**ImageJ Area Counting Protocol for Peroxisomal analysis**

1. Open the .czi image stack in Zen gray software
2. Select the particular stack of interest
3. Export only one of the stack as 12bit tiff file
4. Open that image with ImageJ
5. Fiji>Image>Color >split channel
6. Use Green channel for peroxisomal number denoted by GFP-SKL and red channel for RFP-Positive or Negative total clonal/non-clonal area calculation
7. Save individual green and red images.
8. Make 8 bit image of the selected GFP channel stack: Image>Type>8bit
9. (\*VERY IMP STEP BEFORE STARTED measuring: **Setting the Scale**, one can find the needed information in info section of the original image in this case in the Zen software)

Analyze > Set Scale > Distance in pixels= 512/1024 >Known distance: (Distance on X axis) > Unit of length: µm > Unselect global

1. Analyze > Set measurements > Area, Min & max gray value, Shape descriptions, skewness, mean gray value, perimeter, Ferets diameter
2. Repeat the STEP 8: Image>Type>8bit (this will make the image black and white)
3. Process>Find Edges
4. With the ImageJ wand tool or freehand selection tool, select the interested peroxisome and PRESS M to measure the area of all the peroxisomes.
5. With the freehand selection tool, select the clonal/ non-clonal area and press M
6. Measured results were saved in the excel sheet.

**Sharayu Jangam**

ImageJ peroxisome area calculation:

Different scans of the original images were used to calculate the area of the peroxisomes. The individual scan is divided in GFP-SKL(green layer) and RFP(red layer), converted to black and white and then by finding the edges the area of each peroxisome calculated with freehand selection tool or wand tool. The detail protocol is presented in the supplementary figure.