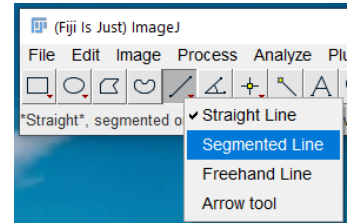


Exercises Session 4 – Automating measurements in Fiji

Exercise 9) Measure western blots (or a dot blot for simplicity) (20 minutes)

- Open a western blot image. (Or use the dot blot image as a first go, it is simpler)
- Use segmented line tool. Draw dot-to-dot style across the bands. Right click to finish.
- Set the line width to cover the bands. (Double click line tool)
- Plot profile (K).
- Move the line up or down and plot profile of background.
- Remove gridlines. (More>> axis options / Draw Grid)
- Adjust range. (More>> Set Range... 0-255)
- Use Process / Image Calculator and combine the 2 graphs with 'min'
- Draw 1 pixel straight lines between each lane. (use draw of Fill to draw the lines – check colours has foreground as black, if not use 'delete')
- Use magic wand to highlight each band. Press T (ROI manager). Set measurements to area.
- Select all ROIs and measure.

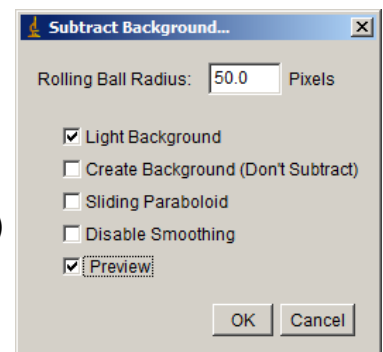


I've made a macro to simplify the process:

WesternsforElliot.ijm

Exercise 10) Rolling ball background correction (10 minutes)

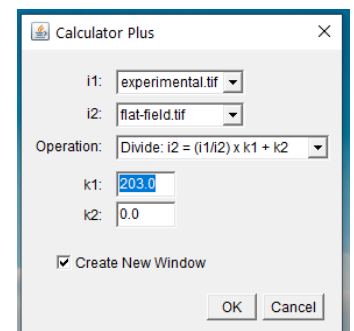
- Open Sample Image > Cell Colony (**File / Open Samples / Cell Colony**)
- Draw a line diagonally from top left to bottom right across image (Try to go through a few blobs)
- Open the Histogram (H) & Plot Profile (K) (use both in Live mode)
- Fix the Y axis range to 0-255 (More>> Set Range...)
- On the Cell Colony image: **Process / Subtract background...** (use Preview)
- Adjust the radius: 50, 10, 2 pixels, & smoothing (Toggle Preview)



What happens when you disable smoothing? Does this matter?

Exercise 11) Image Calculator plus (removes a known background from other images) (5 mins)

- Open the images in the background folder (Exercise images).
- Measure the mean intensity of the background (flat-field) image (how??)
- **Process / Calculator Plus:**
- i1 = Image i2 = background
- Use divide, and add the mean intensity of the background image as k1.



Exercise 12) Manual Cell Counter (5 mins)

- Open Blobs.gif
- Open cell counter (**Plugins / Analyze / Cell counter**)
- Initialize
- Remove some types
- Hit type 1, click on the image...
- Options: Change colours of each type (do yellow)
- Delete mode to remove mistakes. (Type specific)
- Hit type 2, clickety clickety click...
- Keep clicking all the stuff you want to count...
- Results...
- Measure...

