User manual for ‘Stitch’

Stitch Authors

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Description

Stitch uses the ImageJ Grid/Collection stitching plugin to stitch together, in a batch manner, multiple tiff series derived from microscopy. It does so in a batch fashion, meaning it can iterate over many directories containing microscopy tiff file series and do the stitching for each of these directories independently.

More specifically this programme was originally created to stitch together tiffs from the Aurox Clarity microscope coupled with Visionary software. However, if the user has a companion.ome file associated with their tiff series, this programme can be used for stitching tiffs. Alternatively, as long as a set of at least 2 tiffs is present, and a positions.csv file (details below) is present, this programme can be used for stitching tiffs.

Usage

Stitch requires an installed version of ImageJ with the Grid/Collection plugin available and Python 3 or above. Run the programme by right clicking on ‘stitch_RUN.py’ and clicking ‘open with -> python’.

Select ImageJ.exe file:

First browse for and select your ImageJ.exe file (e.g. ImageJ-win64.exe).

Select root folder:

Then select the root folder with your directories containing tiffs to be stitched together (for an example see ‘example_root_folder’ in Figure 1).

OME METADATA ONLY STITCHING.

Only use companion.ome file for stitching?

If you have a companion.ome file (if you are using ome open microscopy) in your tiff series directory (e.g. ‘File1_with_tiffs_to_be_stitched’ in Figure 1) and the metadata in this file is correct, you can simply select the ‘Only use companion.ome file for stitching?’ option. Once selected, the ‘Create stitched tiff using original positions?’ and ‘Enter positions multiplier:’ elements are disabled as they are no longer required. Stitch will run and search for the companion.ome files in the subdirectories of your root folder. If it finds a companion.ome file and this file is next to a series of tiffs, it will use this metadata to stitch the tiffs together and move them to an automatically created FUSED_TIFFS directory (see Fig.2).

You may still select ‘Run imageJ macro’ (explained in detail below) when performing OME metadata only stitching.

If the metadata in the companion.ome files is incorrect or comes from an uncalibrated microscope, this can lead to corrupt stitching output. If this appears to be the case and your tiff series also comes with positional info in a positions.csv file (e.g. as with Aurox Clarity Visionary software output) you can uncheck this 'Only use companion.ome file for stitching?' option and continue with the options below.

STITCHING WITH POSITIONAL INFORMATION FROM MICROSCOPE

With the 'Only use companion.ome file for stitching?' option unchecked, Stitch will check each directory (for example 'File1_with_tiffs_to_be_stitched' in Figure 1) in your chosen root folder for two things: a 'positions.csv' file holding the position tiles for each of your tiff images to be stitched together and also at least 2 '.tiff' files to be stitched together.

Stitch will still check for a metadata companion.ome file (which it can later use for deriving information for the ImageJ macro (see section 'ImageJ Macro'), although this is not a requirement. Only the 'positions.csv' file and at least 2 '.tiff' files are necessary.

The positions.csv file must hold a position name, an x_position and a y_position in the following format:

	A	B
1	pos435,-31269,-16488	
2	pos436,-31269,-16727	
3	pos437,-31269,-16969	
4	pos438,-31269,-17165	
5	pos439,-31315,-17367	
6	pos440,-31404,-17535	
7	pos441,-31404,-17731	
8	pos442,-31404,-17874	
9	pos443,-31568,-17962	
10	pos444,-31568,-18183	
11	pos445,-31461,-18356	
12	pos446,-31461,-18580	
13	pos447,-31490,-18834	
14	pos448,-31520,-19063	
15	pos449,-31520,-19314	
16	pos450,-31537,-19530	

The stitcher will use these positions for stitching.

Enter positions multiplier:

As this positions.csv file contains the same x/y positions as the metadata in the companion.ome file, you might wonder why we need to stitch in this manner and not simply run using the companion.ome file as described above. Stitch prompts you to enter a multiplier number. This number will be used to multiply every position in the positions.csv file by this constant. When we originally used the Aurox Clarity with Visionary software, the positions file gave positions in micrometres (µm). The ImageJ plugin used by Stitch requires positions in pixels (px). Therefore, this multiplier number is equivalent to the µm to px conversion rate. If you open a single tiff of one of your tiff series with imageJ and show image information you can find the conversion number. In

doing this, we have some flexibility over the conversion. This often means that we can tweak this number to produce more accurate stitching outcomes. Hence the requirement for an option to stitch using these options.

For example when using the Aurox Clarity with Visionary software, we found that instead of using the exact µm to px conversion rate (e.g. 3.079), increasing the multiplier number slightly (e.g. 3.1) helps with producing better stitching output. Once you have found the right multiplier value for your microscope and magnification, you can reuse this value each time you want to batch process more tiff series.

(Note the use of a period '.' instead of comma ',' for the multiplier value)

The stitcher needs the positions to be accurate (with a slight tolerance of variation). If you are using a different microscopy setup and the positions are accurate and already in pixels, you can simply set this multiplier value as 1. In this case, you should preferably use the **OME METADATA ONLY STITCHING** outlined above if you have a companion.ome file.

When run, the tiffs within each in directory in the root folder will be stitched together. Using our example, the tiffs in 'File1_with_tiffs_to_be_stitched' will be stitched together to create a 'stitched' tiff, and the tiffs in 'File2_with_tiffs_to_be_stitched' will be stitched together to create another separate 'stitched' tiff.

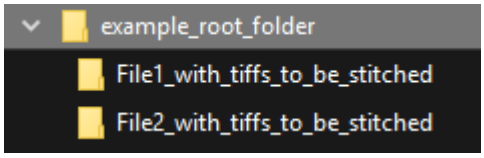

Fig.1

When running, 4 new directories will be automatically created as highlighted in figure 2. If you checked 'Create stitched tiff using original positions?', Stitch will first create a stitched image simply using the original positions in the positions.csv file multiplied by whichever multiplier you choose at the start. These stitched tiffs that are created will be moved to the 'ORIG_FUSED_TIFFS' directory and named after the directory they were derived from. In our example, by selecting 'Create stitched tiff using original positions?' we will therefore create two stitched tiffs in the ORIG_FUSED_TIFFS directory named 'File1_with_tiffs_to_be_stitched.tiff' and 'File2_with_tiffs_to_be_stitched.tiff' with the original positions given from the microscope (multiplied by your chosen multiplier).

However, sometimes these original positions are inaccurate (even after conversion with the multiplier) and lead to less than optimal stitching. Therefore, Stitch will, by default, create a further stitched image using the calculated overlap functionality of the Grid/Collection ImageJ plugin. These stitched tiffs will be moved to the 'FUSED_TIFFS' directory and will be named after the directory they were derived from. Continuing with our example we will therefore create two stitched tiffs in the FUSED_TIFFS directory named 'File1_with_tiffs_to_be_stitched.tiff' and 'File2_with_tiffs_to_be_stitched.tiff' with the new updated overlap, often leading to better stitching outcomes.

Note that if 'Create stitched tiff using original positions?' is left unchecked only the stitched tiffs for the FUSED_TIFFS directory will be created. This can save time and disk space but sometimes the

overlap calculation and subsequent stitching does not go well and it is good to have the ORIG_FUSED_TIFFS available as a backup option.

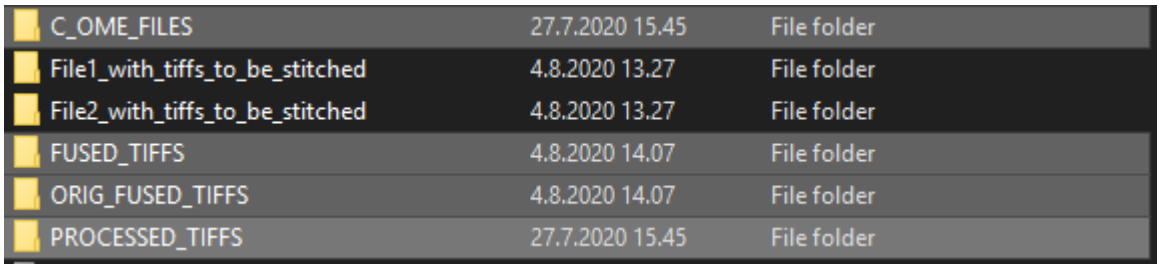

Fig.2

The C_OME_FILES directory temporarily holds relocated 'companion.ome' files (if OME microscopy is being used), whilst the stitching process is running. This leads to better stitching outcomes as the companion.ome file in this author's experience has confused the Grid/Collection plugin if present in the tiff series directory when stitching using this method. The companion.ome files are returned to their original location after the stitching process has ended.

N.B., if you hard quit Stitch, **do not delete** these C_OME_FILES directories as they may still hold companion.ome files! Stitch will automatically return these files upon rerunning.

Run ImageJ Macro?

Stitch comes with the option to run an ImageJ macro on the tiffs created and stored in the FUSED_TIFFS directory. This will be done if the 'Run imageJ macro?' selection is checked.

The Stitch package comes with a default ImageJ macro: 'stitch_macro.ijm'. This default macro uses the companion.ome file associated with each tiff series (if you are using OME microscopy and have companion.ome files) in order to derive information about the tiffs (e.g. width, height, channels, slices, frames). The default macro has been designed for several purposes:

1) To check if the stitched images output in FUSED_TIFFS are 'composite' images (sometimes the Grid/Collection plugin output is composite, other times not)

2) To create channels if not composite (sometimes the Grid/Collection plugin creates a tiff with all channels merged on a single z plane)

3) To assign each channel a default colour.

4) To auto-adjust the brightness and contrast so for easy visualisation

If the stitched tiffs in FUSED_TIFFS are successfully processed by the macro, the new processed tiffs will be saved in the PROCESSED_TIFFS directory.

The user of Stitch is free to use this default macro or to modify it (by modifying the stitch_macro.ijm file) as they see fit.

The default macro will itself not be successful (i.e. will not be able to read the metadata (channel no. etc) in the absence of companion.ome files. However, if you do not have companion.ome files, the stitch macro will still be run as long as the 'Run imageJ macro?' selection is checked. This leaves the option open for the user to edit the 'stitch_macro.ijm' file for their own purposes to be ran on the output stitched tiffs in the FUSED_TIFFS directory.

If you are unfamiliar with macros, you may want to leave this unchecked. However, if you have the same issue with stitched images (they default to grey and all channels are on a single z-plane) then you might attempt to use the default macro. The original stitched tiffs will remain unaltered in the FUSED_TIFFS directory. The output from the macro is saved in PROCESSED_TIFFS

Other Features

- A log file called 'stitch.log' will be created in the selected root folder. Allowing easier identification if errors occur, particularly for users familiar with coding in python.

- The directories with tiffs (e.g. '/File1_with_tiffs_to_be_stitched/') in our examples above don't all have to be directly next to each other in the root folder. They can be kept in different subdirectories and Stitch will still locate and process these directories.

Further Notes

Stitch uses the ImageJ Grid/Collection stitching plugin with default parameters. If you need to alter these parameters for your stitching (e.g. regression threshold, max/avg displacement threshold etc.) this Stitch programme is likely not what you require for batch running stitching.

Please acknowledge the authors of this programme (Jack Morikka and Jorge Fuentes) when you have used this Stitch programme for eventual publication purposes. Equally, please cite the reference below if you use this programme for eventual publication purposes.

References

Stitch uses the ImageJ Grid/Collection stitching plugin which is based on an article by Preibisch et al. 2009:

- Bioinformatics, Volume 25, Issue 11, 1 June 2009, Pages 1463–1465. <DOI - https://doi.org/10.1093/bioinformatics/btp184.>