Channel:Green. The Min Size and Max Size can be useful for selecting specific colonies, these units are in pixels. The max aspect controls the aspect ratio of colonies. It may be useful to adjust this parameter if your colonies are more or less uniformly round than the 2.5 default aspect ratio. The filename can be appended with Suffix and the overlay text can also be customized. The grid settings should usually be the exact same as you used with *Croptacular*. You can batch an entire folder, a single image, or start from current image.



- 8) The first step in counting is to set the threshold. This is based on a upper and lower pixel intensity value. Make the threshold as stringent as possible while still selecting all the colonies. Ideally there will be some space between selected colonies, but the software is capable of segmenting the blobs to a degree. Click OK on the action required box when the threshold is satisfactory.



- 9) To inspect the results, you can zoom in and look more closely at the colony counts. Cancel will abort the plugin. Click OK to continue.

- 10) On the first image, you will be given the option to proceed or return to the setup menu, for example, if you want to change the minimum colony size. Or you may proceed with the batch process. Click OK, then select a folder to keep the receipts and results table. Use the “receipts” folder or create a new folder.



- 11) After the first image, the following images will default to the same threshold as the previous settings. Click OK to use these settings or adjust the settings. If the photos are consistent and the setting is accurate, you only need to click OK on the “Action Required” dialogue box.



- 12) When you have completed all the images in the batch, the results table will save automatically in the same folder as your receipts. If the software crashes or you need to quit, save the results table with a unique name like “Summary-1A” because when you relaunch and start from current image, it will automatically save the results table in the destination folder and overwrite the table from your first try.

COUNT ON-IT™ Summary Cr			
Position	P1100905_COI\	P1100906_C\	P1100907_COI\A3_M
A1	26	8	3
A2	12	12	2
A3	10	12	3
A4	28	8	4
A5	1	5	2
A6	18	9	2
A7	34	9	6
A8	17	3	2
A9	5	8	0
A10	17	9	3
A11	24	9	0
A12	1	9	2
B1	22	11	3
B2	14	9	2
B3	16	14	4
B4	26	5	3

# Count-On-It Algorithm

