

Image J:

Creating overlay of images:

- Open the images to be combined using Image J.
- Adjust the brightness/contrast of each image using going to Image-> Adjust-> Brightness/contrast.
- For combining the images, go to Image-> Color-> Merge channels.
- In merge channels window, red filter for Tritc, blue for dapi and gray for brightfield. And then click ok to create composite.
- Then to adjust the color of the image, go to Image-> Adjust-> Brightness/contrast and do the necessary.

Adding scale bars:

1. First you need to add scale by choosing Analyze-> Set scale. (the parameters are 1, 0.5, 1, um).
2. Then click the picture and go to Analyze->Tools->scale bar.