**Islet Segmentation Overview:**

* Step 1. Prepare for ilastik training.
* Step 2: Train pixel classifier
* Step 3: Segmentation
* Step 4: Measure and export
* Step 5: Peri-islet identification
* Step 6: Cell type enrichment per region

**Notes:**

1. Image format (x,y,c) = (1000,1000,1).
2. Pipeline built under Cell Profiler version 3, ilastik 1.3.3, and R 4.0.2.

**Islet Segmentation Overview:**

Step1: Prepare file for ilastik

* Single channel image is processed in a similar way as described in the cell segmentation workflow.
* Specifically, the steps are:
  + Single hot pixels are removed.
  + Images are sacled up by 2x.
  + Images are saved as .h5 files.
* Note: Thinking back the analysis, we believe scaling up the image by 2x might not be necessary for islet segmentation.

Step 2: Train a pixel classifier for identifying islet vs background:

* The set-up is similar to the nuclei-membrane classifier used for cell segmentation, except the input file is single-channel tiff file.
* H5 files generated from step 1 are loaded in Ilastik and the model is trained with 6 images.
* The trained model is included in the folder, it outputs a pixel probability for being classified as islet.

Step 3: Segmentation using probability map generated from ilastik.

* Probability maps generated from step 2 are loaded in cell profiler for object segmentation.
* In Metadata setup, assumes the input file has name matching this pattern: “<Name>\_164Dy\_CD99.ome\_s2…”. Name metadata is extracted this way and used to save output islet mask. Make sure to change this if your filename has a different pattern.
* Specifically, the steps are:
  + Probability map is resized back to original dimension.
  + Primary object is identified.
  + Objects are converted to images, as islet masks.
  + A threshold module is used to differentiate positive signal vs. negative.
  + The max radius for islet masks is measured and ones with less than 10 pixels in max radius and ones with area less than 50 pixels are filtered out.
  + Islet masks are saved, where islet signal is represented by 255 while background signal is 0.

Step 4: Measure and export

* Cell masks are loaded back into cell profiler for measuring islet location and size.
* This script outputs an excel file.

Step 5: Peri-islet identification.

* In our manuscript, we divided the image into three regions: intra-islet, peri-islet, and exocrine. Islet measurement generated from step 4 indicates intra-islet region.
* To identify peri-islet region, we expanded islet mask by 30μm outward, and subtract it by the islet mask. In this way, we will get a ring around the islet, indicating peri-islet region.
* All other pixels will be exocrine region.
* The script demonstrating this workflow is included.

Step 6: cell type enrichment per region

* One analysis we performed in the manuscript is to find the number of cells in each of the three regions, normalized by region area. An R script that performed this analysis is included here.