1. Where do you see image analysis fitting into your future project? Are there specific techniques and/or types of data you hope to collect from your images?  
     
   Given that I am still doing rotations, I’m not entirely sure what my future project would be. If I happen to join the lab of Soham Ghosh, however, I would use fluorescence microscopy quite frequently to image mesenchymal stem cells. I would be looking at actin and lamin fibers within the nucleus and how they change when the cells are grown on different substrates.
2. What was the most challenging part of working through the example in CellProfiler? Why was it challenging?  
     
   This was probably trying to figure out how to make cell profiler label actin and myosin as objects correctly. It didn’t seem very intuitive that you couldn’t just give it a specific hexadecimal color code and say, “treat all colors brighter than this color as signal and all colors darker than this color as background”. I think I found a workaround by first measuring overall image intensity then setting the threshold as the upper quartile of that distribution, but I wish that it could have been finer tuned than that because a lot of “background” was still included in the primary objects.
3. 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? Include references.  
   A collage of images of blue and green spots

   Description automatically generated  
   Fluorescence microscopy image of HCT116-RAD21-mAC cells with Hoechst staining of the nucleus and mClover expression (co-expressed with RAD21, a subunit of cohesion). The images show that the researcher’s auxin inducible degron of cohesion works. They used time-lapse wide-field fluorescence microscopy using a DeltaVision OMX microscope. Images were deconvolved using the built-in software SoftWoRx and brightness/contrast was increased in Photoshop.

Rao *et al*. (2017). Cohesin Loss Eliminates All Loop Domains. *Cell*, *171*(2), 305–320.e24. https://doi.org/10.1016/j.cell.2017.09.026

1. Describe an image analysis platform we haven’t discussed in class. What types of images can be analyzed and what types of analyses are possible? Include references.  
     
   Cytomine – open-source web-based platform that can do image analysis and annotation. Is useful for sharing with a large team of researchers since it is hosted on the internet instead of a PC. Can analyze multi-gigapixel images. Uses machine learning algorithms to detect tumors, determine tissue boundaries, and count objects such as cells marked with a tagged antibody.

Rollus *et al*. (2016). Collaborative analysis of multi-gigapixel imaging data using Cytomine. *Bioinformatics (Oxford, England)*, *32*(9), 1395–1401. https://doi.org/10.1093/bioinformatics/btw013