





- Field Of View VS Individual Objects
- Independent Channel

We saw previously that we could get some measurements from the overall population, or from individual objects, within this population. In this short video, we will see why it could be necessary to have independent channels. when you want to detect objects.

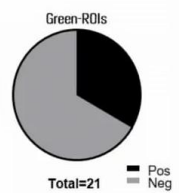
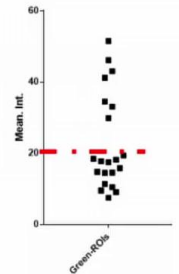
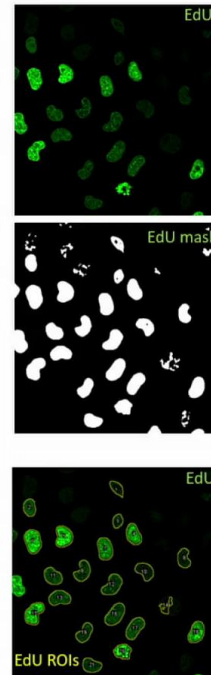
Notes

Summary



0m 04s

# Getting Positive Cell Number



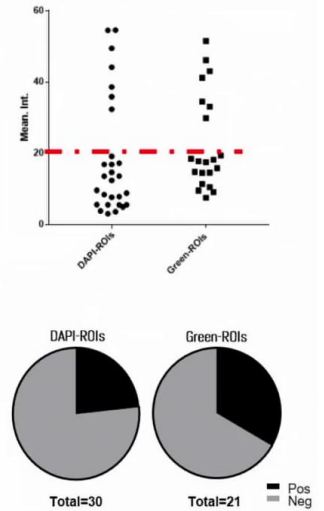
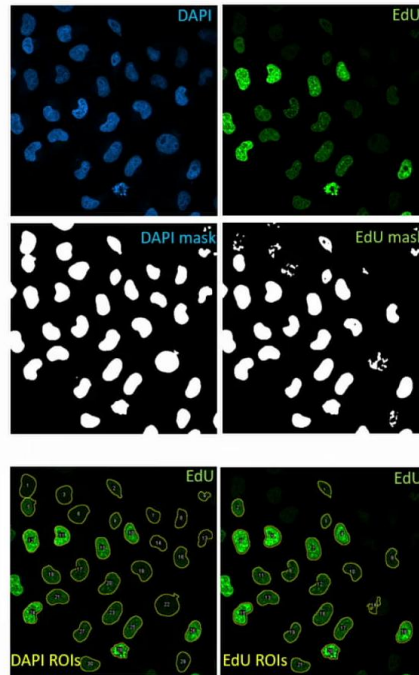
Suppose we have stained some cells with Edu (let's simplify it to a cell division marker) and we want to know how many cells are 'Positive' for this staining. As we saw previously, we could threshold the image, maybe clean it a little bit, then get some ROIs and measure their intensities. We could make a nice graph and get our answer! Do you think we did a good job ? Do you see any issue in our workflow? You can guess that there is a problem, otherwise I would not insist so much...

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# Getting **Unbiased** Positive Cell Number



To better understand the issue, let's do the same steps, except that now we will have an independent channel. Here DAPI, that stains all the cells. We make a new mask, and you already notice something... You can see the ROIs we obtained from the DAPI image and those obtained from the EdU image. You see all the red marks? They are the missing cells from the EdU image. The problem is, by detecting the cells on the channel we wanted to measure, we have missed all the cells with very low intensities!! Due to this mistake, we have underestimated the number of negative cells. And thus we have overestimated the number of positive cells.

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# Conclusion



Ok! This is the end of this short video. I hope I convinced you to remain skeptical about your results, and to always have a channel that is independent from the effect you are trying to measure ! Thank you and goodbye !

Notes

Summary



1m 56s