Automation of FtsZ-ring analysis-Assignment A

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1 Image data inspection

Question 1:Which format does the image data belong to?
The file "FTSZ-61.5x.nd2", belongs to the Nikon microscopy image storage format. ND stands for Neutral density filter, which modifies and significantly reduces the intensity of colors and all light wavelengths leaving the HUE of the captured color unchanged. That specific filter is either a clear or gray, and it provides utilities that minimize the overexposure. Our image data is a z-stack, where every stack out of the 41 are sections of 3D image captured along the z-axis. The image was captured by Camera model Andor Zyla VSC-00981 based on the MetaData. The bit depth is 16 and consists of 1350 x 1352 pixels. The image depicts cells bounded with fluorescent green markers with visible spots of the FTSZ rings, which intensity varies across the slices.

Question 2: What is your plan in extracting the FtsZ-ring diameters from the image data and how do you plan to describe your results?
In order to extract the the FtsZ-ring diameters from the image data, i am going to draw straight lines in the rings after importing the image in the Fiji application[1]. Then i am going to import each line inside ROI manager, keep the drew line(Fig.1) so i can keep drawing lines to the next rings without making overlaps and automate the KymoResliceWide plugin which produces kymographs of maximum intensity to run every time i cross section a ring. This procedure is going to happen after running a macro which also includes saving the ring picture in ".tif" format, so i can import everything to python and calculate the distances. The distance calculation will happen between the high local intensity peaks of each image using the built in function "peak_local_max" from scikit-image and then calculate the euclidean distance between those peaks while adding some constraints. Further statistical analysis will be applied, by calculating means and standard deviations in order to get further insights about the cells.

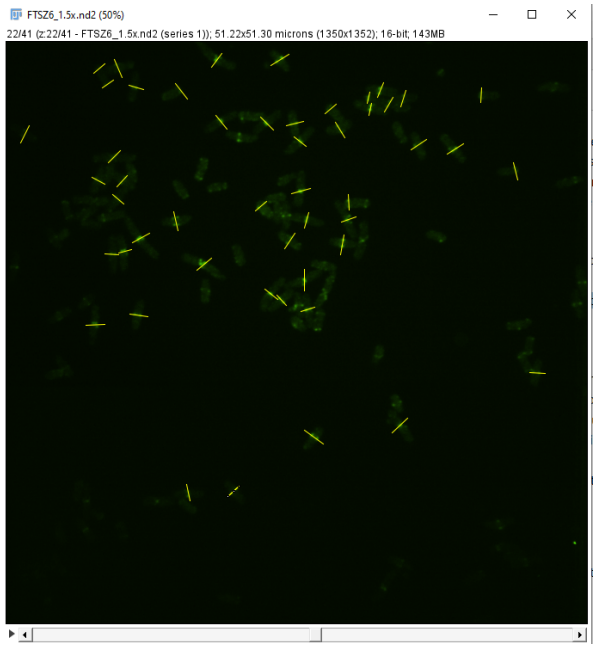


Fig. 1: Cross-sectioned cells in Fiji app using straight lines

2 Image processing workflow

Question 5:Are all the cells at the same stage of division? (i.e. having the same FtsZ-ring diameter within a tolerance). If not, please describe the FtsZ-ring diameters distribution in this image data and your thoughts about the general state of the bacteria
After calculating the standard deviation of the distances and the mean (Table.1), taking into account a threshold of 1, it is clear that not all the cells are in the same stage and their divisions are not synchronised . I tried to minimize the evaluation of the distances of the wrong peak identification like Fig3 with a distance threshold so the low quality images/peak identification would not affect our analysis that much. Based on the frequency plot (Fig.2) a lot of cells had similar value of approximately 9.5 showing a similar division state. But most of the rings were not perfect, having elliptical or other shape, showing different division states across the population. We are not sure of the experimental procedures that these cells underwent, but if the growth conditions, fixation methods, staining and other environmental factors were not robust, they could be underlying reason behind the spotted variability. Another causal effect could be the heterogeneity of the cell populations[**?**], which results in unsynchronised cell states.

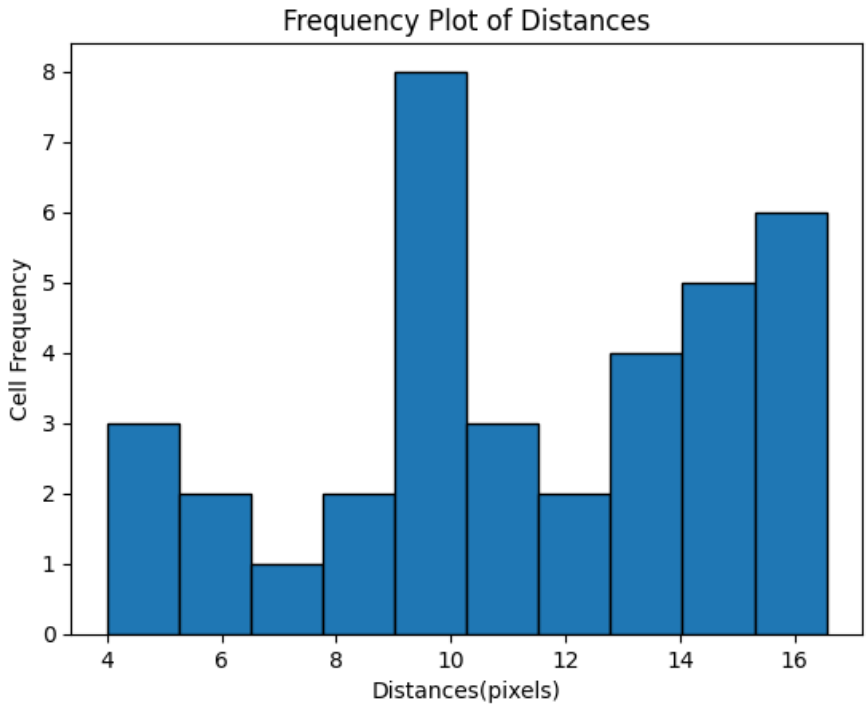
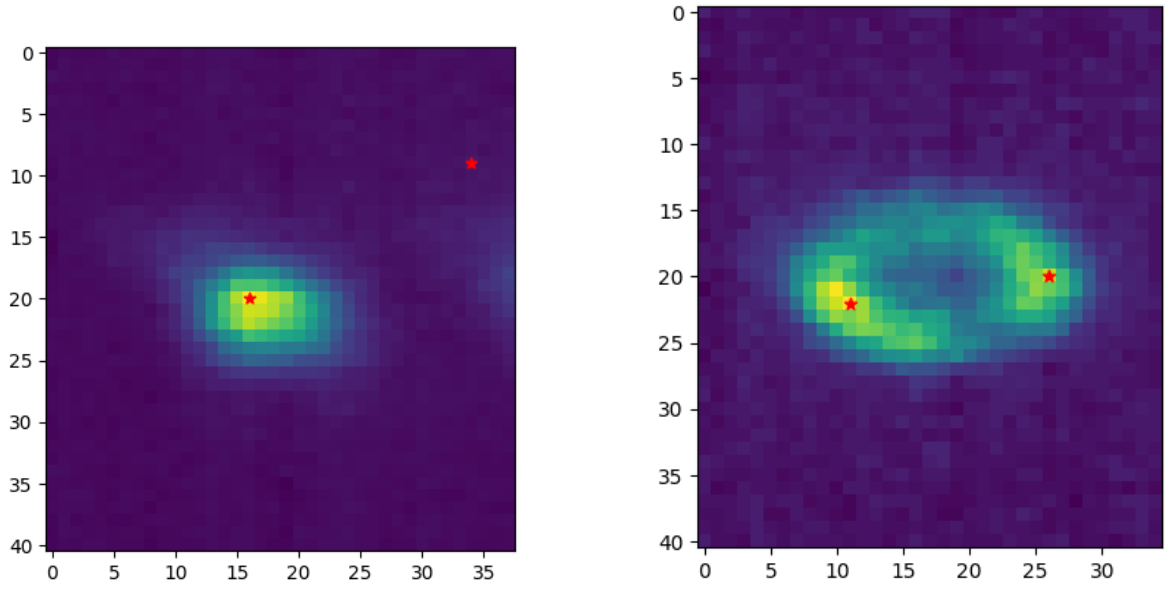


Fig. 2: Frequency plot of ring diameters across the cells

Table 1: Approximate Statistics

Mean	Standard Deviation	Metric
11.3	3.63	Pixels
0.43	0.137	Microns

Bonus question: Is the diameter calculation method ideal? Identify the limitation(s) of this approach or in the image data, and propose workarounds.
The diameter calculation is not ideal, while the built-in function seems to not be really accurate for this task. Every pictures seem to have different levels of fluorescence intensity, validating that the FtsZ ring is a dynamic structure that can change its shape with circular, elliptical or other forms during the cell division process. We can observe that some images have perfect rings (Fig.3b), some others do not (Fig.3a). That makes the distance calculation and the peak identification difficult for the built-in function, while noise and image artifacts can also affect it. One solution that would fix this problem would be higher resolution voxels, while also making a more robust technical experiment that could reduce the variability. On the computational part, we could make a model that classifies sub-forms of the ring , and then use a different method of calculations for each group instead of using one global distance parameter for all of the rings.



(a) Example of a non-ring FtsZ image, with false peak identification (red spots)

(b) FtsZ ring and peaks (red spots)

Fig. 3: Comparison of FtsZ Images

References

1. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. Fiji: an open-source platform for biological-image analysis. *Nat. Methods 9*, 7 (June 2012), 676–682.