Assignment A: Automation of FtsZ-ring Analysis

Course : Image Processing and Quantitative Data Analysis
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Date : June 5th 2023

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1.0 Introduction and general instruction

FtsZ, a bacterial homolog of tubulin, is involved in bacterial cytokinesis or cell division (Bi & Lutkenhaus, 1991). A FtsZ-ring is formed at the future site of division in each cell and its diameter decreases as the cell approaches division (See Figure 1). The overarching goal of this assignment is to understand the cell division stages by looking at the diameters of the FtsZ-ring. In other words, we want to know if all the cells in the image data are at the same stage of cell division.

Image data: FTSZ6_1.5x.nd2

This is a 3D data set from a confocal z-stack, where the protein E. Coli FtsZ was labeled with a fluorescent protein and imaged with the spinning disk confocal microscope. The cells are fixed and you will see cells that are at different stages of the cell division cell cycle, some have no rings yet, others larger rings and some very small rings.

General instruction

We proposed the following workflow: First extract the cross-section of the FtsZ-rings and export the cross-sections as image files by writing an ImageJ macro. Secondly, import these image files to Python and compute the diameters of the FtsZ-rings. Lastly, present your results (e.g. distribution of FtsZ-rings diameters using tables or histogram) and relate the findings to the biological knowledge about FtsZ and cell division you can find from the literature.

Bonus points if you can identify the limitation(s) of this approach or in the image data, and propose workarounds.

ImageJ - Extract and export cross-section of FtsZ-ring

1. Install KymoResliceWide plugin (plugin website : https://imagej.net/plugins/kymoreslicewide). Before starting ImageJ you will need to copy the .jar plugin file in the plugins folder of FIJI (Fiji.app\plugins)
2. Launch ImageJ and open the image file:FTSZ6_1.5x.nd2 (See Figure 2 for cropped view of a cell along its thickness (Z-axis))
3. To understand how KymoResliceWide works: Locate bacteria with a clean ring, then draw a line across the ring and use the plugin KymoResliceWide the get a slice along the line and z-direction (See Figure 3)

4. Now that you get an idea how KymoResliceWide works, develop an ImageJ macro that runs the kymoreslicewide plugin for a line you have drawn and placed in the ROI manager, and that for each line a ring is exported as a separate *.tif file with a unique name.

   (Optional) Also save the lines using the ROI manager for later use or inspection. Give the files unique names (e.g. use the ROI names from the manager). You can also loop over the ROIs in the manager and apply the operations for example see

   https://github.com/ScienceParkStudyGroup/studyGroup/tree/gh-pages/lessons/20190409_ImageJ-Macro_Franka

   To explore more methods with the roiManager, see
   https://imagej.nih.gov/ij/developer/macro/functions.html

Python - Automatically calculate the peak-peak distance as the diameter of the FtsZ-rings

1. Use python to import these images exported from ImageJ.
2. Find the pixels with peak intensities of each of the images, and compute the distance between the peaks.
3. Assign the distance as the diameter of the FtsZ-ring (See Figure 4)

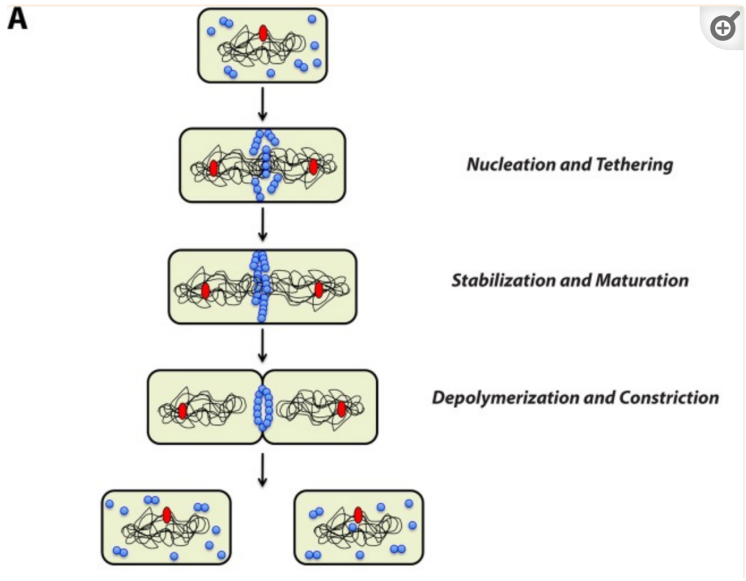

Figure 1. Graphic illustration of the movement and position of FtsZ molecules at stages of a cell division. FtsZ molecules are indicated with blue spheres. At stages prior to cell division, the FtsZ molecules form a ring (FtsZ-ring) and the diameter of this ring reduces as it approaches division. Image adapted from (Huang et al., 2013).

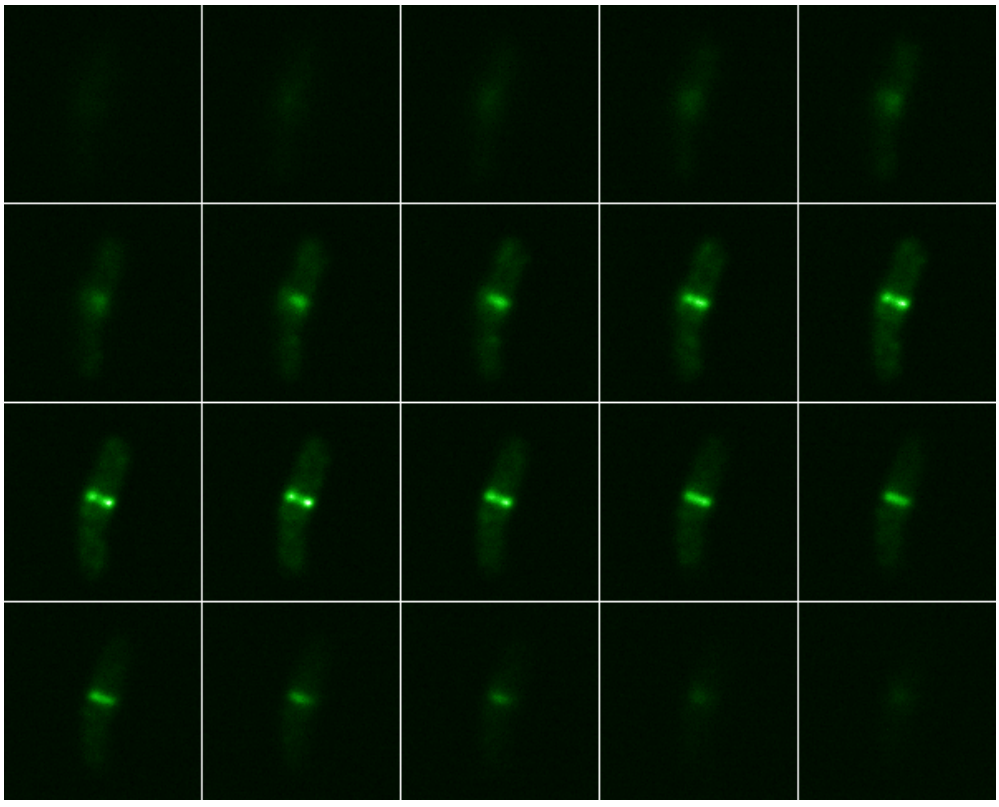

Figure 2 A cropped view of a cell along its thickness (Z-axis). The bright pixels (rendered in green) indicate the FtsZ-ring of the cell.

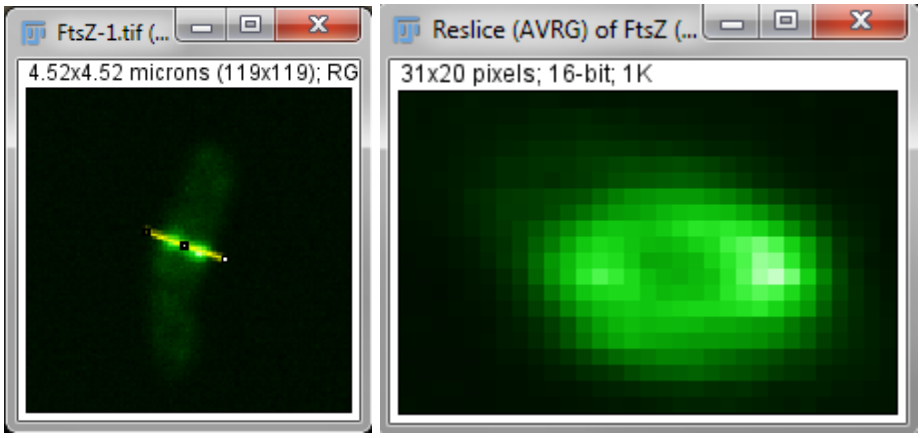

Figure 3 Extract cross-sectional image of a FtsZ-ring of a cell. Draw a line across the FtsZ-ring and generate a cross-section (Kymograph) using plugin KymoResliceWide.

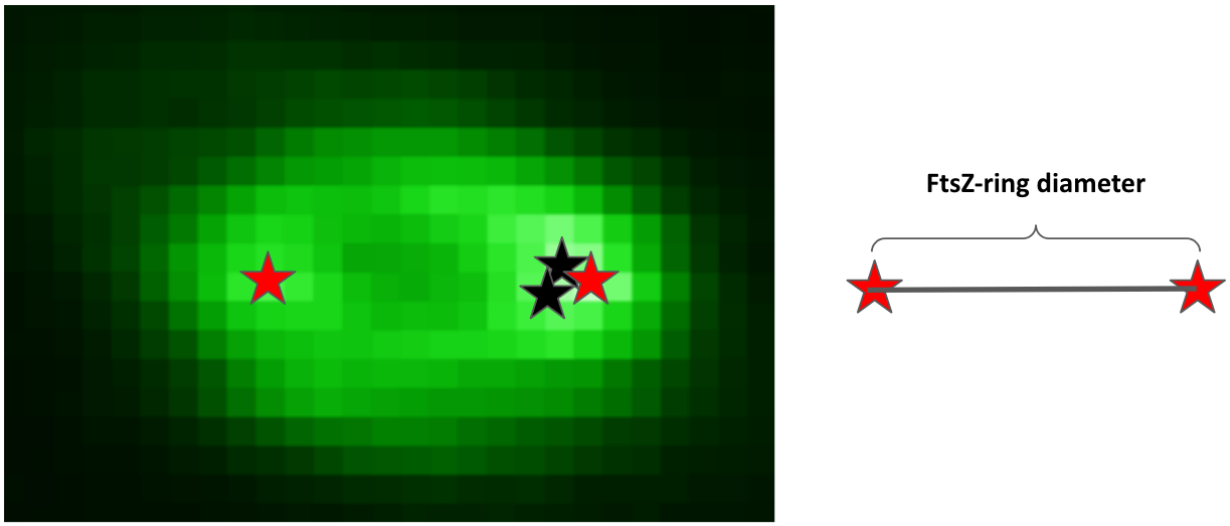

Figure 4 Illustration of finding FtsZ-ring diameter. Find the pixels with the peak intensities that form a ring (red stars) and compute the distance between peaks. (Note: you may need to set some rules so that the pixels with high intensity and close proximity (black star)to the brightest pixel (red star) will not influence the computation of diameter.)

2.0 Tasks and questions (Total 10 points)

2.1 Image data inspection (2 points)

Image data: FTSZ6_1.5x.nd2

- Question 1: Which format does the image data belong to? Briefly describe the observation on this image data. You can use either text, figures or graphs to support your description.
- Question 2: What is your plan in extracting the FtsZ-ring diameters from the image data (See Introduction > General Instruction for hints) and how do you plan to describe your results?

2.2 Image processing workflow (5 points)

- Question 3: With code comments in the ImageJ script, briefly describe the workflow used in extracting the cross-section of the FtsZ-rings and export them as image files.
- Question 4: With code comments in the Python script, briefly describe the workflow used in computing for the diameter of the FtsZ-rings.

2.3 Data analysis and discussion (3 points)

- Question 5: Are all the cells at the same stage of division? (i.e. having the same FtsZ-ring diameter within a tolerance (e.g. 1 standard deviation) that you specify). If not, please describe the FtsZ-ring diameters distribution in this image data and your thoughts about the general state of the bacteria (e.g. why their divisions are not synchronized). Remember to include referred citations.
- Bonus question: Is the diameter calculation method ideal? Identify the limitation(s) of this approach or in the image data, and propose workarounds.

2.4 Submit your work in 3 formats

1. One PDF that contains text-based answers, figures and/or tables. Don't forget about the references.
2. Two text scripts: ImageJ macro and Python script
3. A zipped folder of all exported cross-sectional images

3.0 Reference

Bi, E., & Lutkenhaus, J. (1991). FtsZ ring structure associated with division in Escherichia coli. Nature, 354(6349), 161–164. https://doi.org/10.1038/354161a0

Huang, K. H., Durand-Heredia, J., & Janakiraman, A. (2013). FtsZ ring stability: of bundles, tubules, crosslinks, and curves. Journal of bacteriology, 195(9), 1859–1868. https://doi.org/10.1128/JB.02157-12