PROTOCOL FOR IMAGE OVERLAY IN IMAGEJ

1. Make sure each file you want to overlay has a name that you will recognize. For example the DAPI stained image should have DAPI in the file name. This will be important to ensure each color is correctly added.

2. Open ImageJ64. Open each image that you want to overlay in the program. To do this, go to FILE → OPEN and click on the correct file. Do this for each image.

3. Every picture must then be converted to 8-bit. To do this click IMAGE → TYPE and select 8-bit.

4. Next we will merge the pictures to create the overlay. Click IMAGE → COLOR and select Merge Colors.

5. A pop-up menu will then allow you to assign a picture to a color. Traditionally, DAPI is blue, FITC is green, and rhodamine is red. Once assigned, click OK.

6. Your picture will then be displayed.

7. To save your picture you must covert the image type to RBG Color. To do this, click IMAGE → TYPE and select RGB Color. Next flick FILE→SAVE and you will be allowed to name your new file and save where you like.