

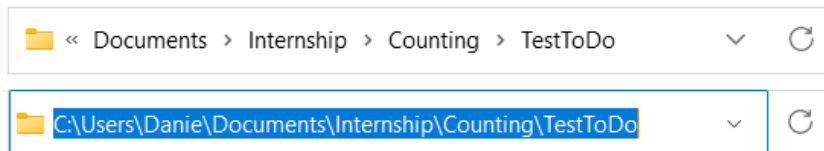
ImageJ set_environment protocol

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This script automatically configures an environment for analysing images. It tracks which images have already been analysed, so it will always open a new one for you. Press a hotkey once to start analysing, press it again to save your data and get a new image. The environment will consist of an image, Multipoint Tool, Results and ROI-manager. The Multipoint results, ROI results and image changes (ROI and Multipoints) are saved. By following these steps, you will learn how to use this script and install it.

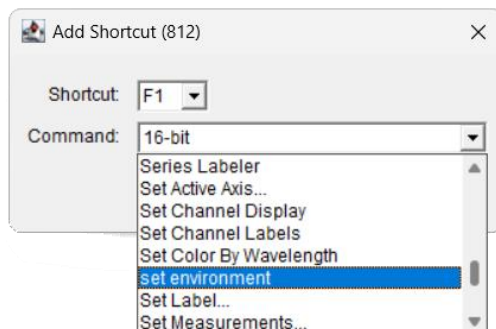
1. Download set_environment.ijm and place it in the “fiji-win64 > Fiji.app > plugins” folder.
2. Open ImageJ.
3. Drag the script (“set_environment.ijm”) from the folder to the ImageJ window.
4. **Personalize** the script.

- a. First, go to your file explorer and make three folders.
 - i. Folder with all pictures that should be analysed.
 - ii. Folder for saving the analysed images.
 - iii. Folder for saving the Multipoint data.
 - iv. Folder for saving the ROI data.
- b. Copy the file path by clicking on the address bar in the file explorer:



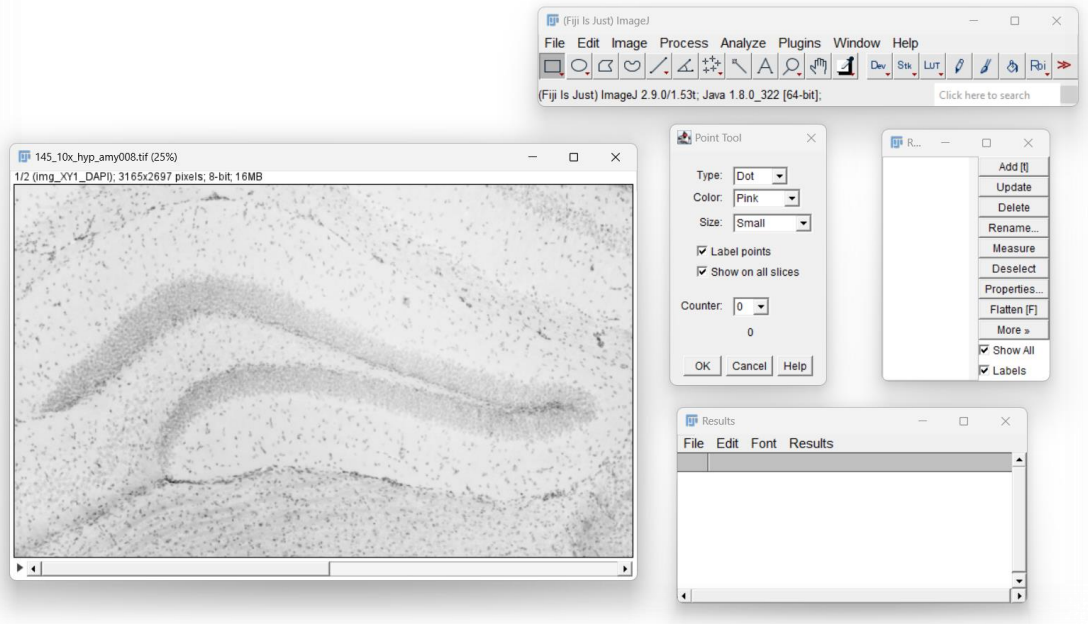
- c. Paste this copied path in the code in between quotes, make sure to use slashes “/” instead of backslashes “\” and to put a slash at the end:


```
16 | do_folder = "C:/Users/Danie/Documents/Internship/Counting/TestToDo/"
```
 - d. Do the same for “done_folder”, “measurements_folder” and “ROI_folder”.
 - e. Save the script (Ctrl + S) and restart ImageJ.
 - f. If you want to better understand how the code works, you could read everything behind the slashes “//”, since this explains what the code does.
5. Install a **hotkey**.
 - a. In ImageJ, click Plugins → Shortcuts → Add Shortcut...
 - b. For Shortcut choose a hotkey that you would like to use, for example F1 (used by pressing Fn + F1).
 - c. Select the Command, this should be “set environment”.



- d. Press “OK” and restart ImageJ.

6. Set the environment by using the script.
 - a. Open ImageJ and press the installed hotkey, for example F1.
 - b. The following tabs should appear.



- c. Draw the ROI with your preferred tool and use the Multipoint tool  to count the cells with different counters.
7. **Save** the results.
 - a. Press the installed hotkey, for example F1.
 - i. The script will automatically save the image with the ROI and the points.
 - ii. It will also measure the points that were made with the point tool and save them in a separate file (.csv) inside the “measurements_folder”.
 - iii. Then the ROI data will be saved as well, including: “Area”, “Mean”, “Min” and “Max” in the “ROI_folder”.
 - iv. After saving the results, the script will automatically open the next file from the “do_folder”, that is not already in the “done_folder”.
8. **Troubleshooting:**
 - a. If the message “There are no images open” appears, it is possible that you closed all images but did not use the hotkey for this. In this case it is best to restart ImageJ.
 - i. Another possibility is that you may be pressing the wrong keys, for example Fn + 1 instead of Fn + F1.
 - b. Sometimes the Point Tool will not reset. This is not due to this script, but due to the ImageJ program itself. If this happens, press “OK” in the Point Tool and open the Point Tool again.