Read Me: Intensity Profile analysis

1. Create Mask using FIJI - make sure to convert the image to 8-bit - ROI in black and BG in white, and save the image in Tiff file.

   Example:

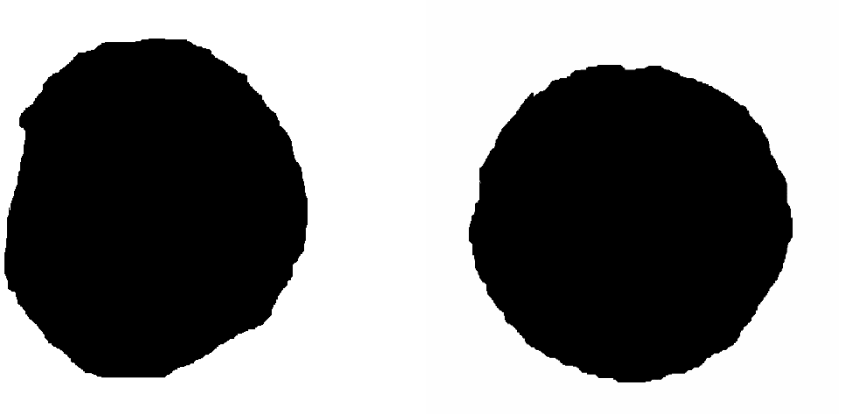


Figure 1: Masks required for the programme, the masks were contour from the DAPI or Hoechst staining images. The images are 8-bit, ROI is in black with the white background.

2. Convert the image of interest to 8-bit using FIJI and save the image in tiff file
   Example:

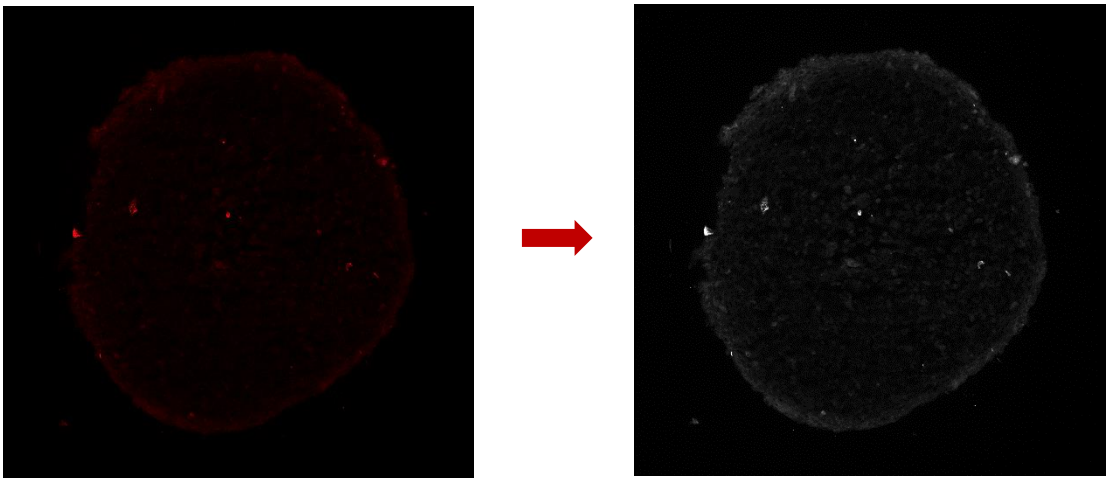


Figure 2: Converting the original image of interest (left) to 8-bit (right) using FIJI.

3. Run ***spheroid_mask_smoother*** function on Matlab

   Using this command:

```
[inner_spheroid_masking]=spheroid_mask_smoother(directory,filename,step)
```

```
where inputs are
 - directory is the directory where we keep the masking image.
 - filename is the filename of the mask we are working on.
 - step is the interval of the degree that we are selecting e.g. one every
x degree.
```

Example output:

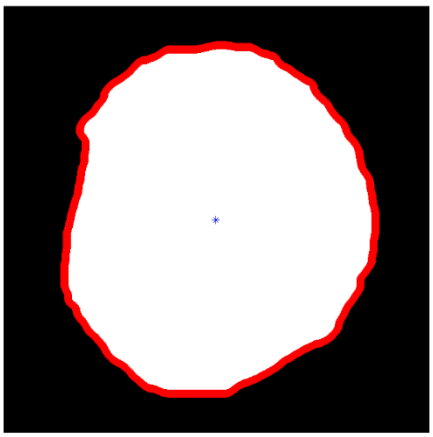


Figure 3: Points with x-y coordianates indicate the edge of the mask (the edge of the spheroid). In this example the points were taken by drawing the line every 1 degree from the centre to the edge.

4. Run ***intensity_profile_individual*** function on Matlab
   Using this command:

```
[intensity_profile,
linear_eqn]=intensity_profile_individual(inner_spheroid_masking,directory_i
m,directory_mask,image_file,mask_file,step,calibration)
```

```
 where the inputs are
 - inner_spheroid_masking is the output from the function
   spheroid_mask_smoother.
 - directory_im is the directory where we keep the images.
 - directory_mask is the directory where we keep the masks.
 - image_file is the filename of the image we are working on.
 - mask_file is the filename of the mask that we are working on
   -> In case of invasion study, this will be the invasion mask.
 - step is the input for intensity measurement frequency i.e. measuring
   every x um along the line
 - calibration is the calibration factor converting um to pixel (unit
pixel/um)
```

Example outputs:

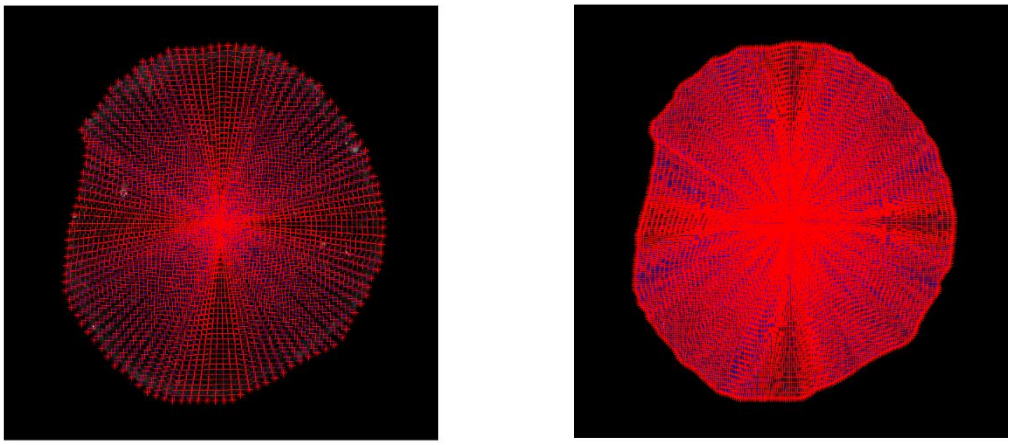


Figure 4: The data points selected to record the signal intensity along each line. In this example the line were taken every 40 degree (left) and 10 degree (right), the data points were recorded every 10 μm along the line.

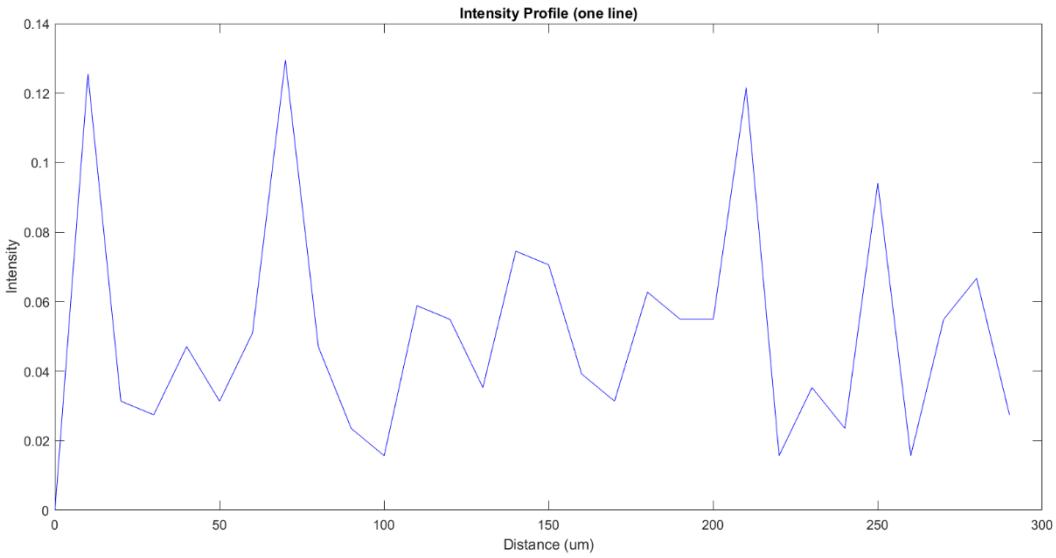


Figure 5: Example of intensity profiles of one line, the data were taken every 10 μm distance along the line.

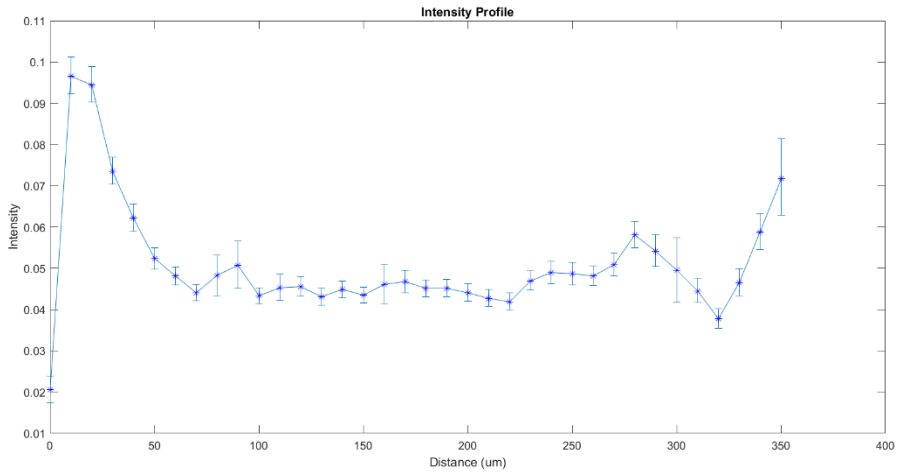
5. Run ***average_intensity_profile*** function on Matlab
   Using this command:

```
[output]=average_intensity_profile(intensity_profile)
```

   The input is intensity_profile which is the parameter created from the previous function explained in 4.

   Example output:

A)



B)

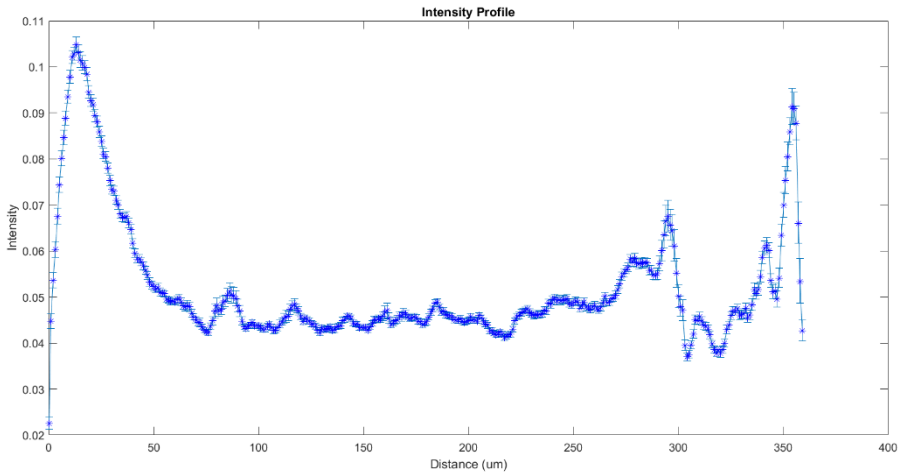


Figure 6: Example of average intensity profiles, the data were taken from (A) every 10 degree lines and every 10 µm distance along each line, (B) every 1 degree lines and every 1 µm distance along each line.