Cell Counter Script

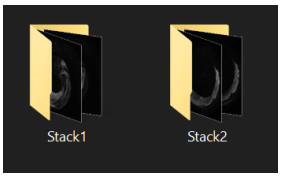
What Is This?

Due to the variety of images you might encounter, this cell counter uses multiple methods to produce different counts. You will have to use your intuition and knowledge of how the count is produced to determine which count is most accurate to your images depending on the nature of the images. I've included some recommendations for this below.

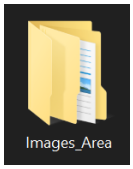
Prerequisites

- This script is designed to count the number of white objects in an image (cells). The script will generate an .xls (Excel) file with the generated areas.
- Other colours of stains will interfere with the script, as it will likely interpret the colours as darker (black) and will not include them in the calculated area.
    - I recommend only using black and white images, or something where the stain is significantly brighter than the background.
- Images for processing must be contained in their own separate subfolders (any images within the same folder will be considered as part of the same stack and will treated as a single image), which should be combined into one folder.

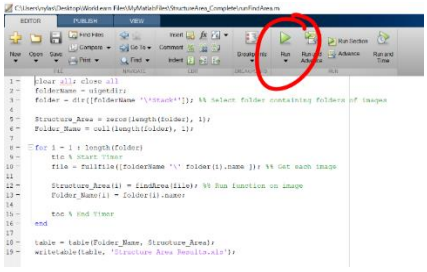
How to Run



- Collect the images as .tif files.
- If the images come from a Z stack, combine all these images into their own folder.
    - It is important that when you name these folders, you must use the word "Stack" somewhere in the name. This is because the script uses this specific word to flag folders to process. Without this word (or altering the script), the script will not detect the folders.
    - It does not matter what you name the folder containing all the sub folders.



- Put all these subfolders of images into one single folder.
- Open the runCellCounter script. Run the script.
- The script will prompt you to select a folder. You must select the folder that contains the series of subfolders of images.
- The script will generate an excel file with the results into the current directory. This should appear in the left column of files in the main MATLAB window, called "Cell Counter Results".
    - If you plan on running this script multiple times, keep in mind that you will need to move or rename the results file or else you may overwrite the data.



If You Encounter Errors

- Check that your images are in the appropriate folders. If you are trying to work with one folder only containing images, try putting those images into subfolders within that same folder.
- Check that your subfolders are named appropriately. They MUST contain the word "Stack" for the script to flag them as valid folders.

    - ALL files containing the word “Stack” will be flagged. If other files contain this word, the cell counter will try to use them, and this will cause errors.
- Check that your images are .tiff files. The script uses this file extension to flag the images for analysis. Any other type of image will not work.

What Does Each Count Mean (Which One Should I Use)?

The Cell Counter will give you 4 different numbers in a table – Mean Count, Mean Count 2D, Count 2D, and Count 3D. It is important to understand how each number is produced to find the best option for your images.

Count	**How it Works**	**Recommended Images**
Count 2D	The image is preprocessed using an equalized histogram and threshold to produce binary images of just the cells. The number of cells on each plane is counted and totalled.	Images where cells are only present on a single plane benefit best from this count. If the cells overlap planes, I would depend more on the Count 3D, as this count will not distinguish cells present on multiple planes and will double (or triple) count them.
Count 3D	Each preprocessed image is combined into a single 3D image. A distance transform and watershed are used to break up objects with significantly different centers. Finally, the number of volumes present in the 3D image are counted.	This count is only reliable if you have a significant number of planes to work with (each cell should show on at least 2-3 of the planes). Think of the 3D image like stacking slices of swiss cheese – if the holes line up too much, its hard to distinguish if they came from the same bubble. The cell counter works very similarly; if you cannot form a “volume” out of the stacks, it will struggle to interpret it as an object. As many stacks as possible is recommended, I would be wary if you have less than 20-30 (depending on how many cells you have to count).
Mean Count 2D	This is the average number of cells found on each plane. It is NOT an accumulative count of all the cells.	This is not an accumulative count. Could be interesting to compare same-plane cell densities, but if you have images in which no cells are present, this number is easily skewed.
Mean Count	This is the average between Count 2D and Count 3D.	I would avoid using this count unless you do not have many stacks (<30), but your cells tend to overlap. It is not as reliable as the other cell counts, due to the slightly arbitrary nature of the product.