

## About

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## Purpose

Convert a hard-drive folder of .tif files and perform the following:

- Split 2 channel files into \_ch1.tif and \_ch2.tif channel separated files.
- Perform slice-by-slice stack alignment on one channel and apply transformations to the other.  
This requires 'MultiStackReg' to be installed (<http://bradbusse.net/downloads.html>).
- Optionally save 8-bit versions.

## Installation

- Drag and drop bAlign\_Batch\_v6.py into your Fiji 'plugins' folder.
- Make sure you have 'MultiStackReg' plugin installed, you can download it from:  
<http://bradbusse.net/downloads.html>  
<http://robertcudmore.org/software/download/MultiStackReg1.45.jar>

## Running

- Select 'bAlign Batch v6' in Fiji plugin menu
- Select the hard-drive folder with .tif files to convert
- Fill in options
- Press 'OK'

## Options

### Channels

- Get number of channels from scanimage 3.x or 4.x header.
- Otherwise, assume all stacks have this number of channels.

### ScanImage4

- Remove linear calibration
  - Crop all images
- Note:** Each image is cropped using the specified rectangle (in pixels).  
If your stacks have a mixture of 1024x1024 and 512x512 images you want to put them into separate folders and run bAlignBatch6 on each folder, specifying the proper rectangle (in pixels).

### MultistackReg

- Run multistack reg (requires additional plugin)
    - If 2 channels then align on this channel
  - Start alignment on middle slice
    - Otherwise, start alignment on this slice
- Note:** if you specify a slice below the bottom of a stack (for any of the stack you are converting), the FIRST slice of the stack will be selected for alignment.

### Save 8-bit

- Save another folder with 8-bit copies of your channel separated .tif stack
- Note:** These 8-bit versions are saved in a different folder. The main output folder still contains your channel separated originals with the original bit-depth.

Align Batch Options

Source Folder: /Volumes/ThreeT/huganir\_lab/scanimage\_4/  
Number of .tif files: 2

**Channels**  
☒ Get Number Of Channels From ScanImage 3.x or 4.x header  
Otherwise, Assume All Stacks Have This Number Of Channels:

**ScanImage4**  
☒ Remove Linear Calibration From ScanImage 4.x

**Crop**  
☒ Crop All Images (pixels)  
Left   
Top   
Width   
Height

**MultiStackReg**  
☒ Run MultiStackReg  
If 2 Channels Then Align On This Channel

**Align On Middle Slice**  
☒ Start Alignment On Middle Slice  
Otherwise, Start Alignment On This Slice

**Save 8-bit (at end)**  
☐ Save 8-bit

Cancel OK