

## **Collagen Segmentation Overview:**

To test whether collagen enrichment is associated with disease duration or islet composition changes, we identified “relative islet collagen area”, which is the percentage of collagen positive region around and inside of the islet.

Specifically, this is done in several steps:

1. we expanded islet mask (generated from islet segmentation) by 50 $\mu$ m
2. Identify collagen-positive region using IdentifyPrimaryObjects module in cell profiler, by Otsu thresholding method to determine a cutoff to divide pixels into collagen-positive vs. collagen-negative.
3. Lastly, the pixels that covered by expanded islet mask and collagen mask are identified as relative islet collagen region. And the area is normalized by expanded islet cell mask.

Please see the CellProfiler script for all the details.