Assignment B: RhoA activation and inhibition analysis

Course : Image Processing and Quantitative Data Analysis
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Date: 7th June, 2023

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1.0 Introduction

Fluorescence resonance energy transfer (FRET) is a technique based on Förster Resonance Energy Transfer for studying molecular interactions with spatial and temporal resolution inside living cells. The protein of interest here is RhoA, which belongs to a group of GTPases that play pivotal roles in cell activities including cell cycle and cytokinesis (Yoshizaki et al., 2004, Zhang et al., 2020). RhoA activity can be activated by histamine and suppressed by mepyramine, and this activity change can be measured with FRET. Two fluorescent molecules (donor and acceptor) are bound to two sites of the protein. When RhoA is activated, these two sites come close to each other, and the energy transfer from the donor to the acceptor molecules will lead to a higher emission level from the acceptor site (see Figure 1).

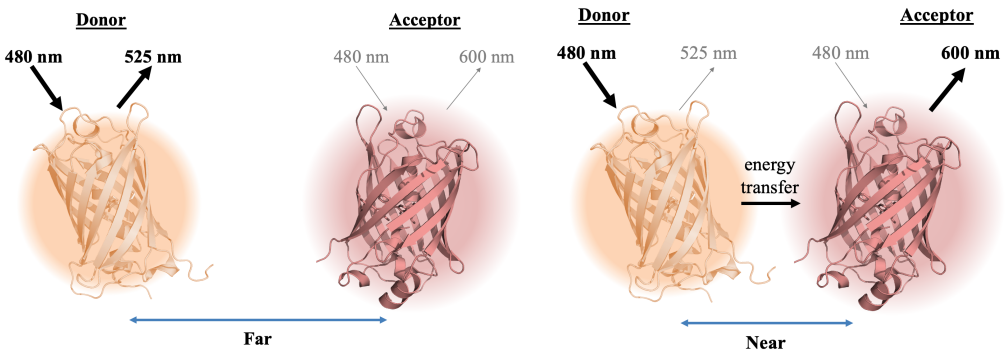

Figure 1 FRET principle. Donor and acceptor are excited at 480 nm. When the protein sites are far apart, the donor emission level is higher than the acceptor emission. When the fluorescent molecules of the donor and acceptor

sites of the protein are in near proximity to each other, energy transfer from donor to acceptor leads to higher emission level at acceptor site.

Image data: W47-SGFP2-mScarlet-I-01-1_2channels.tif

This is a time-series dataset used in (Bindels et al., 2016), where there are visible 4 HeLa cells. The cells were perturbed with histamine at 15th and mepyramine at 45th timepoints (see Figure 2). The excitation wavelength was set at 480 nm and the emission spectrum between 550 to 800 nm was recorded. To form the FRET channels (donor and acceptor), a dichroic mirror with two bandpass filters was used where the donor emission was filtered to 525 ± 40 nm, and acceptor emission to 600 ± 37 nm.

The goal of this assignment is to describe the RhoA activity to of the cells subject to perturbations, using the FRET ratio (R), which is defined as the ratio of acceptor (A) to donor emission (D) signals, i.e. R = A/D.

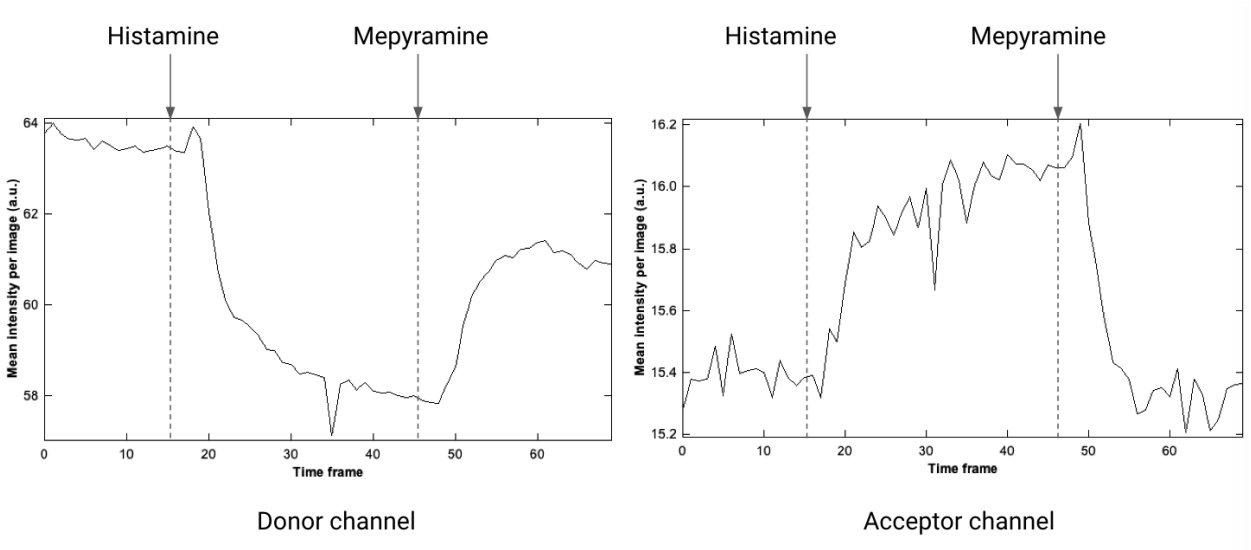

Figure 2 Mean intensity per frame over all timepoints. (Left) Donor channel and (right) acceptor channel. The administration of histamine and mepyramine are shown at the same timepoints for both channels.

General instruction

This assignment is Python-focused, but you are encouraged to use ImageJ to inspect the image data (for Question 1 and 2). Design a FRET ratio calculation workflow (draw a flowchart) and make sure you understand the key components in the workflow. You can learn about the workflow from the provided starting script. Then, complete the provided starting script at spaces between "#Start coding here" and "#End coding here".

2.0 Tasks and questions

2.1 Image data inspection (3 points)

Image data: W47-SGFP2-mScarlet-I-01-1_2channels.tif

- Question 1: What is the shape of the data? What does each index represent? Briefly describe the observation on this image data. You can use either text, figures or graphs to support your description.
- 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?
- 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.

2.2 Image processing workflow (5 points)

Starting script: IPQDA_23_ASS_B_Starting_Script.ipynb

With code comments in the Python script, briefly answer the following:

- Question 4: What is the purpose of calculating the mean of the z-projection of each image stack?
- Question 5: The image data is inherently noisy. A thresholding step is recommended in the starting script to reduce the image noise. Briefly explain the reason behind the threshold value you have used in the solution.
- Question 6: How does the function “AffineTransform” work?

2.3 Data analysis and discussion (2 points)

- 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.
- 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)

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. One text script: Python script
3. A zipped folder containing an animated gif of the FRET ratios images

3.0 Reference

Yoshizaki, H., Ohba, Y., Parrini, M.-C., Dulyaninova, N. G., Bresnick, A. R., Mochizuki, N., & Matsuda, M. (2004). Cell Type-specific Regulation of RhoA Activity during Cytokinesis. Journal of Biological Chemistry, 279(43), 44756–44762. https://doi.org/10.1074/jbc.m402292200

Zhang, J. Y., Nguyen, A., Miyamoto, S., Joan Heller Brown, McCulloch, A. D., & Zhang, J. Z. (2020). Histamine-induced biphasic activation of RhoA allows for persistent RhoA signaling. 18(9), e3000866–e3000866. https://doi.org/10.1371/journal.pbio.3000866

Bindels, D. S., Haarbosch, L., van Weeren, L., Postma, M., Wiese, K. E., Mastop, M., Aumonier, S., Gotthard, G., Royant, A., Hink, M. A., & Gadella, T. W. J. (2016). mScarlet: a bright monomeric red fluorescent protein for cellular imaging. Nature Methods, 14(1), 53–56. https://doi.org/10.1038/nmeth.4074