mocoi_R2G

Iterative movement correction for calcium imaging. Corrects an image stack with activity dependent fluorescence (e.g., GCaMP data in green channel) based on movement correction of a simultaneously acquired, non-activity dependent fluorescent imaging stack (e.g. tdTomato or mRuby co-label in the red channel).

Required files:

moco package for ImageJ (translation-based movement correction plugin developed by R. Yuste Lab)
Download from http://www.columbia.edu/cu/biology/faculty/yuste/methods.html. Follow instructions in `ReadMe.txt` to install.

batchStitchGnR.m (MATLAB script)

Calls `StitchTiffs_greenChan .m` and `StitchTiffs_redChan.m` for each experiment directory.

StitchTiffs_greenChan.m - (MATLAB script)

Stitches green channel data from each 2-channel stack in `"raw"` directory into one continuous time-stack for entire session. Saves `StackInfo.mat` , which contains file info from original stacks, including `frameRate, numFrames, trigTime, trigDelay,` and `savFile_name`. Saves to directory: `<root_dir>/stitched/` .

StitchTiffs_redChan.m - (MATLAB script)

Stitches red channel data from each raw 2-channel stack in `"raw"` directory into one continuous time-stack for entire session. Saves to directory: `<root_dir>/ stitched_redChan/` .

Mocoi_R2G.ijm - (ImageJ macro)

1. Creates two directories: `<root_dir>/registered/` and `<root_dir>/moco results/` .
2. Creates "seed" reference image by applying iterative movement correction to first 1000 frames of red channel, using average of `nFrames_seed` frames as initial reference. Creates `<root_dir>/registered/ref_img.tif` , the average projection of this movement corrected stack.
3. Applies iterative movement correction to red channel and saves results of each repeat in `<root_dir>/moco results/`. Briefly, stitched stack is corrected with moco, using `ref_img.tif` as reference, then averaged to generate reference for next repeat. The last three parameters described below are used to test whether to repeat. Creates `<root_dir>/registered/reg_<savFile_name(1:end-4)>red.tif` , the movement corrected stack from the red channel.
4. Results are summed to calculate total translation (dx,dy) for each frame, and then applied to the green channel. Creates `mocoi_Results.txt` , which records in each row the total translation (dx,dy) for each frame. Creates `<root_dir>/registered/reg_<savFile_name(1:end-4)>.tif,` the movement corrected stack from the green channel.

Default Parameters (use 'Plugins>>Macros>>Edit…' to change)

```
nFrames_seed = 100;    // nFrames to AVG for for initial ref image
max_error = 1;         // threshold abs(dx)+abs(dy) per frame for repeat
err_tolerance = 0.001;        // threshold cumulative error (pixels) for repeat
max_rep = 10;          // threshold #iterations for considering only max_error for
repeat
```

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