Removing Uncorrected Background Images and Montage Images Using Fiji

Download the open source software Fiji from http://fiji.sc/wiki/index.php/Fiji It's essentially the same as ImageJ, but comes with many pre-installed plugins and can update itself automatically.

Introduction

When the image capture program Velocity corrects uneven background illumination, it (irritatingly) save both corrected and uncorrected versions. Fiji can be used to remove only the uncorrected files, leaving the corrected images for montaging.

Removing Uncorrected Images

Drag the folder of tiff files created in Velocity to the main menu of Fiji. You will be prompted if these are an image series, click yes at defaults and the images will open as a stack of images. Using the < and > keys, scroll through the series and conform the images alternate between uncorrected and corrected forms. Usually, image1,3,7, etc. are uncorrected.

To remove them, go to Image / Stacks /Tools / Slice Remover. Specify the first (1) and last number of the stack as appropriate. Choose increment as 2. Press 'Okay' and all the odd numbered files are removed.
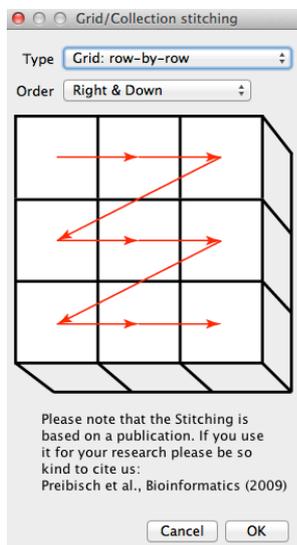
Saving Corrected Images

To be usable for montaging, the images need to be saved as individual files, not a single stack file of images. To do this, go to the Save / Save As / Image Sequence menu and save into a new folder. Have no other files in this folder as the program will try and incorporate them into your montages, with what can only be described as 'sub-optimal' results.

Proceed to the next page to complete the montaging process.

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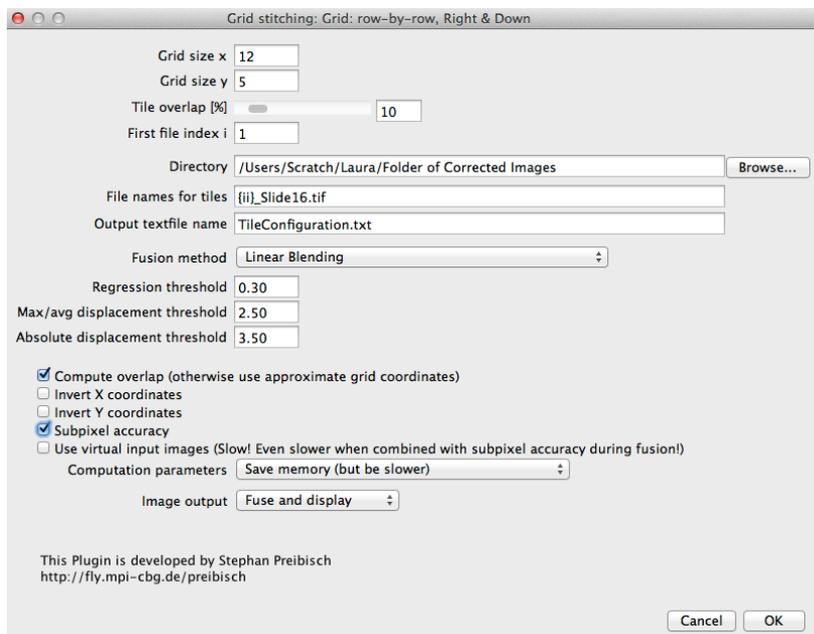
Select Files to Montage

Go to the menu Plugins / Stitching / Grid Collection Stitching and choose the option which suits your files.

The choice you make will depend upon how you collected the images. Choosing 'Unknown Position' should work in most cases if you're unsure, but may take a lot longer to calculate the montage. It is much faster choosing Grid Row by Row and then entering the grid dimensions at the next step.

When the window on the left appears, choose the folder containing your images to montage. Make sure only the images to make into each montage are contained in each folder.

The {ii} is the placeholder for the incrementing part of the image series name. The unchanging part of the file name needs to be entered as appropriate. For example an image series: slide_01.tif, slide_02.tif, etc. Would be entered as slide_{ii}.tif.

Choose the setting which are appropriate to your requirements and press 'Ok'. The process may take a minute or two, depending upon the number and complexity of the images and your computer's processing power.

Go to the menu Image / Colour / Stack to RGB to merge layers into one. Save the montaged image.