Protocol for auto\_track and verify\_paths

ImageJ/FIJI Cell Selection

1. Open raw ND2 images using ImageJ/FIJI using Bio-Formats Importer under Plugins>>Bio-Formats
2. Ensure that “Open all series” and “Concatenate series when compatible” are checked.
3. Hit OK.
4. Wait for file to open. Timelapse files can be around 1 GB or larger, this takes time to load into memory so be patient. I would suggest moving the ND2 file to your hard drive before attempting to open them.
5. Hit “t” to open ROI Manager
6. Hit “SHIFT+C” to open B&C (brightness and contrast menu)
7. Change Z slider to the middle plane (for 7 steps this is z=4/7)
   1. For most image stacks the Color order is: Trans, GFPbi, RFPbi (C=0, C=1, C=2 respectively). NOTE: the Trans channel only has an image at the “Home” position, the rest of the z-planes will be blank (all 0s).
8. Hit “Reset” button in B&C window.
9. Change to channels one and two (remember, indexing starts at 0 in ImageJ/FIJI) using C slider.
10. Adjust contrast for each channel by first hitting “Reset” button then user Minimum and Maximum sliders to adjust contrast to your liking.
11. Navigate to the GFP channel (typically middle channel)
12. Draw a Rectangular ROI around the cell containing the two GFP foci you wish to track
    1. By default the Rectangular ROI drawing tool should be selected, if not, it is the box icon on the far left of the ImageJ/FIJI window
13. Use the white boxes on the edges of the ROI to resize to your liking
    1. NOTE: Ensure that ONLY TWO foci are in your ROI over all timepoints. This will prevent errors in auto\_track and tedious corrections in verify\_paths. Be sure to scrub through all timepoints.
14. To confirm your ROI hit “t”. Three hyphenated numbers should appear in the ROI Manager.
    1. To view all your ROIs on the current image file check the box next to “Show All” in the ROI manager. Uncheck the box next to “Labels” to turn off the numbers that typically bock the view of your foci.
15. When all ROIs are selected, hit the “More>>” button in the ROI Manager and select “Save…” from the pop-up menu.
16. IMPORTANT: Click on the name of the image you are currently viewing. If you are unsure of which file you are currently viewing, the filename appears at the top of the main window. REMOVE the .nd2 portion of the name and then hit “Save”.
    1. NOTE: If more than one ROI has been created for the image the file will save a .ZIP, otherwise it will be saved as a .ROI.
17. After saving the ROI(s) press “Delete” in the ROI Manager. A pop-up window will appear stating “Delete all items on the list?” Press “Yes”.
    1. NOTE: If an ROI happens to be select at the time you press “Delete”, that ROI will be removed from the list without any request for confirmation. Hit “Delete” again to delete all ROIs in the list.
18. Close the image window, and repeat above steps until all images have been screened for cells containing 2 GFP foci.

NOTES for this section

1. Typically you will select cells with a medium sized bud. However cells with large or misshapen buds are okay to track.
2. Do NOT select cells in which one of the foci stretches into a filamentous line.
3. Do NOT select cells in which the foci separate into mother and daughter buds. This cell is undergoing anaphase and should not be tracked.
4. Foci that join may be difficult to track but should be selected anyway.
5. If you are unsure if you should select a cell, go ahead and selected it anyway. The program verify\_paths.m has a feature that will allow you to quickly discard difficult to track cells.

ImageJ/FIJI timelapse\_bioformats\_split\_dir.ijm section

1. IMPORTANT: The macro (type of program) you will run in ImageJ/FIJI requires that each ND2 file in a directory is followed by either a .ZIP or .ROI file. ND2 files that did not contain any suitable cells need to be removed from the directory prior to running the macro. Josh was lazy when he wrote this macro, so you must follow this file structure. If you wish to update the macro to correct for this admitted oversight, you are more than welcome.
2. Select “Plugins>>Macros>>Run”
3. Locate the ImageJ Macro named “timelapse\_bioformats\_split\_dir.ijm”
4. You will be prompted to choose a source directory. Select the directory that contains your ND2 and ROI/ZIP files. REMEMBER: each ND2 file MUST be followed by a ROI/ZIP file. Remove any ND2 files without corresponding ROI/ZIP files prior to running the script.
5. The script will loop through all the images and automatically crop and divide the ND2 files into separate channels. The source directory will contain three .TIF files for each cell selected from the ND2 file.

MATLAB auto\_track.m Section

1. Open MATLAB 2015 (or later version)
2. Add auto\_track directory to path
3. Add bfmatlab folder to path (bfmatlab must be added last for a reason I do not understand)
4. Change directory to the same directory containing the cropped GFP.tif files you created using the timelapse\_bioformats\_split\_dir.ijm script.
5. Run auto\_track by typing auto\_track and hitting enter. Folders with plane-picked .tif images will be created.
6. NOTE: The auto\_track.m program is NOT perfect. Paths of foci must be verified.