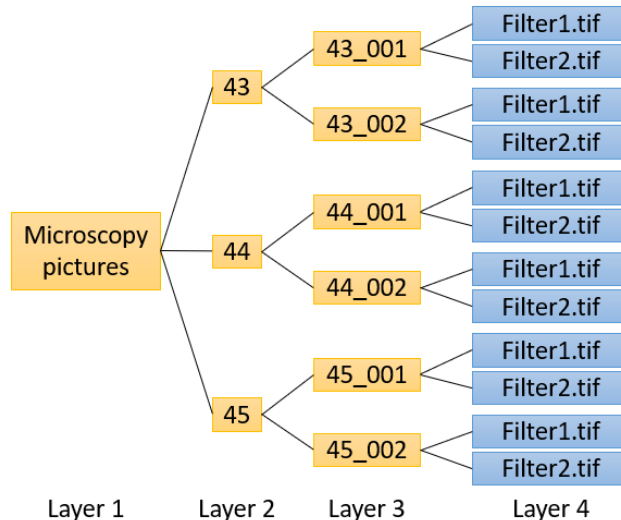


ImageJ invert_images protocol

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This script (invert_images.ijm) stacks images, reverts image colours and saves them.

1. First, make sure that the to be stacked and inverted pictures are inside the same folder, but without other pairs that must be stacked, for example:



- a. Layer 2: folders named after animal numbers.
 - b. Layer 3: folders named after the brain slices.
 - c. Layer 4: files, one brain slice may be captured with multiple microscope filters (for example DAPI or TxRED), resulting in multiple pictures for one slice.
 - i. Maybe another not-needed filter was applied as well, for example Cy5 and maybe the pictures are in RGB and in black and white. The unnecessary images inside the slice folders will be filtered in the next step.
2. **Filter** unnecessary images (for Windows computers).
 - a. Open the windows PowerShell inside the folder that resembles Layer 1. Do this by going to your file explorer, right-click on the Layer 1 folder > Open in Terminal.
 - b. If you only want pictures with TxRED or DAPI in the name, remove everything else by running this code (copy, paste and press Enter):

```
(Get-ChildItem -File -Recurse) | where-Object {$_.Name - NotMatch "TxRED|DAPI"} | Remove-Item
```
 - c. If you only want black and white pictures, remove everything that has RGB in the name, by running this code (copy, paste and press Enter): :

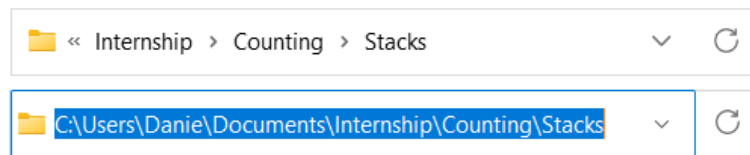
```
(Get-ChildItem -File -Recurse) | where-Object {$_.Name - Match "RGB"} | Remove-Item
```
 - d. Your PowerShell will look similar afterwards:

```
Windows PowerShell
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Install the latest PowerShell for new features and improvements! https://aka.ms/PSWindows

PS C:\Users\Danie\Documents\Internship\DG_blackwhite_nonfiltered> (Get-ChildItem -File -Recurse) | Where-Object {$_.Name -NotMatch "TxRED|DAPI"} | Remove-Item
PS C:\Users\Danie\Documents\Internship\DG_blackwhite_nonfiltered> (Get-ChildItem -File -Recurse) | Where-Object {$_.Name -Match "RGB"} | Remove-Item
PS C:\Users\Danie\Documents\Internship\DG_blackwhite_nonfiltered> |
```

3. Download invert_images.ijm and place it in the “fiji-win64 > Fiji.app > plugins” folder.
4. Open ImageJ.
5. Drag the script (“invert_images.ijm”) from the folder to the ImageJ window.
6. **Personalize** the script.
 - a. Before proceeding to this step, check whether you are left with the images you want to have.
 - b. Go to your file explorer and make one new folder where all stacks will be saved.
 - c. Copy the file path by clicking on the address bar in the file explorer, for example:



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

```
13 | stacks_savingfolder = "C:/Users/Danie/Documents/Internship/Counting/Stacks/"
```
 - e. Copy the path of the folder resembling Layer 1 as well and follow steps c. and d. for this folder as well. Paste this folder behind “mainfolder”.

```
12 | mainfolder = "C:/Users/Danie/Documents/Internship/DG_blackwhite_nonfiltered/"
```
7. Press “Run” in the script window.
 - a. The images will now be reversed, stacked, and placed in your “stacks_savingfolder”. The images will be named after the names of the folders that resemble Layer 3.
8. **Troubleshooting:**
 - a. If an error pops up, check whether there are three layers of folders and one last layer with files as portrayed above.