Robust Identification of Islets with Variable Morphology in H&E-Stained Pancreatic Tissue Using HALO AI™

INTRODUCTION

The insulin-secreting beta cellsand glucagon secreting alpha cells that comprise the pancreatic Islets of Langerhans play a fundamental role in glucose homeostasis and are therefore critical in the study of the hyperglycaemic metabolic disorder, diabetes. Many environmental toxicants and chemotherapeutics have been associated with the development of diabetes or have been shown to disrupt pancreatic beta cells in animal models.[1-10] Histopathological evaluation of islets in pancreatic tissue is critical for understanding mechanisms of metabolic disease and evaluating the underlying cause of toxicity; however, the variable morphology of islets in hematoxylin and eosin (H&E) stained tissue can make manual identification and evaluation a laborious and time intensive task for the pathologist.

In this application note, we train the HALO AI™ VGG convolutional neural network (VGG-CNN) to identify islets within pancreatic tissue sections following H&E staining. We demonstrate that it is possible to build a robust classifier to accurately segment islets from surrounding exocrine tissue, irrespective of stain or morphological variability. This study highlights the potential for HALO AI to simplify the pathological evaluation of pancreatic tissue in metabolic research and toxicological pathology.

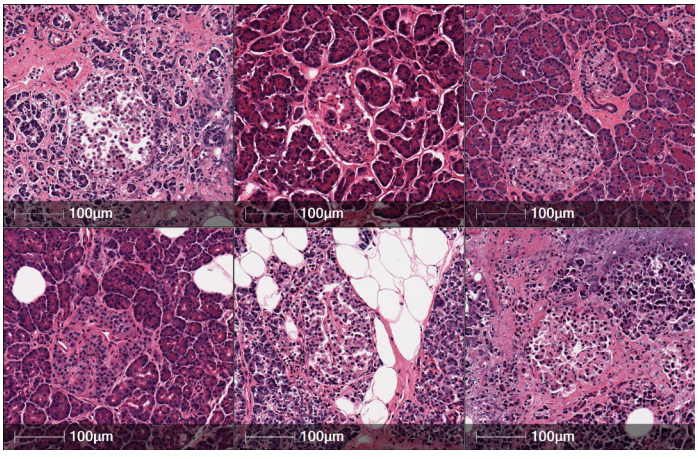

Figure 1. Variability of islet visualisation in H&E pancreas tissue sections. The top row displays examples of islets within the training set and the second row displays those in the test set.

METHODS & RESULTS

Thirty (30) H&E images of human pancreas tissue acquired from the open source Genotype-Tissue Expression (GTeX) Program* were used for training and testing HALO AI's VGG-CNN. Tissues were from both male and female patients and contained varying degrees of autolysis and saponification. Islet visualisation varied across both the training and test sets as shown in **Figure 1**. To ensure the classifier would be able to accurately detect islets across a large cohort, as much variability as possible was incorporated in the training dataset. The classifier included only two classes, islet and background. For this application, both exocrine tissue and white space were placed into the background class. Twenty five (25) images were used for training and approximately 70,000 iterations were required for the VGG-CNN to converge on a solution. HALO's real-time tuning window was used whilst the classifier was training to visualise the progress of the classification training. Following training, the classifier was used to analyse whole slide images in the test set **(Figure 2)**. The classifier was able to segment islets accurately despite considerable slide-to-slide variability in stain intensity and morphology among slides in the test set.

The probability of a pixel belonging to a certain class is generated by the CNN in order to help define the class boundaries. Here, the default probability threshold of 50% was used. However, it is also possible for the user to assign a custom threshold within the HALO interface. In this application, it may be useful to assign a higher probability threshold (e.g. 80%) to achieve higher accuracy of classification at the borders of the islets **(Figure 3)**.

CONCLUSIONS

Islets in H&E sections can be extremely variable and therefore time consuming to locate, evaluate and count manually. Here, we demonstrate that the HALO AI VGG-CNN is a robust and automated method for islet detection in H&E stained pancreas tissue sections. The trained classifier was able to handle significant variability in islet morphology, stain color and intensity.

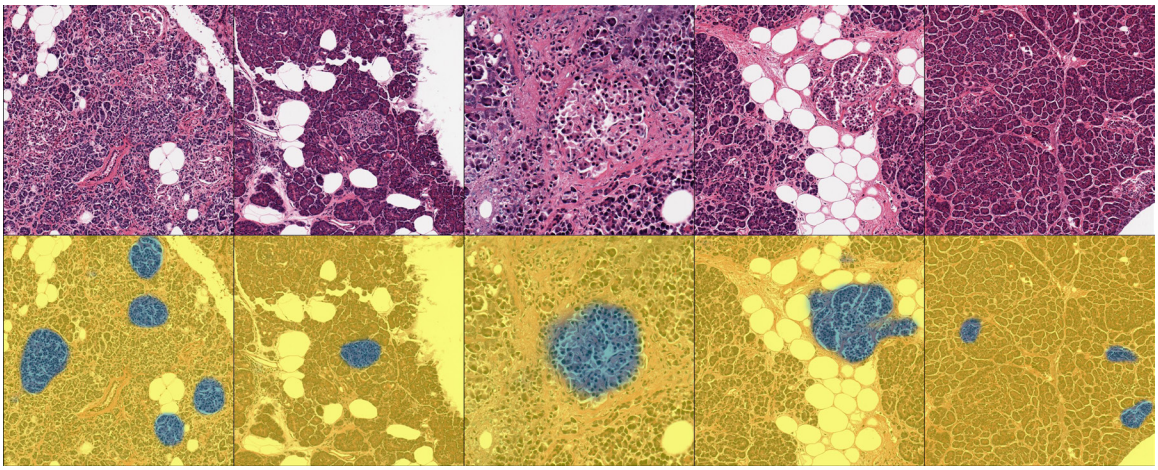

Figure 2. HALO AI classification of islets in H&E tissue sections. The original tissue is shown on the top row and the second row displays the corresponding HALO mark-up image with islets shown in blue and negative areas in yellow.

To shed light on the underlying mechanisms of diabetes and other metabolic disorders, it is common to stain pancreatic tissues with specific cell markers, such as insulin and glucagon, using immunohistochemistry (IHC) and immunofluorescence (IF). While this application note has used H&E images for islet detection by HALO AI, it is important to note that the VGG-CNN network can be trained in the same manner with IHC and IF-stained images. Additionally, if IHC has been performed on serial sections, the classification defined by HALO AI on the H&E images can be superimposed on the serial sections.

Once HALO AI has been used to detect the islets, one of HALO's cell quantification algorithms can be used to quantify up to 5 markers in bright field, and 30+ markers in fluorescence.

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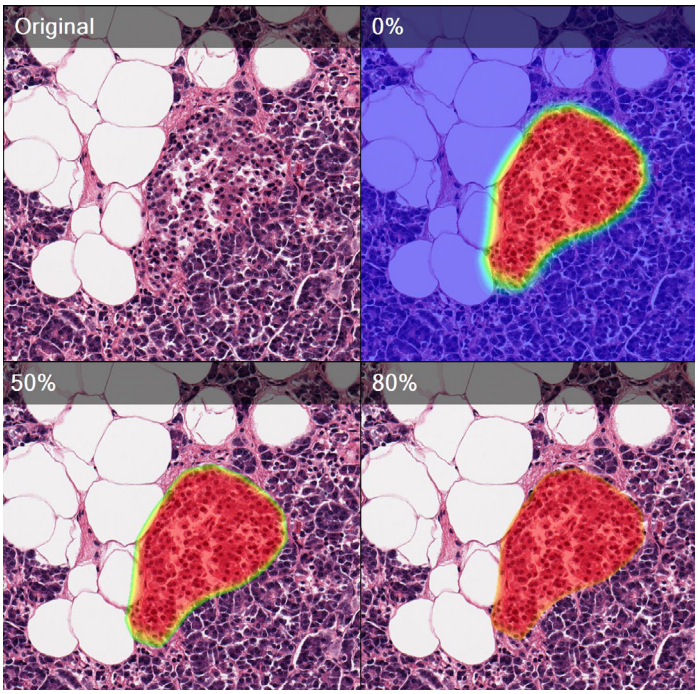

Figure 3. Setting a probability threshold in HALO AI for islet detection in H&E images. When the probability threshold is set to 0% the user will see a heat-map showing areas of the tissue with the highest probability of belonging to the chosen class (islet in this instance) in red and the areas with lowest probability in blue. When the probability is set to 50%, only areas in red and yellow are included in the islet class and at 80% only areas in red are included.

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For specific information about the image analysis methods used in this application note, please email our Application Support Team at info@indicalab.com.