RhoA activation and inhibition analysis - Assignment B

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1 Image data inspection

Question 1: What is the shape of the data? What does each index represent? Briefly describe the observation on this image data.

For our image "47-SGFP2-mScarlet-I-01-1_channels.tif[1], after importing and running image.shape function we obtain 4 values (70, 2, 260, 348). Those values represent a time series with 70 stacks, 2 channels , 260 as height and 348 as width of the image. After opening the image file with Fiji [2]we can easily observe 4 cells, in the first channel of the Donor the overall intensity seems to be higher than the Acceptor channel. If we briefly check the time-series, we can observe that after the Histamine activation (15th stack and after) and mepyramine inhibition(45th stack and after) the Acceptors/Donors intensities fluctuate.

Question 2: There are two channels (donor and acceptor) in the data but their positions are misaligned due to technical limitations. If they are not aligned in the image processing steps, will the calculated FRET ratio be reliable? Why and why not?

If two positions are misaligned, then when we will try to compare the intensity signals between the channels it is possible to get wrong information as the comparison will be done into different regions. Based on that we will have to make a correction to one of the channels and align them properly so their x,y,z positions match in order to obtain the correct ratio.

2 Image processing flowchart

Question 3: Build a flowchart to describe the workflow towards extracting the FRET ratios. In the figure description or in the main body of the report, briefly explain each step.
Observing the Flowchart (Fig.1), we start the procedure by importing the image data to our python script followed by channel split, to separate Donor and Acceptor in order to make their z-Projections. The mean Z-projections of each channels give us a clear idea of the intensities and the areas of all the frames across the z-axis. Based on the projections we can create a background mask and apply it to the channels in order to separate the cells from the background and reduce unwanted noise in the low intensity parts. Moving to the pre-processing part, background subtraction and smoothing is done based on the background information we obtained, while alignment is performed with Affine transform function two translate the 2 channels. The alignment improves the quality of our FRET ratios[3] as they are calculated from the same exact positions . After receiving the corrected frames from all procedures above, the FRET ratio is calculated between the channels (ratio = Acceptor/ Donor) , and the ratio frames are generated and saved into a .Gif with an addition of a look up table (LUT) and a label showing mepyramine and histamine frames.

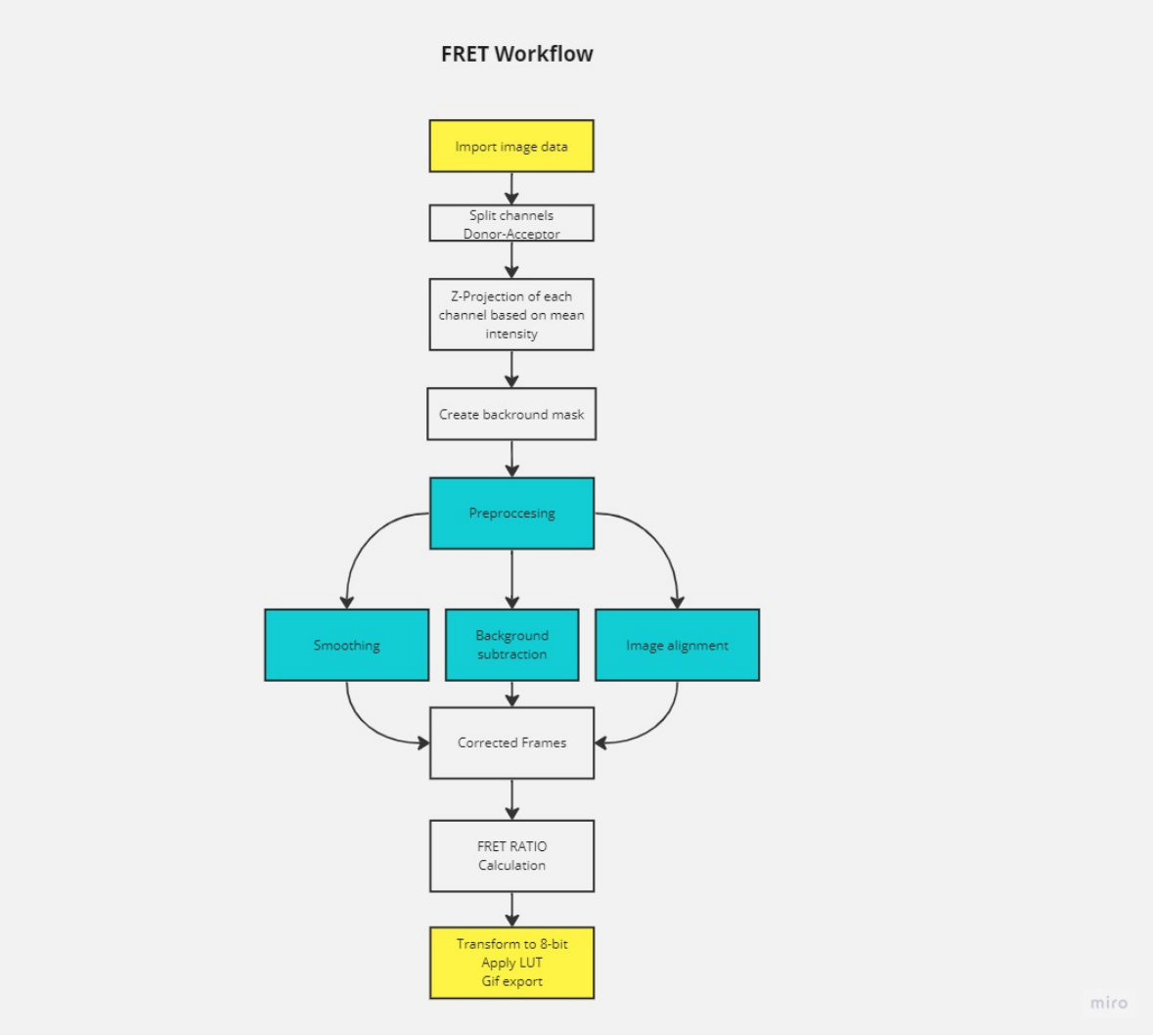

Fig. 1: Workflow towards extracting FRET ratios

Question 7: Describe the changes in FRET ratios with respect to the time when histamine or mepyramine was administered. Are there timepoint delays in the FRET changes? Briefly explain what you think that caused this observation.

Looking at the mean FRET ratio against time (Fig.2), we can see that the changes in the ratio dont happen exactly on the time points that the histamine and mepyramine was delivered (15th and 45th frame respectively). Probably for both compounds the delay includes the time to reach their target site. Additionally, the downstream signaling triggered by histamine/mepyramine may involve other molecule interactions, enzymatic reactions and transcriptional changes, which will influence the registered signalling time-point. Based on those observationswe can conclude that the observed changes in the FRET ratio do not only include the compound kinetics but other factors as well.

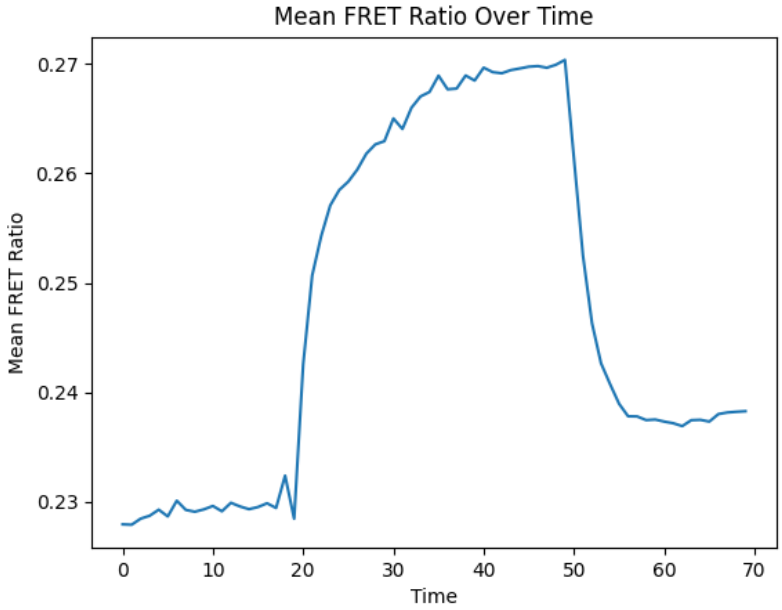

Fig. 2: Mean FRET ratios(x-axis) against time(y-axis)

Bonus question: The calculated FRET ratios describe the RhoA activities in all 4 cells. What would you do differently if the interest is to look at cell-to-cell differences? Propose a simple image pre-processing workflow (hint: it involves cropping the hyperstack)

After applying the background mask and the pre-processing we already did for this assignment, i would draw suitable region of interest (ROI) within the hyperstack that contains the cells of interest and crop the hyperstack to retain only the selected ROI. I would extract all the important information like intensities and FRET ratios, and then i could do statistical tests in order to see the different RhoA activity across different cells.

References

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2. 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.
3. Yoshizaki, H., Ohba, Y., Parrini, M.-C., Dulyaninova, N. G., Bresnick, A. R., Mochizuki, N., and Matsuda, M. Cell type-specific regulation of RhoA activity during cytokinesis. *Journal of Biological Chemistry 279*, 43 (Oct. 2004), 44756–44762.