Length, Width and Volume calculation macro guide (for use by VE Lab members)

ImageJ macros are a powerful tool to do repetitive tasks. They can be written to perform a variety of functions like z-projecting your images, stitching them side-by-side etc. Their main usefulness comes from the fact that the user can specify a folder with images, and the macro program will do the processing on all the images inside the folder saving valuable time and giving reproducible results.

What you should know:

The user is expected to be familiar with ImageJ/FIJI. They should know the concepts of z-stacks, z-projection, channels, image histograms, thresholding and plot profiles. Basic programming knowledge and understanding of for loops, if else statements, while statements is required. Diving headfirst into ImageJ and exploring all the options and plugins on an image is the best way to get started.

Before starting:

Make sure ImageJ/FIJI is installed (https://imagej.net/downloads). The BAR plugin also needs to be installed (https://imagej.net/plugins/bar).

The 15x50 rectangular ROI and Left half ROI are provided in the accompanying folder. These ROIs may need to be modified depending on the size of the image. They are ideal for 512x512px images. Download them as well and specify their paths in the code.

For adapting these macros for your use, make sure to go through the code and change the input and output folders according to your machine. Also remember to modify the ROI paths (as mentioned before).

The macro code was developed on FIJI/ImageJ 2.9.0/1.53t; Java 1.8.0_322 [64-bit]

This document does not provide a step-by-step guide or explanation on how the macro code works. For that please refer to the macro code files, they contain annotations explaining the code. This document aims to give an overview of the macro code and the theory behind it.

The macro codes used are of three types:

1. **DAPI/Spindle width macro**: Both codes are essentially the same. The difference is the colour channel on which they work. DAPI width macro works on the blue channel (channel 3) and spindle width macro works on the red channel (channel 1). For detailed explanation refer to the DAPI width macro code file.

   Inputs: They use z-projected .tif files and a 15x50 pixels rectangular ROI as input. The ROI is centred on the image, where the DAPI/spindle width is maximum.

   How it works: This macro measures the plot profiles inside the rectangular ROI in the y-direction (from top to bottom). The code calculates the plot profile. It then calculates the threshold value for separating the signal from background. Different thresholding methods can be used depending the type of image histogram- bimodal, trimodal, unimodal etc. (Go through the code to identify the specific methods used). All the values in the plot profile above the threshold value are saved as a list (only the values corresponding to the hump). The start and end x-axis values (first and last numbers) of the list give us the start and end point of the hump in microns. The difference between the start and end values gives us the length/width of the hump. That's the DAPI width when calculated for the blue channel and Spindle width when done for the red channel.

2. **Spindle/Centrosome/Half centrosome volume macro**: Again, they are all essentially the same. Spindle volume code works on red channel and centrosome volume code works on the green channel (channel 2).
   Half centrosome volume code has 3 lines of extra code that modify it to calculate volume only on the left half of the image; giving us the left centrosome volume. Right centrosome volume can be calculated by subtracting the left centrosome volume from the total centrosome volume.

   Inputs: Spindle and centrosome volume code use the unaltered .czi files as the only input. Half centrosome volume code uses the unaltered .czi files and a rectangular ROI file (that covers the left half of the entire image).

   How it works: This code works by thresholding the entire z-stack of the image. It then calculates the foreground (black) area for every z-slice. It then adds up all the areas from every slice and multiplies it by the z-depth to give us a crude volume estimation. Think of integration but larger step sizes. It does this for one channel at a time. Spindle volume code does this for the red channel and centrosome volume code does this for the green channel.
   The half volume code does exactly the same thing but inside the half ROI. In effect, it calculates the area only for the left half of the z-slices.

3. **C2C/P2P length macro:** These macros use the BAR plugin, before proceeding further, make sure you have it installed and working correctly. The C2C macro works on the green channel and the P2P macro works on the red channel. For detailed explanations refer to the C2C macro code file with annotations.

   Inputs: These macros require a .tif file with a line ROI already drawn from the left centriole to the right centriole, spanning the entire image. If centrioles are not visible, use your best guess (in these cases C2C distances will not be calculated, but P2P can be calculated).

   How it works: This code draws the plot profile along the predefined ROI in the .tif file (The line ROI should be made on the .tif file and saved before the code is run).
   In the C2C macro (green channel), it finds the peaks using BAR plugin. Then it prompts the user to identify the centriole peaks (on the plot profile window) and draw another line ROI starting from the left centriole peak ending at the right centriole peak. User input is needed as centriole identification requires manual supervision. It then calculates the distance between the two peaks and saves it as inter-centriole distance.
   In the P2P macro (red channel), the valleys are identified instead of the peaks and the user is prompted to draw a line ROI starting from the left valley (left spindle pole) ending at the right valley (right spindle pole).

All the macro codes, annotations as well as this user guide were written by Agastya Singh (IISER Bhopal) as a part of his MS Thesis project work undertaken at VE lab in IIT Bombay from August 2022-April 2023.

Good luck!