

Image Processing and Quantitative Image Analysis

Assignment C

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1 Image data inspection

1.1 Question 1

The FISH-tagged ARAG mRNA can usually be found in three places; the nucleus, the endoplasmic reticulum (ER) and the cytoplasm. This happens because after it is transcribed from DNA in the nucleus, is then exported to the cytoplasm or the ER.

2 Image processing workflow

2.1 Question 2

There are many differences between Figure 1 and 2 for each of the three treatments. In both cases, we are trying to identify the fluorescently labeled molecules. The main difference is how we approach this problem.

In Figure 1 (left) for instance, we are detecting the spots using the local maximum in the smFISH channel, represented by a red circle. The problem is that the spot regions are too dense, hence more than one spots identified as a single spot. In Figure 1 (right), instead of finding the local maximum, we decompose these dense regions to solve this problem, and this is achieved by using the `detection.decompose_dense()` function.

The function decomposes the smFISH channel by estimating and removing its background. Then, it creates a reference median spot from the unique pre-detected spots. A Gaussian signal is fitted on the reference spot. The function then detects candidate dense regions in the denoised image and the fitted Gaussian signal is used to fill as many spots in the candidate regions as possible.

2.2 Question 3

Spots: They are single molecules of mRNA. Each spot represents a local maximum in the smFISH channel, meaning it has a higher intensity than its neighbors.

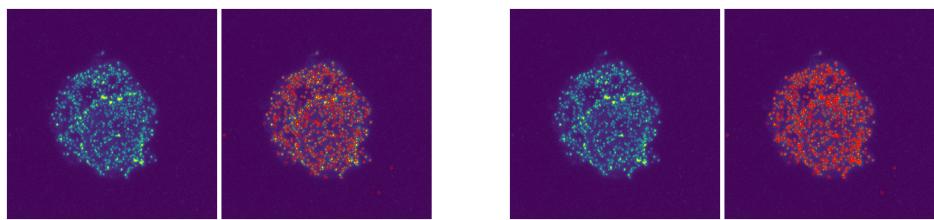


Fig. 1: DMSO - Spot detection before (left) and after decomposing the regions (right)

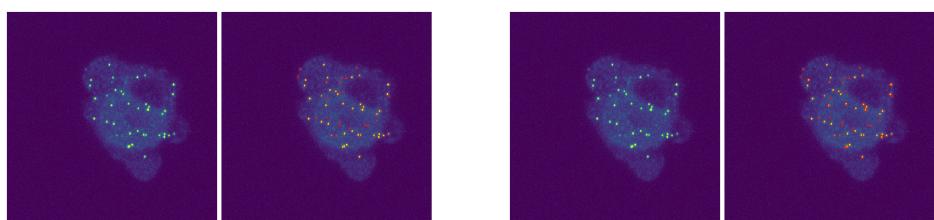


Fig. 2: JQ1 - Spot detection before (left) and after decomposing the regions (right)

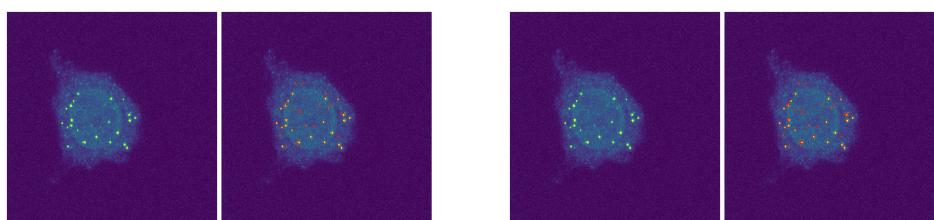


Fig. 3: TSO - Spot detection before (left) and after decomposing the regions (right)

Clusters: They are regions where multiple mRNA molecules are localized together. Clusters are detected based on a specific radius and a minimum number of connected spots.

From a biological perspective, clusters are important because they represent different biological functions. One example would be a cluster of mRNA molecules which shows a region of active transcription, where multiple mRNA molecules are being produced from the same gene.

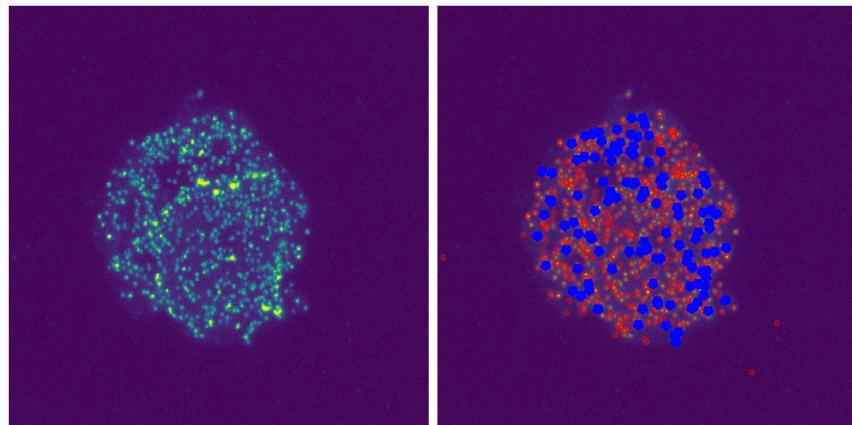


Fig. 4: Spots vs Clusters for DMSO

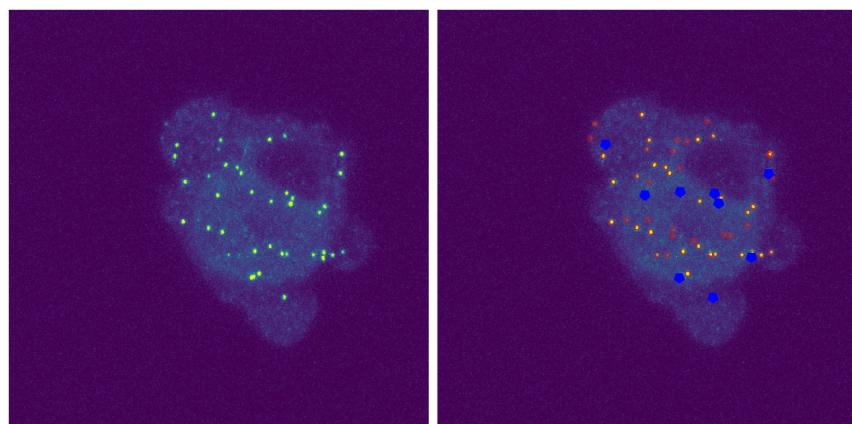


Fig. 5: Spots vs Clusters for JQ1

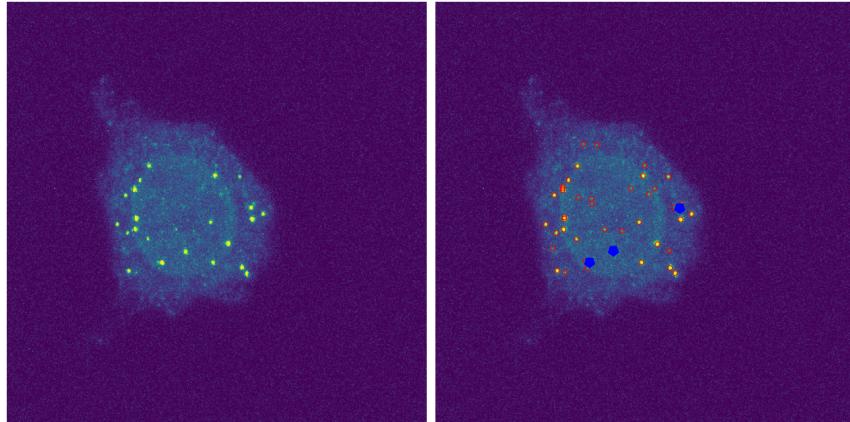


Fig. 6: Spots vs Clusters for TSO

3 Data analysis and discussion

3.1 Question 4

Table 1 provides the summary of the results for each condition. What we can see is that DMSO has the highestt number of RNAs, inside and outside the nucleus, as well as the most foci and transcription sites. This indicates that DMSO might be connected with higher RNA activity.

Figures 7-9 show the spatial distribution of the detected spots and clusters within each cell. Again, DMSO has a more dense distribution of RNAs and foci, which supports the results from Table 1.

Metric	DMSO	JQ1	TSO
Cell Area	50332	50198	40652
Nuc Area	19590	16821	14371
Nb RNA	940	59	42
Nb RNA in Nuc	298	24	17
Nb RNA out Nuc	642	35	25
Nb Foci	72	5	1
Nb Transcription Site	39	4	2

Table 1: Summary of cell data for different conditions

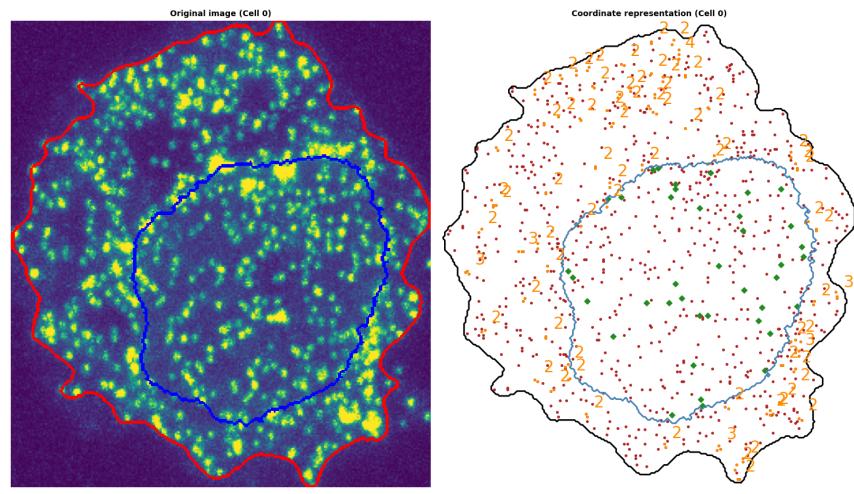


Fig. 7: Cell information - DMSO

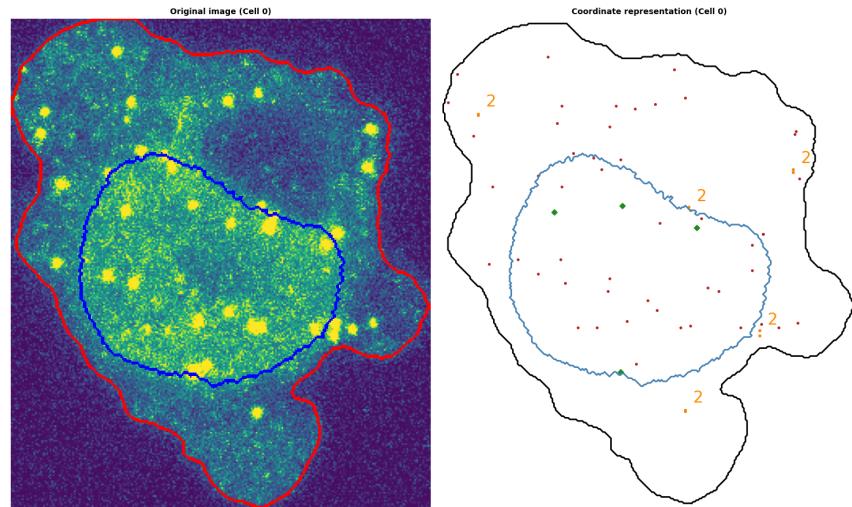


Fig. 8: Cell information - JQ1

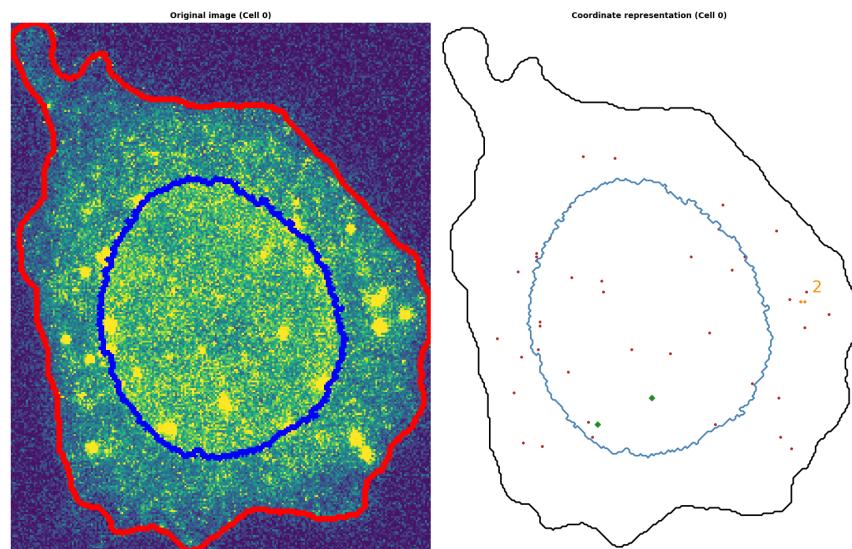


Fig. 9: Cell information - TSO

3.2 Question 5

The number of mRNA in the nucleus and cytoplasm isn't the same for each condition. How well the drug performs can be observed by how much it reduces the mRNA count or if it transfers a load of mRNAs from the nucleus to the cytoplasm. The less mRNA counts it achieves, the better the drug.

3.3 Question 6

Based on the results of this experiment, it seems that JQ1 has a greater treatment effect in contrast to DMSA and TCA. This results from the fact that it reduces the number of mRNAs in both the nucleus and cytoplasm.

3.4 Question 7

Making a conclusion based on one cell per condition is not an valid approach, because it does not consider all variability of the cells and may not represent the total population. To make valid assumptions about drug efficacy, we need to analyze a large number of cells for each condition which would allow us to have a get accurate estimation.

References