Analyzing treatment effect on breast cancer cell line using FISH - Assignment C

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1 Image data inspection

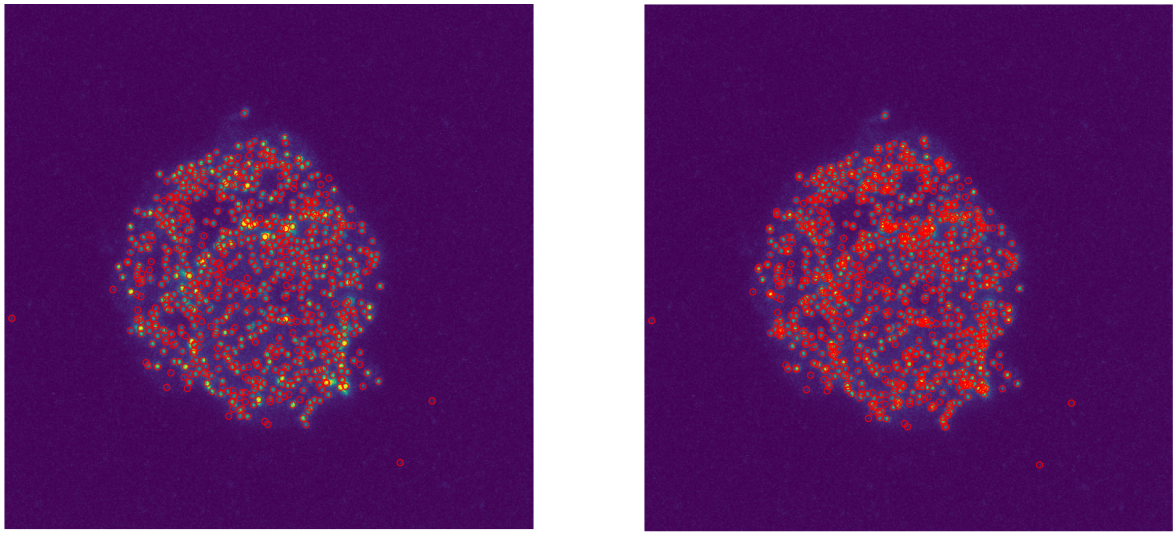
Question 1: What could be the reason that the FISH-tagged ARAG mRNA is not confined to the nucleus?

The main reason that the FISH-tagged ARAG mRNA is not only spotted in the nucleus is probably because after the mRNA is transcribed, it is transported to other cellular parts in the cytoplasm for localisation but also for protein synthesis and other post-transcriptional modifications. Another reason could be that these spots are experimental artifacts, as the smFISH method is a difficult and sensitive procedure, so further investigation of the ARAG mRNA should be conducted to make accurate assumptions.

2 Image processing workflow

Question 2: Explain the difference between Figure 1 and 2 in the notebook "5 - Detect spots.ipynb" by elaborating the function detection.decompose_dense().

Observing the two Figures bellow (Fig.1) which are our control analysed cells, after using the function "detection.decompose_dense()" (Fig.1b), we were able to detect more spots in the high brightness and dense areas than the original detection method, which identified many parts as a single fluorescent molecule (Fig.1a). That result was generated by the decomposition function that is included in the big-fish package [3], which combines a variety of methods, including smFISH channel denoising, Gaussian signal fitting, and a calculation of the median intensity of the single predicted spots, to use it as a threshold in order to obtain a similar number of spots on the high density areas.



(a) Spot detection before region decomposition (770 spots)

(b) Spot detection after decomposing the regions(1047 spots)

Fig. 1: Comparison of pre and post spot decomposition detection for DMSO-control

Question 3: What is the difference between spots and clusters (FIgure 3 in notebook "5 - Detect spots.ipynb")? From a biology perspective, why does it matter to find the clusters?

Spots in smFISH refer to individual fluorescent signals that represent the presence of a single hybridised mRNA molecule[1], whereas clusters observed in Fig. 2 refer to the presence of multiple mRNA molecules withing a region. Clusters can give us great insights into the aspects of gene expression regulation. Depending on the area that the clusters are formed, they could either be transcription or translation sites, while complex forming and other cellular procedures could be involved, including mRNA decay sites named Foci[2].

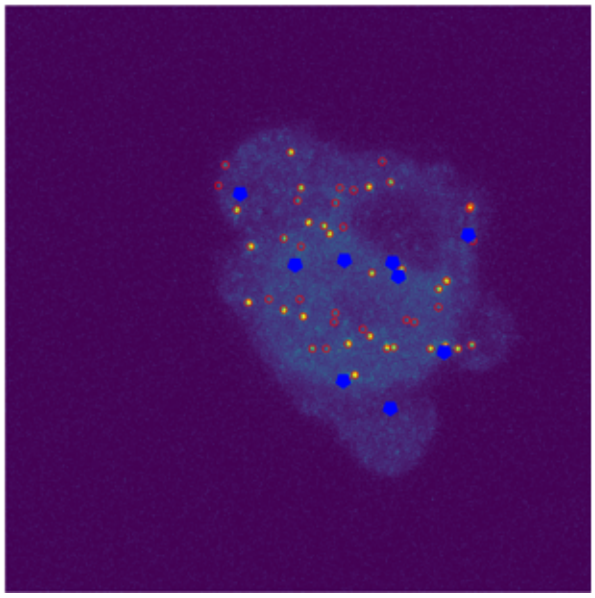
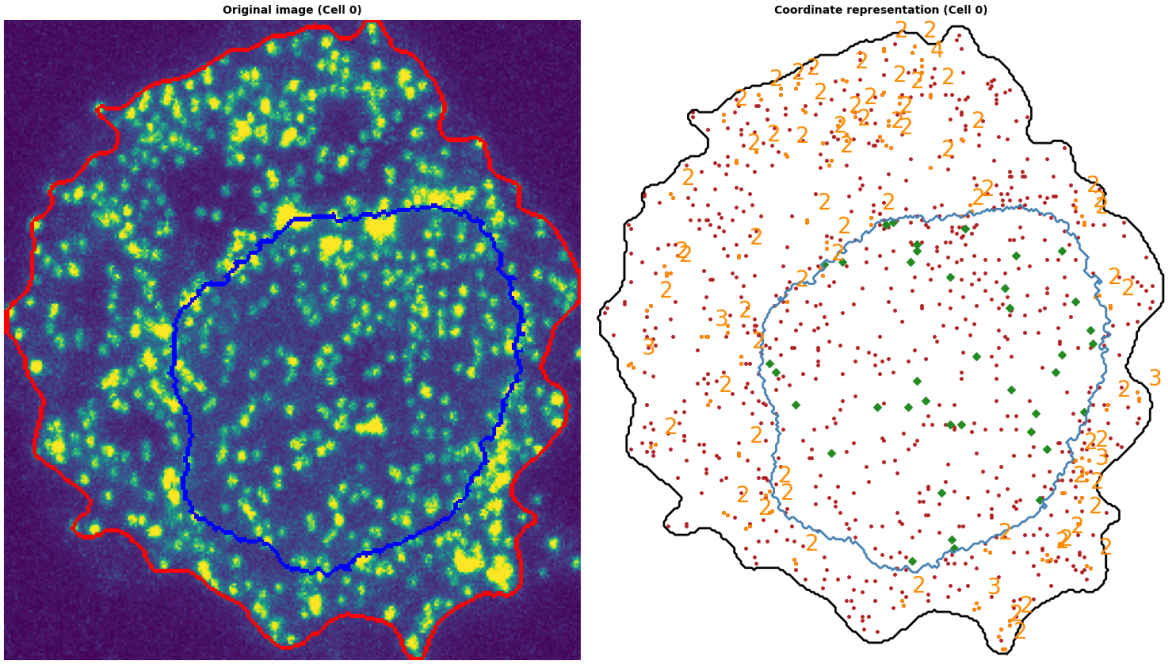


Fig. 2: smFISH of ARAG gene. Spots(Red) and cluster detection (Blue), after JQ1 effect.

3 Data analysis and discussion

Question 4: Combine Figure 4 and Table 1 (a pair for each condition) in notebook "6 - Extract cell level results.ipynb" in a presentable way and describe the results.

We quantified and extracted cell information of our 3 FISH-tagged ARAG mRNA conditions, using various big-fish modules [3] for cell and nucleus segmentation and analysis. Our control cell (Fig. 3a) , based on the analysis results on table3b, showed greater number of nuclear mRNA abundance (nb_rna_in_nuc) in comparison to the JQ1 (Fig. 4) and TSA (Fig.5) affected cells, possibly representing lower transcription ratio of the specific ARAG gene. Similarly, looking at the general cell area, the obtained data from the DMSO (control) showed higher mRNA counts(nb_rna_out_nuc) from the two other conditions, and also significantly less transcription sites and foci areas for both JQ1 and TSA. That observation could possibly mean that after some period of time the total mRNA molecules could progressively decay for the 2 drug affected cells, as the transcription sites seem inhibited. It is clear that in order to validate this theory we would need time-lapse image data and multiple cell analysis. The cell (cell_area) and nucleus (nuc_area) area were also measured in order to investigate the role on the localisation of the ARAG and the possible effects of its inhibition.

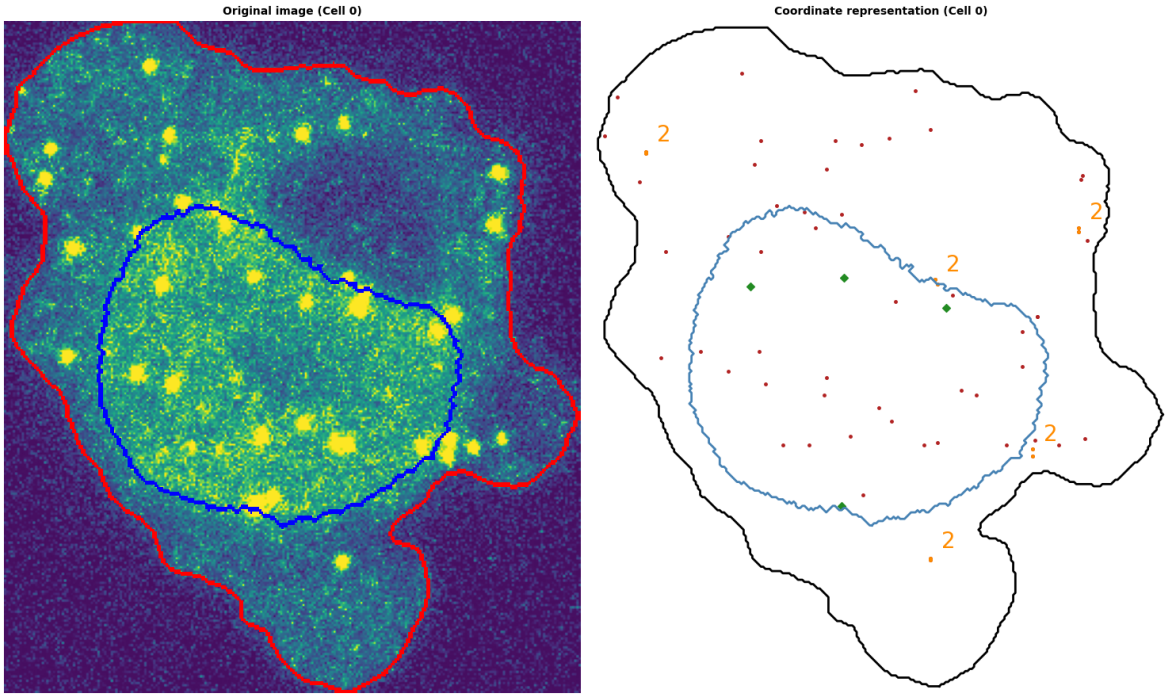


(a) Representation of the cell's coordinates in FISH-tagged ARAG mRNA,including transcription sites(Green), Foci sites("2") and other cellular compartments (Red spots). Also depicted the cell boundary (Red on the original-Black on the coordinate representation) and nucleus boundary(Blue) lines.

	cell_id	cell_area	nuc_area	nb_rna	nb_rna_in_nuc	nb_rna_out_nuc	nb_foci	nb_transcription_site
0	1	50332	19590	940	298	642	72	39

(b) Extracted nuclear and cytoplasmic mRNA quantification of the cell

Fig. 3: DMSO (control) single cell segmentation and analysis with Big-FISH package.

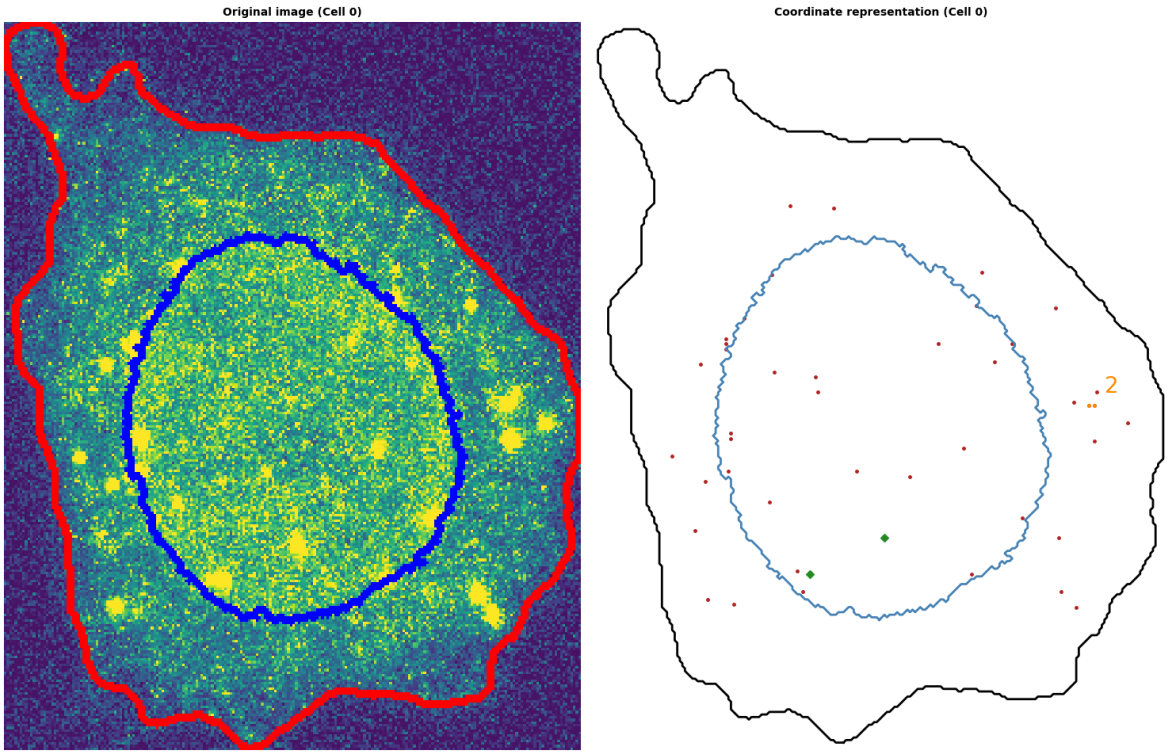


(a) Representation of the cell's coordinates in FISH-tagged ARAG mRNA,including transcription sites(Green), Foci sites("2") and other cellular compartments (Red spots). Also depicted the cell boundary (Red on the original-Black on the coordinate representation) and nucleus boundary(Blue) lines.

	cell_id	cell_area	nuc_area	nb_rna	nb_rna_in_nuc	nb_rna_out_nuc	nb_foci	nb_transcription_site
0	1	50198	16821	59	24	35	5	4

(b) Extracted nuclear and cytoplasmic mRNA quantification of the cell

Fig. 4: JQ1 drug affected single cell segmentation and analysis with Big-FISH package.



(a) Representation of the cell's coordinates in FISH-tagged ARAG mRNA,including transcription sites(Green), Foci sites("2") and other cellular compartments (Red spots). Also depicted the cell boundary (Red on the original-Black on the coordinate representation) and nucleus boundary(Blue) lines.

	cell_id	cell_area	nuc_area	nb_rna	nb_rna_in_nuc	nb_rna_out_nuc	nb_foci	nb_transcription_site
0	1	40652	14371	42	17	25	1	2

(b) Extracted nuclear and cytoplasmic mRNA quantification of the cell

Fig. 5: TSA drug affected single cell segmentation and analysis with Big-FISH package.

Question 5: Are the mRNA counts in the nucleus and in the cytoplasm maintained around the same proportion in each condition? If not, how would you describe the drug efficacy with respect to the spatial information of the mRNA counts?

The mRNA counts of the nucleus/cytoplasm ratio was similar between the two drugs (~1.45) with the TSA showing even lower number in total counts. However this ratio was different in the DMSO-control cell being around x1.5 higher. Based on our previous theories in Q4 and our current calculations, we could measure the drug efficacy based on the spatial information of the mRNA counts, with lower ratio numbers showing greater drug effect, relying also in the total counts of the cell and maybe taking into account the nucleus/cytoplasm surface, if we could prove that different cell area sizes could affect the abundance by measuring more cells from each condition.

Question 6: Write a conclusion about which drug has a greater treatment effect (based on your answer for Question 5)?

Because we only have 1 cell from each condition and we cannot be sure on how area affects the localisation of the specific mRNA, thus based on just the spatial information and the total counts we could chose TSA as the most effective drug, showing also less spotted transcription sites and foci's.

Question 7: Do you think making a conclusion based on one cell per condition is an ideal approach? Why and why not?

Deriving conclusions from only one cell per condition is not considered as the best way to get quality and trustworthy results. Optimally, we should include more cells with a possibly a greater variety of areas and time-points. Then we could truly measure if the nucleus/cytoplasm area affects plays a role on the abundance or if the difference is an effect of the drug. We would also validate our mRNA count measurements and retrieve a clear and solid idea of how each drug affects the expression of the ARAG gene and the cell in general.

References

1. Chen, J., McSwiggen, D., and Ünal, E. Single molecule fluorescence &ltem&gtin situ&lt/em&gt hybridization (smFISH) analysis in budding yeast vegetative growth and meiosis. *Journal of Visualized Experiments*, 135 (May 2018).
2. Cougot, N., Babajko, S., and Se
raphin, B. Cytoplasmic foci are sites of mRNA decay in human cells. *Journal of Cell Biology 165*, 1 (Apr. 2004), 31–40.
3. Imbert, A., Ouyang, W., Safieddine, A., Coleno, E., Zimmer, C., Bertrand, E., Walter, T., and Mueller, F. FISH-quant v2: a scalable and modular analysis tool for smFISH image analysis.