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Comp Bio

Image Processing Module

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1. *Where do you see image analysis fitting into your future project? Are there specific techniques or types of data you hope to collect from your images?*

Although my project is yet to be defined, I work in molecular biology and can imagine that I will be doing plenty of RNAseq experiments. Gene expression can vary between cell types and in response to stress levels. Using techniques like RNAscope, I could label the RNA transcripts and then use cell profiler to identify which cells have taken up those transcripts. Cell profiler would be a great tool to quantify the number of cells containing different fluorescently labeled transcripts as well as to see if any colocalization is happening.

1. *What was the most challenging part of working through the example in CellProfiler? Why was it challenging?*

The example was straight forward (and designed to be that way), but I did run into some issues when generating my own pipeline. The most challenging part was figuring out what order to add modules to get the desired effect. I got a pretty cool 3D looking cell when I added the enhance or suppress features. I figured I would be able to use the identify primary objects module after, but cellprofiler wanted to identify the background instead. I then had to use the first image that was uploaded and play with settings in the module in order for it to identify the cells I wanted to measure. So, it appears that using this program can be a lot of guess work until I become proficient.

1. *Find an image from a recent paper you read. What type of data did they collect from the image and what technique/software did they use to analyze it?*

[This paper](https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0035157&type=printable) has some cool images of cancerous lung tissue . In figure 8 they show images of normal lung and lung tumor side by side. They used ESAM (antibody) staining. It says that they used a CellProfiler algorithm to identify ESAM-positive cells. They also use CellProfiler to quantify and score single cells for ESAM by defining cells with DAPI+ nuclei and ESAM+ immunofluorescence after a flat illumination field correction.

Mehan MR, Ayers D, Thirstrup D, Xiong W, Ostroff RM, Brody EN, et al. (2012) Protein Signature of Lung Cancer Tissues. PLoS ONE 7(4): e35157. <https://doi.org/10.1371/journal.pone.0035157>

1. *Describe an image analysis platform we haven’t discussed in class. What types of images can be analyzed and what types of analysis are possible?*

Orbit is a free open source software that is most useful for large format images such as entire slides of tissue. It can be hooked up directly to a microscopy image server or on a cluster or local computer. This software is cool because it takes your whole slide image and creates a series of tiles that can be looked at through different resolutions. This “tiling” capability allows you to identify a region of interest ( single cell) and send it to CellProfiler for processing and then can be read back into orbit where they can be visualized.

The features include tissue quantification, object and cell segmentation object classification and defining region of interest. It is built for large images up to gigapixels.