

Step 1: Make 5 folders:

1_raw_images
2_max
3_VNC
4_VNC_quality_checked
5_data

Step 2: Drop your image files into folder "1_raw_images"

Step 3: Launch FIJI, then drag and drop the following scripts to open them

1_files_to_max.ijm
2_worm_to_VNC.ijm
3.0_quality_check.ijm
3.5_find_settings.ijm
4_measure_puncta.ijm

Step 3: Run script 1_files_to_max.ijm

1. Select "1_files_to_max.ijm" when it prompts you to "Choose the folder with your lif/image files"
2. Select "2_max" when it prompts you to "Choose the folder to save your max projections"
3. Step 4: Run script 2_worm_to_VNC.ijm
4. Select "2_max" when it prompts you to "Choose the folder where your uncropped max projections are"
5. Select "3_VNC" when it prompts you to "Choose the folder to save your cropped VNC images"
6. A window will pop up with 2 settings you can change:
 - i. Line width: This will change the thickness of the line you trace over the VNC
 - ii. Hide file name: When checked, this will rename the window of images when you open them in case you are worried about bias from reading the genotype
7. Your images will open one at a time. Draw a line along the VNC, not including any areas that are out of focus. If the image seems too low quality, press ok without selecting a region on the image. If you do this, the true file name will print to the log so you can keep track of which images were not used.

Step 5: Run the script "3.0_quality_check.ijm"

1. Select "3_VNC" when it prompts you to "Choose the folder for images you want to check"
2. Select "4_VNC_quality checked" when it prompts you to "Choose the folder to save images that passed quality checks"
3. A window will pop up to let you decide if you want to hide file names if you are concerned about bias.
4. Your cropped and straightened VNC images will be displayed one at a time. A window will pop up giving you 3 options:
5. Keep: Save the image to the next folder (passed quality check)
6. Toss: Do not save this image to the next folder (failed quality check)
7. Crop: Select the region of the image you would like to be saved to the next folder (use this to remove out of focus regions of an image if mistakes were made while tracing the VNC)

Step 6: Run "3.5_find_settings.ijm"

1. A window will pop up allowing you to decide how many images you want to use to test different settings (if you input 0, it will let you test on all your images).
2. Select "4_VNC_quality_checked" when it prompts you to "Choose the folder with your cropped VNC images"
3. Enter settings that you think would work well, then press ok.
 - i. A good starting place is:
Minimum accepted puncta size = 10
Sigma = 0.5
Radius = 2
Method: Phansalkar
4. Change the settings around, then press ok to test different options. When you are satisfied with the settings you choose, uncheck retest, then press ok. Repeat this for any other images that open up.
5. The settings you chose as the 'best' for each image will be printed to the log window. Compare the settings, and pick what seems like a happy medium.

Step 7: Run "4_measure_puncta.ijm"

1. Enter the settings you decided in the previous step.
2. Check "Manually check each ROI" if you'd like to double check how well your settings work (I'd leave this unchecked when you're actually running it to collect data since it will drastically slow down the process).
3. Press ok
4. Select "4_VNC_quality_checked" when it prompts you to "Choose the folder with your cropped VNC images"
5. Save your table to "5_data" when the script is done running

Step 8: Graph/Analyze your data.

1. On rare occasions the full width half max will be calculated to be greater than the width of the ROI due to a poor fit for a normal distribution. When this happens, a 1 is recorded in the "discard" column, so you can filter out those points while graphing. Pixel width is also recorded in case you need to note scale later.
2. If you want to rerun the script with different settings, if you traced the VNCs properly, you can just start from step 6 or step 7.