**Instructions to run “NMJ Analyser”**

Instructions before Running the Macro:

* Make sure to create two folders i.e.; 1) The folder which only contains the images you want to analyse and 2) An empty folder where you wish you save your results.

Instructions for Running the Macro:

1. Open Imagej>Plugins>New>Macro
2. In the Macro window, go to Files and click “Open”.
3. Now select the “NMJ Analyser” macro file to open.
4. Then to run the macro either click on the “Run” option or press “Cntr+R”.
5. Then select the folder where you saved your images to analyse and next select the folder where you wish to save results.
6. Then next a pop-up window with the name “ Have ROIs already be defined appears”, where you select the 1st option “ROIs have not yet been drawn” if you are running the macro for the 1st time for that set of images.
7. Then next a pop-up window with the name “ Segmentation: Default or Advanced Mode” appears where you select the Default mode of zebrafish NMJ analysis and in advanced mode, you can choose your parameters as defined in the article.
8. After selecting any mode you will have to choose the option “Assisted mode” to define ROIs or “Manual mode” if you already have saved ROIs.
9. If you choose “Assisted mode” then each image’s z-projection will appear where you will have to make your ROIs as per the instructions present on the screen.
10. Then after defining your ROIs, click OK and your analysis will start.