Image Processing and Quantitative Image Analysis
Assignment A

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1 Image data inspection

1.1 Question 1

The format of the data is a nd2 file. This format belongs mostly to Nikon. ND stands for neutral density filter, which reduces the brightness of the image by 50%. It is used also in microscope images. The specific image shows some cells at different stages of division. The protein FtsZ(Filamenting temperature sensitive mutant Z), which is a prokaryotic homologue of the eukaryotic protein tubulin, has been highlighted with a fluorescent marker of green colour. In the middle of each cell, there is a ring formed where the cell division takes place later. Just before the cell division, the diameter of this ring decreases.

1.2 Question 2

In order to extract the ring diameter from the image, two steps are required; extract the cross-section of the rings as separate images and process them in a way to measure the ring.

In order to extract the cross-section, using FiJi, an external plug-in by the name of KymoResliceWide was used. After manually splitting the cell in the ROI with FiJi's line tool, the plug-in is able to get a slice of the ROI and save it as a new image.

Afterwards, the generated image gets imported in a Python script, in which the diameter is calculated by finding the pixels with the highest intensities. Using some additional filters in this process, the two points, which determine the diameter of the ring, are acquired and the diameter can be calculated by finding the Euclidean (1) distance of the two points, as we are in a Cartesian plane.

$$\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \tag{1}$$

The results will be described using tables and the findings will be validated by the biological knowledge about FtsZ and cell division found in the literature.

2 Image processing workflow

2.1 Question 3

After the user draws the line in the ROI, the macro is usable. First of all, it adds the ROI to the ROI Manager tool of FiJi. After that, in order to rename the new image, we count the number of images existing in the ROI Manager, so we can have an ascending name list. Next, we run the KymoResliceWide plug-in. There are three check boxes when selecting this plugin: a) Rotate 90 degrees, b) add ROI to Overlay and c) Ignore image calibration, as well a drop down list of Intensity value across width. We don't want to check any box and we also want to set the intensity to maximum, so the macro has to be adjusted with these options. Finally, we save the image as .tif, with its corresponding name from the ROI Manager. The macro also closes the open window, for user experience quality. Finally, the macro was hot-keyed to "q", so the user only has to draw the line and press q, and the image gets saved immediately.

2.2 Question 4

The Python script imports the images exported from FiJi, finds the pixels with peak intensities in each image, and computes the distance between these peaks using the Euclidean distance, which is the diameter of the FtsZ-ring. The script uses the skimage library to perform image processing tasks, such as thresholding the image to create a binary image, applying the binary image as a mask to the original image, and finding the pixels with peak intensities in the masked image. In order to set the threshold, Otsu's method was used. This method calculates one unique threshold value (per image), which is applied to the entire image. In order to ignore the background of the image (the region around the ROI), the image was converted to binary. This was achieved by setting as False all the pixels that had a value of 0. This resulted in a binary image, where the only pixels that were True were only the cell pixels.

3 Data analysis and discussion

3.1 Question 5

Cells are not in the same stage of division. This is observed from the varying diameters of the FtsZ-rings in the image data (Table 1). The distribution of FtsZ-ring diameters was analyzed using a histogram (Figure 1), which shows the frequency of different diameter sizes. This distribution provides insights into the general state of the bacteria, such as the stages of cell division they are in and why their divisions are not synchronized. The absence of synchronization could be due to various factors, such as differences in cell age, environmental conditions, or genetic factors. The FtsZ protein, which forms a ring at the site of future cell division, plays a crucial role in bacterial cell division. The diameter of this ring decreases as the cell approaches division, which can be used as a

marker for the stage of cell division [1]. The stability of the FtsZ ring, which can be influenced by factors such as the bundling and crosslinking of FtsZ filaments, can also affect the timing of cell division [2].

Image	Diameter
Sample 1	14.142136
Sample 2	14.035669
Sample 3	16.124515
Sample 4	15.652476
Sample 5	5.830952
Sample 6	11.704700
Sample 7	16.031220
Sample 8	14.000000
Sample 9	14.317821
Sample 10	14.000000
Sample 11	10.049876
Sample 12	17.117243
Sample 13	7.071068
Sample 14	5.099020
Sample 15	9.055385
Sample 16	13.152946
Sample 17	7.211103
Sample 18	14.000000
Sample 19	10.198039
Sample 20	8.000000
Sample 21	4.123106
Sample 22	15.033296
Sample 23	13.601471
Sample 24	18.973666
Sample 25	14.142136
Sample 26	7.000000

Table 1: Results table.

3.2 Bonus question

The diameter calculation method has a lot of limitations. First of all, if the FtsZ ring is not a perfect circle, then the method encounters a lot of problems. In Figure 3a, we can see that the two most intense pixels are not correct, as one of them is outside of the cell. This happens because we've set the minimum distance between the two points to 3. In case we change that, not only will the two points be incorrect, but another image might break down. In Figure 6a, we can also see that, even if the second point is inside the cell, it is very close to the other point (minimum distance), and is in no case the correct diameter of the ring. Each image is unique, and we have to treat them independently, or find

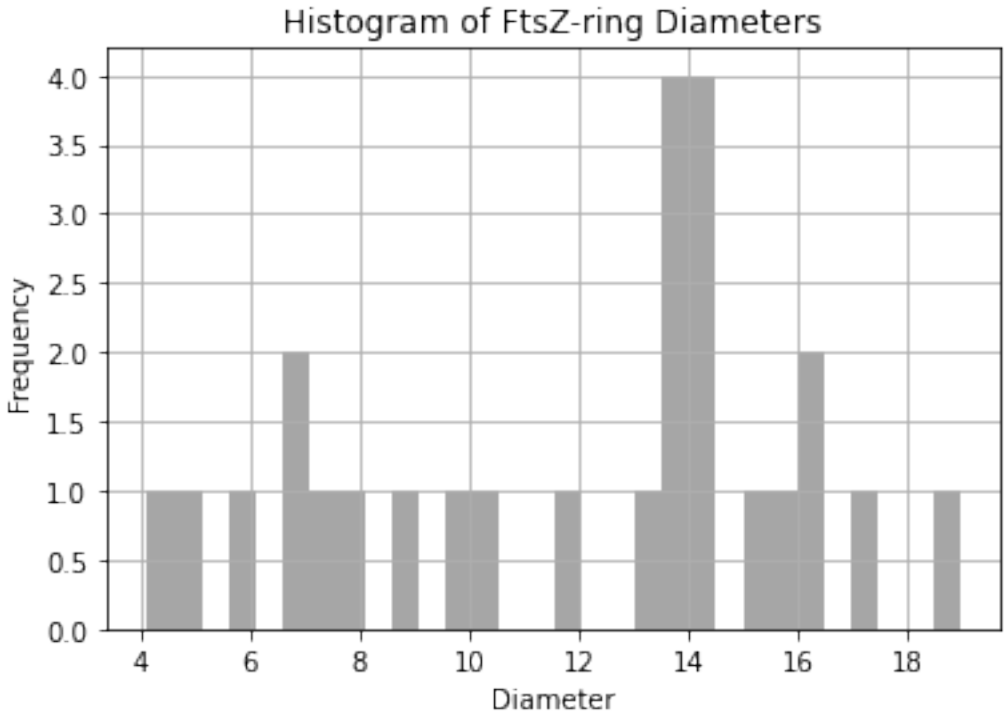
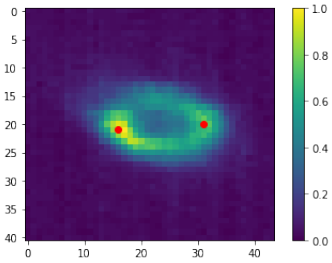
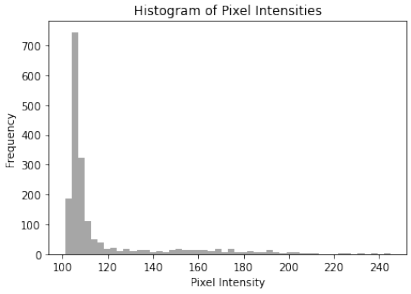


Fig. 1: Results histogram. All the diameters calculated are plotted for distribution identification. Note that there are faulty diameters, although no correction could be applied.

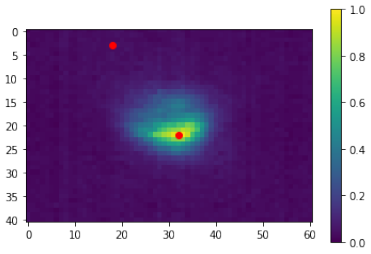


(a) ROI with the two peak intensities.

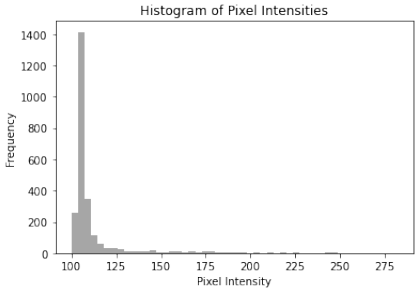


(b) Pixel intensity histogram.

Fig. 2: ROI sample 1

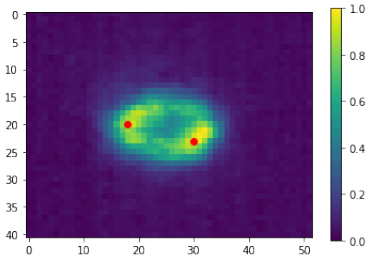


(a) ROI with the two peak intensities.

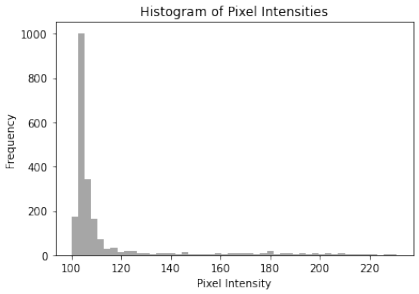


(b) Pixel intensity histogram.

Fig. 3: ROI sample 2

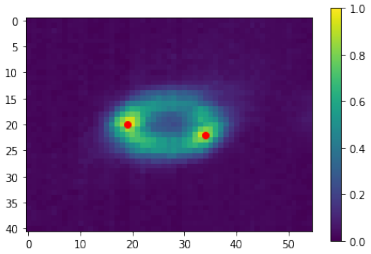


(a) ROI with the two peak intensities.

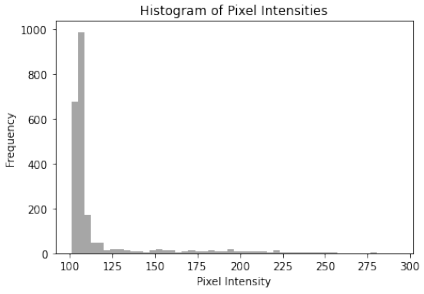


(b) Pixel intensity histogram.

Fig. 4: ROI sample 3

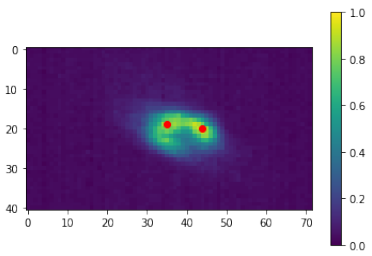


(a) ROI with the two peak intensities.

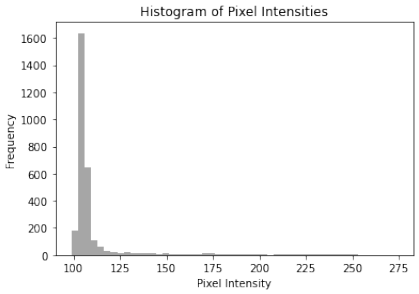


(b) Pixel intensity histogram.

Fig. 5: ROI sample 4



(a) ROI with the two peak intensities.



(b) Pixel intensity histogram.

Fig. 6: ROI sample 5

another, more smart way to calculate it (CNNs for instance). This method is very user-dependant as well, as it could be improved by processing the original image (adjust brightness, contrast etc). The more experienced the user is the better the outcome.

References

1. E Bi and J Lutkenhaus. Ftsz ring structure associated with division in escherichia coli. *Nature*, 354(6349):161–164, 1991.
2. KH Huang, J Durand-Heredia, and A Janakiraman. Ftsz ring stability: of bundles, tubules, crosslinks, and curves. *Journal of bacteriology*, 195(9):1859–1868, 2013.