

**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 scaled 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 30 $\mu$ m 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.