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**MEndR Platform 2D Fibre Analysis Script Usage Guide:**

*Preparation:*

Drag and drop the file Pruning\_.bsh into the Fiji plugins folder. This should be located in the folder entitled Fiji.app or something similar.

*File Separation:*

Your images should be stored in a folder structure such as Experiment -> Condition where Condition contains your images.

Open File\_Seperator.ijm and select the Condition folder. The standard window for opening images in Fiji will open. Make sure to select the tickbox that says separate channels. Do this for each of your Condition folders. After you are done you should get a new folder for each Condition entitled Condition\_separated containing folders for each image with 3 images inside each folder. The images are for the GFP, SAA, and Nuclei channels.

*Preprocessing the Images:*

Now close the File\_Separator script and open the Preprocessing\_Fibres.ijm script. Go into each Condition\_separated folder and run the script on each folder, for each image file. This will produce an image for each of your original files containing the unaltered stacked SAA signal, the GFP and Nuclei signals stacked together, masks for the nuclei and GFP channel (note only nuclei overlapping with GFP signal are masked), and an edge detection algorithm to help differentiate between overlapping GFP fibres. It is important to note this edge detection algorithm performs poorly for very complex networks of small fibres so the original Z-stack should be opened during fibre selection in the next step to help differentiate between individual fibres.

*Fibre Measurement:*

Take all of the images produced by the preprocessing script and place them in a folder inside the Condition\_seperated folder called Input. Also create a folder entitled output. Open the Measure\_Fibres.ijm script. It will prompt you to select two different folders. First select the input folder containing the images, and the the output folder. The image will open as well as a window for ROIs, Results, and a window with a button prompting you to select at least ten fibres. Outline all the fibres needed (be careful in regions where fibres overlap). For every fibre you outline, make sure to press the t key when done to actually save the ROI into the ROI manager. Once you are done click the okay button. The next image in the input folder will then open. You now repeat the exact same process. When you have completed all images in the folder you will be notified. Now navigate back to the output folder in the Condition\_separated folder you were analyzing. The output folder will now contain a text file named results and ROI files with your ROI information for each analyzed image saved. To go back and inspect the fibres you selected just open the appropriate image file in Fiji and then drag and drop the ROI file for that image onto the Fiji tool bar.