ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Region Of Interest

Image Processing & Analysis for Life Scientists

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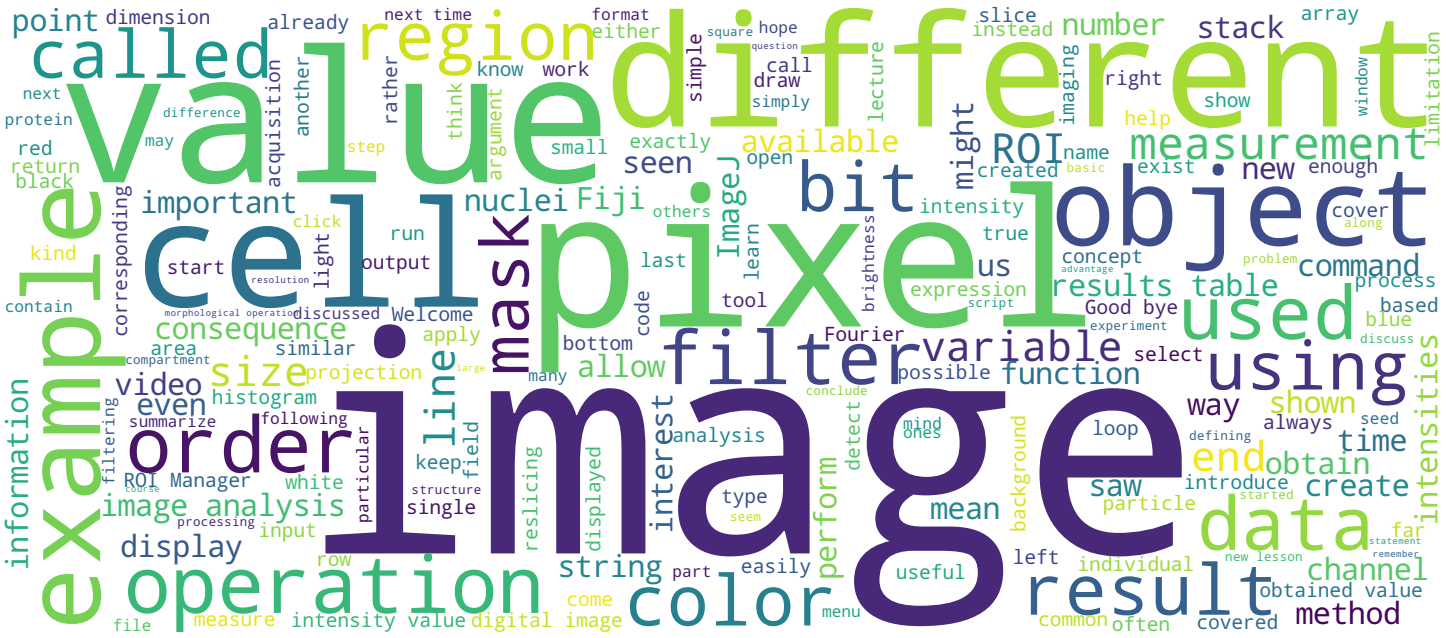

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Outlook

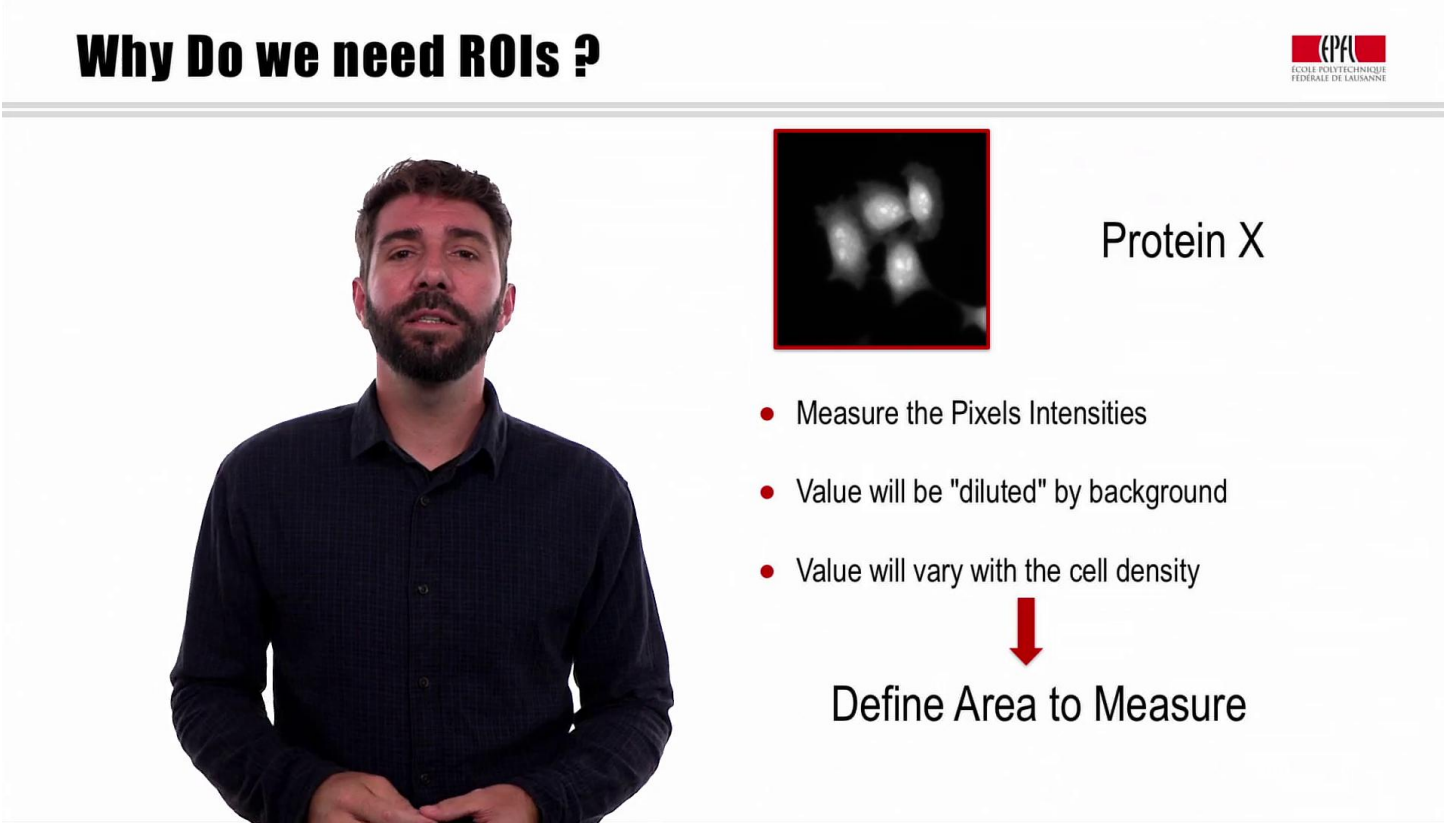
- Motivation
- Region of Interest (ROI) Operation
- Limitations

Welcome to this new lesson. Together we'll learn a bit more about Regions of Interest, or, as we like to call them, ROIs. We will see how similar and different they are from masks. Why we need them to make accurate measurements. And also how to manipulate them; before we conclude with the limitations that exist within Fiji.

Notes

Summary

0m 05s

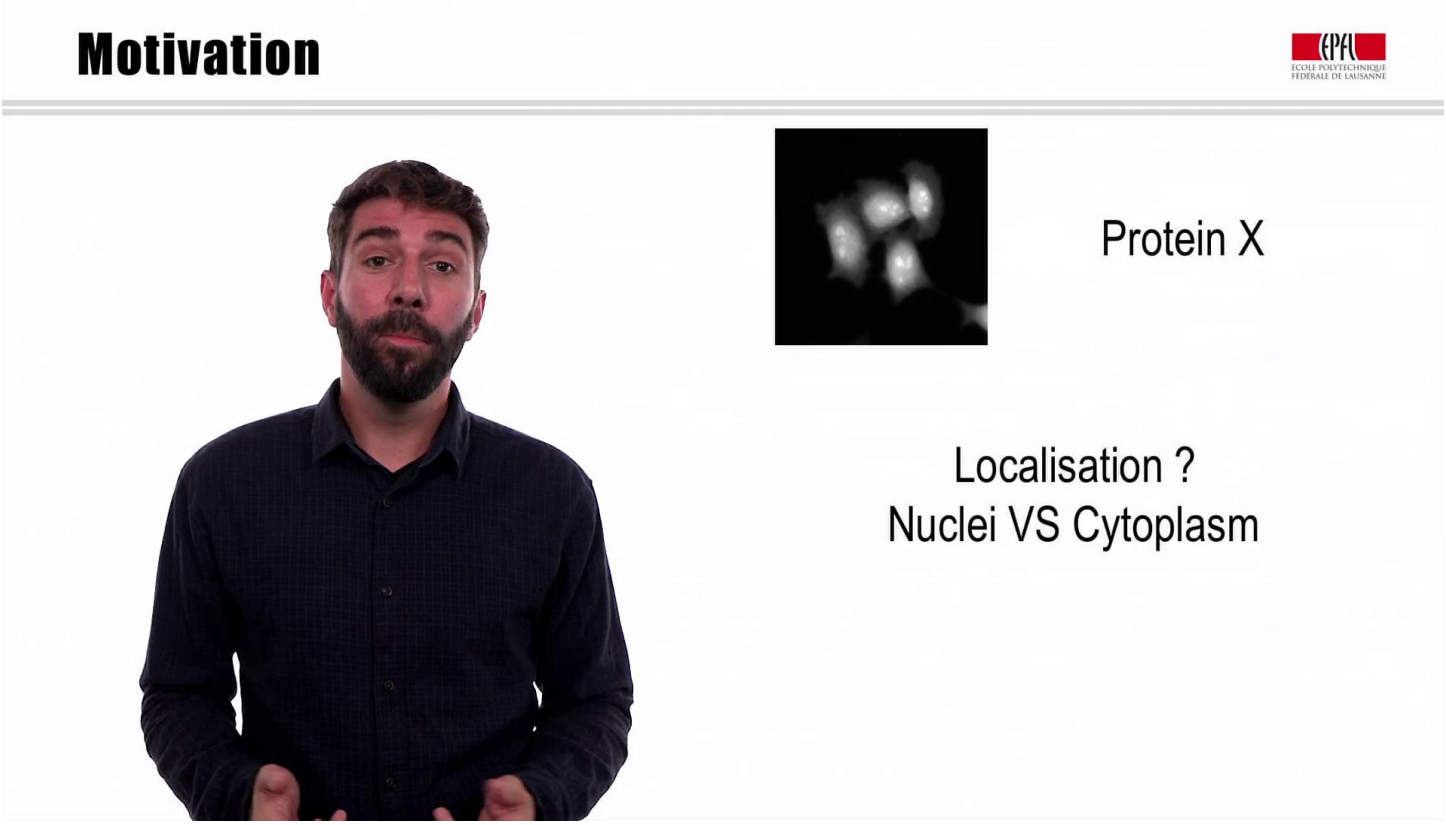


To start, let's consider a protein of interest called X. It is expressed within the cell. And we would like to compare different experimental conditions. If we measure the intensities over the overall image, the first consequence is that the obtained value will be diluted by the background. Because these pixels have intensities close to 0. And the consequence of this consequence is that the obtained value will vary if we have more or less cells in the field. So we need to define a region, an area, before doing the measurements, like a mask as seen before.

Notes

Summary

0m 28s



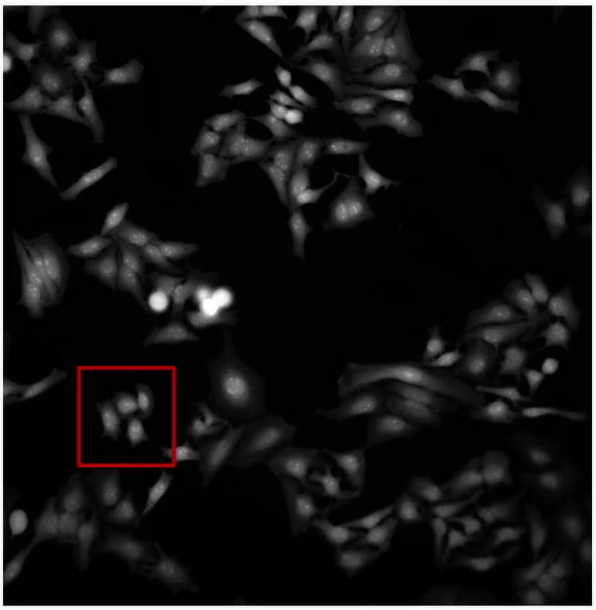
Now let's add another level of complexity. We will also want to look at the signal localisation. Do we have different values between the nuclear or the cytoplasmic regionsx? You might think that we just have to draw the different compartment. Because it's not so difficult to recognize the nuclei on the image. And we can also draw quite accurately the contour of the cells.

Notes

Summary

1m 14s

Motivation



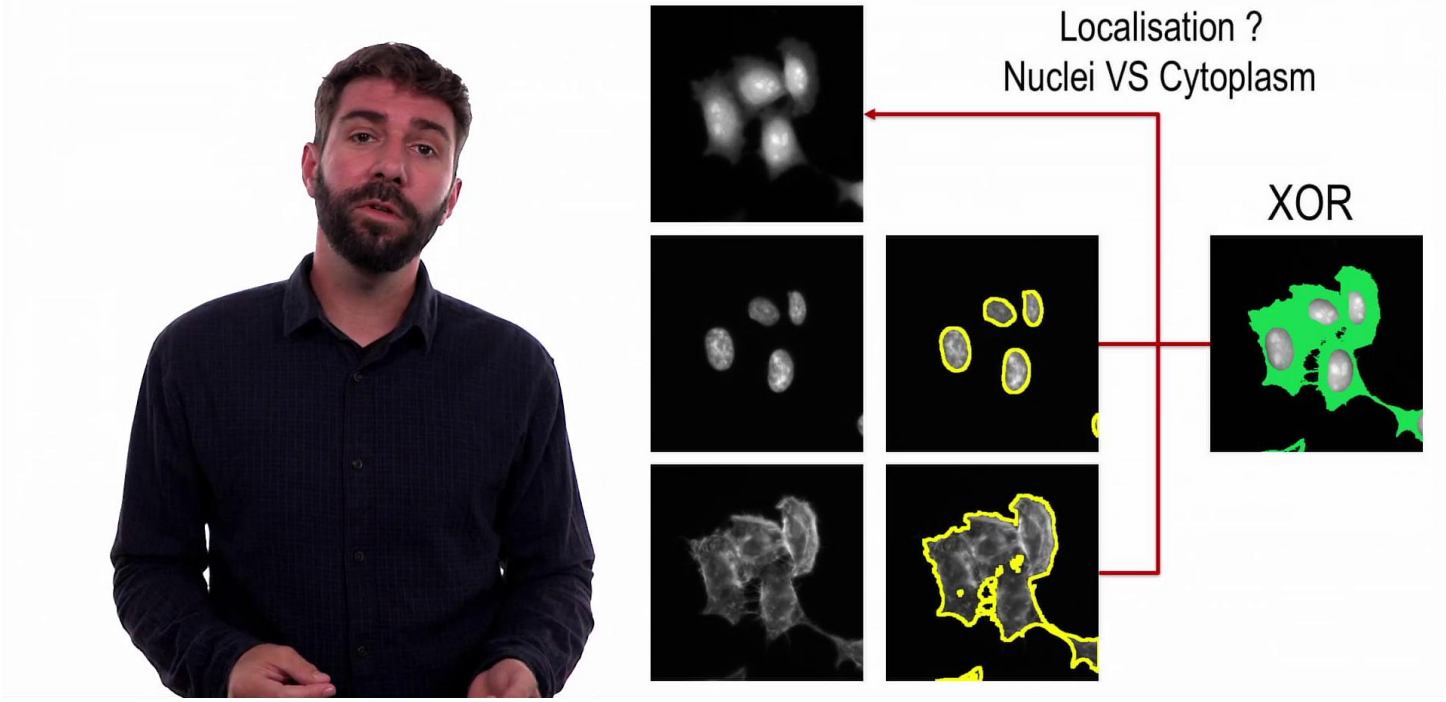
But what if your image is not just this small region?

Notes

Summary

1m 43s

Motivation



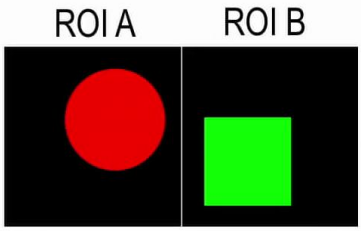
We saw during the previous weeks that we can segment the images and get the masks, and finally get some measurements on some other images. Considering we want to distinguish nucleus from cytoplasm values, the region at the bottom is not exactly what we need. Fortunately, we can manipulate ROIs like we do with masks and create a new one defining the cytoplasm. To do, so we just have to subtract what the nuclei and the whole cell region of interest have in common.

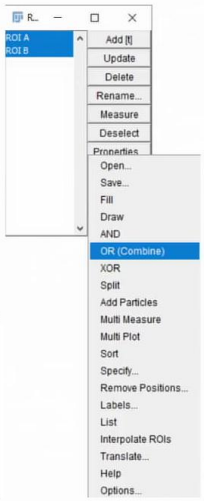
Notes

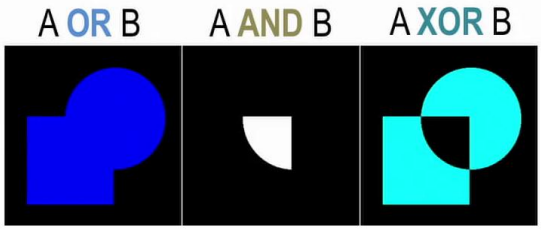
Summary

1m 49s

ROI Operations







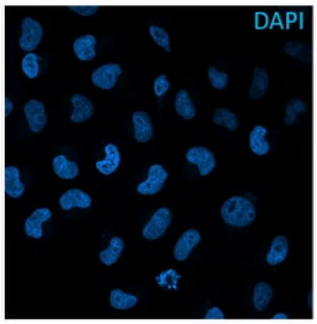
This is done using the so called XOR operator. This operation is accessible in the ROI Manager when you click on More.

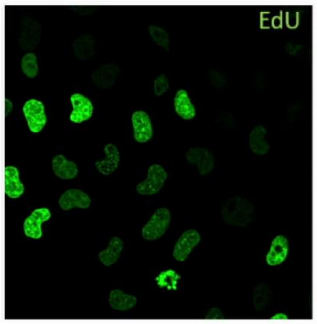
Notes

Summary

2m 28s

Individual ROIs





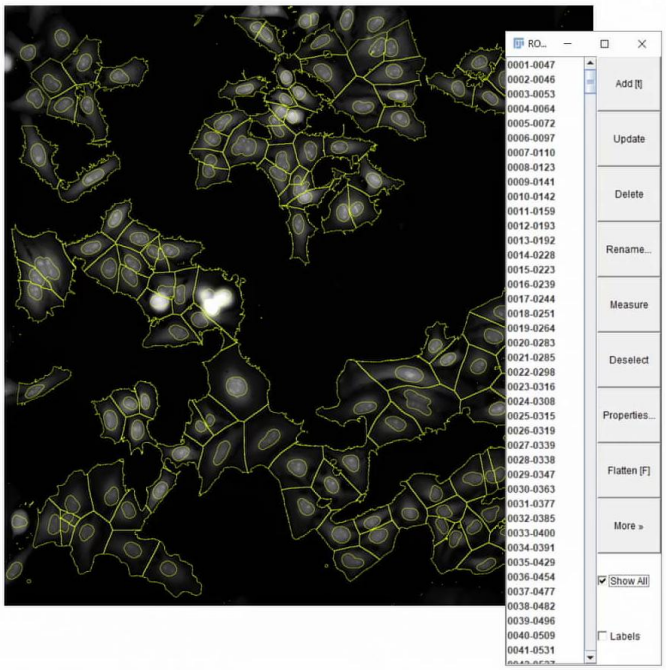
But now, what if we wanted to go even further. You can see on these 2 images that the cells can have various levels of intensities. So perhaps it's not enough to look at the value within the population. But we should look at the values for each individual cell. So now, instead of using a single mask, we can make individual ROIs for each cell and their compartments.

Notes

Summary

2m 39s

Motivation



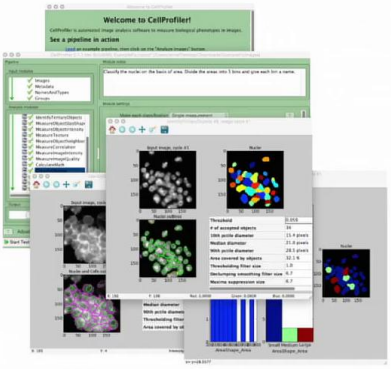
And you might foresee a small problem with this large image. Indeed we will populate the ROI Manager with different ROIs per cell, for hundreds of cells. And as a consequence we will obtain a results table crowded with unrelated ROI measurements. Now, even if it's possible in ImageJ to relate the ROIs one to another, it will involve tedious work with ROIs and result table manipulations.

Notes

Summary

3m 12s

CellProfiler



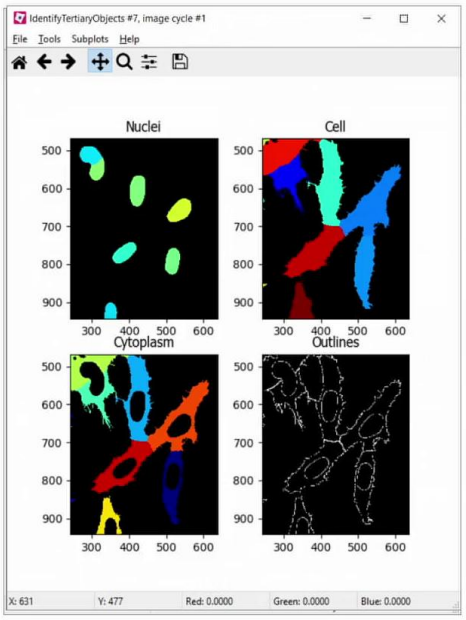
So, for this kind of image analysis scenario, we encourage you to have a look at cell cellprofiler.

Notes

Summary

3m 44s

Cell Profiler



It's an open software freely available that will allow you detect the nuclei as primary objects, use them as seeds to detect the cells as secondary objects, and finally create cytoplasm ROIs by combining the 2 others. Doing so, we will be able to export results table where all the objets and measurements are tagged and can be more easily manipulated.

Notes

Summary

3m 51s

Conclusion

- Need for ROIs
- ROI Manipulation
- CellProfiler an adapted solution for Complex Objects Relationships

Here we are! This is the end of the lesson about the ROIs. We saw together why one might need regions of interest, and how we wan manipulate the created one. Finally, we also saw that other software exist to handle complex relationship between your objects. Good bye! And see you next time!

Notes

Summary

4m 21s