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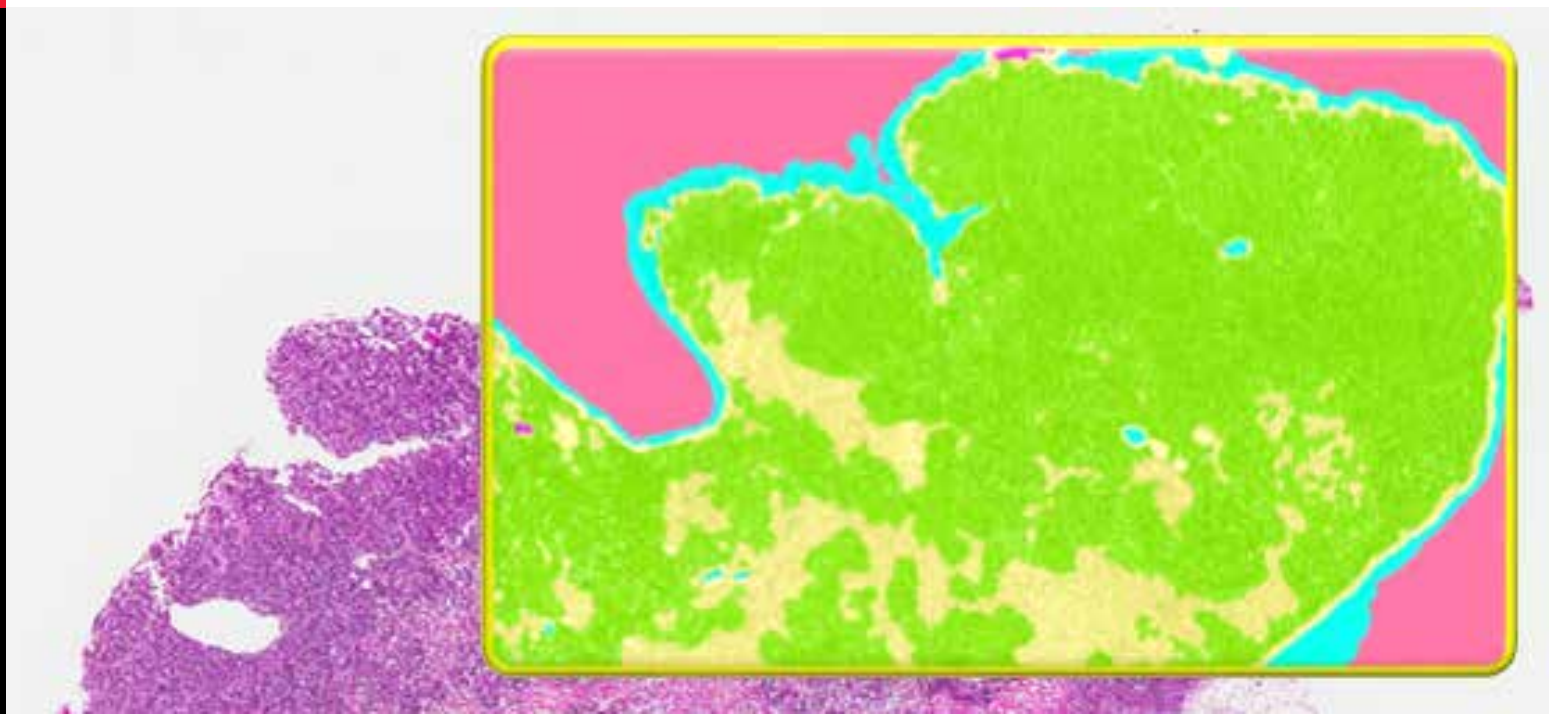

Use of Aperio GENIE Image Analysis Tool for Histopathology Pattern Recognition

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Abstract

Image analysis is a rapidly evolving and increasingly utilized tool in histology. It enables high throughput analysis of a large number of samples, offering the potential for increased precision, plus reduced workload for researchers. The ability of slide scanners to create high resolution whole slide images (WSI) of tissue sections gives rise to the need for quick and effective identification of areas of interest within these images. This review focuses on the use of Aperio GENIE, from Leica Biosystems, as an image analysis tool for automatic identification of different tissue morphologies and cell cohorts within WSI.

Introduction

Aperio GENIE is a general purpose, interactive, adaptive tool for automatically finding and classifying regions of interest (ROIs), such as tumor regions, in heterogenous tissue across large numbers of digital slide images. Instead of manually annotating areas of interest in all digital images, Aperio GENIE allows users to annotate a few representative contrasting areas within a slide set, which can be used as a training set. The training set is used as a reference by the algorithm to detect and distinguish these areas within the rest of the slides. Combined with other quantitative image analysis tools, Aperio GENIE enables highly automated whole slide analysis of immunohistochemistry (IHC) or *in situ* hybridization (ISH) staining in tumor areas (e.g. in entire tissue sections across large sets of slides).

Workflow

The typical workflow in Aperio GENIE follows two logical steps. Firstly, the workflow begins with the user creating a training set of images that Aperio GENIE will use to learn to identify tissue classes. Digital slides containing examples of the structures of interest are added to the training set. Example structures within the tissue are annotated by the user within the image viewer. All of the slides containing the annotated ROIs are added to a training montage that is used by the algorithm to distinguish the structures. The greatest advantage of this training step is that it enables the algorithm to learn based on the expert user's input. In addition, the algorithm is able to learn from multiple slides, allowing for differences in tissue heterogeneity and staining intensity, from slide to slide.

Secondly, a training macro is created and run on the montage images; this macro provides feedback to the users on how accurately the algorithm agrees with the expert's annotation.

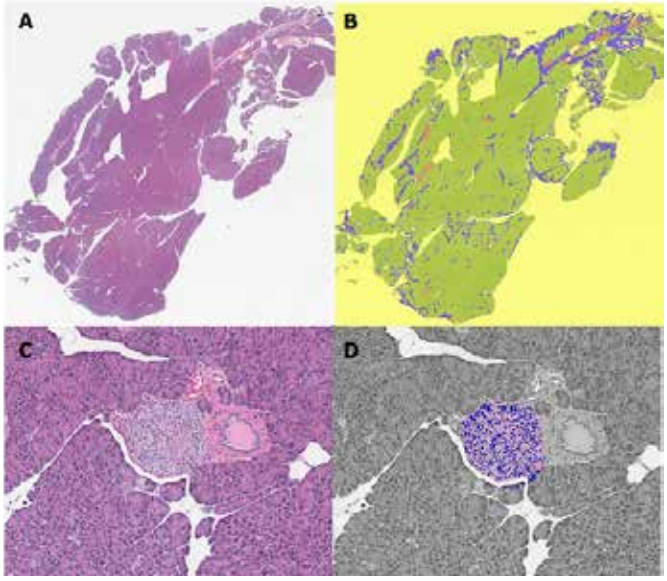

Figure 1: *Images A & B illustrate automatic detection and annotation of islets in pancreatic tissue stained with H&E using Aperio GENIE. Image A is the original digital slide observed at low magnification and, B shows the Aperio GENIE mask, where green is normal tissue, blue areas are ducts and red indicates pancreatic islets. Images C & D show the same tissue at higher magnification and illustrate the subsequent quantification of islet cells by Aperio Nuclear Algorithm, following automatic islet detection using Aperio GENIE.*

The algorithm reports the specificity and sensitivity, enabling users to ascertain if the algorithm has been trained optimally (Figure 2). Once created, the classifier can be applied to a region of interest within a slide (Figure 3), an entire digital image, or across sets of slides, enabling truly automated batch high-throughput analysis.

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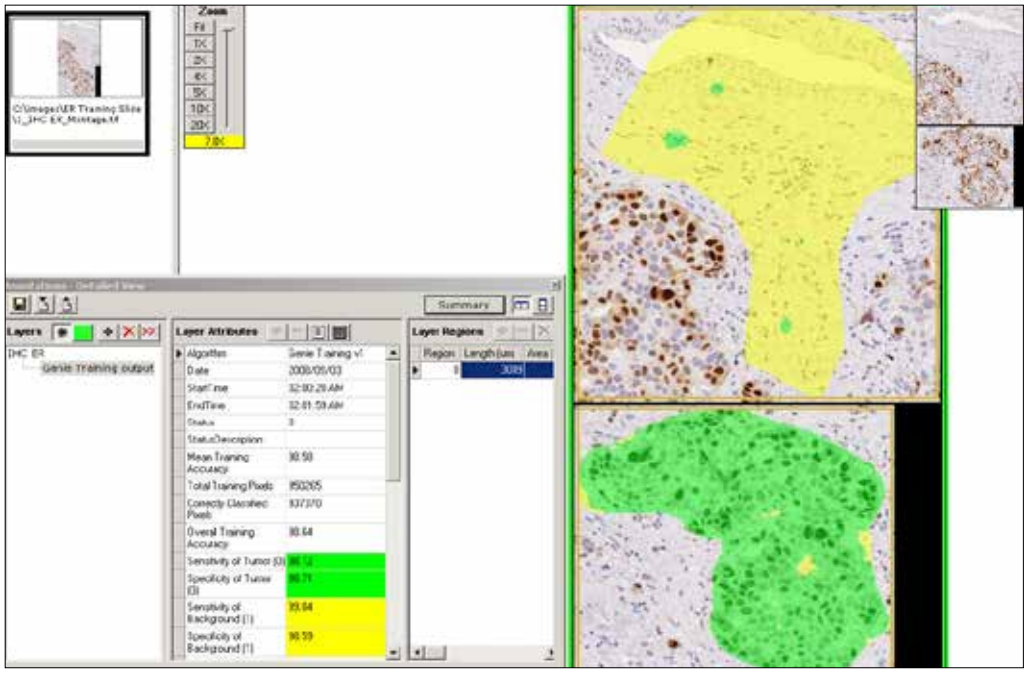

Figure 2: *Illustrates the output from Aperio GENIE Classifier. Areas in green have been identified as tumor and those in yellow are considered non-tumor. Resulting Sensitivity and Specificity demonstrates that there are high levels of concordance between the algorithm and the annotations made by the expert reviewer.*

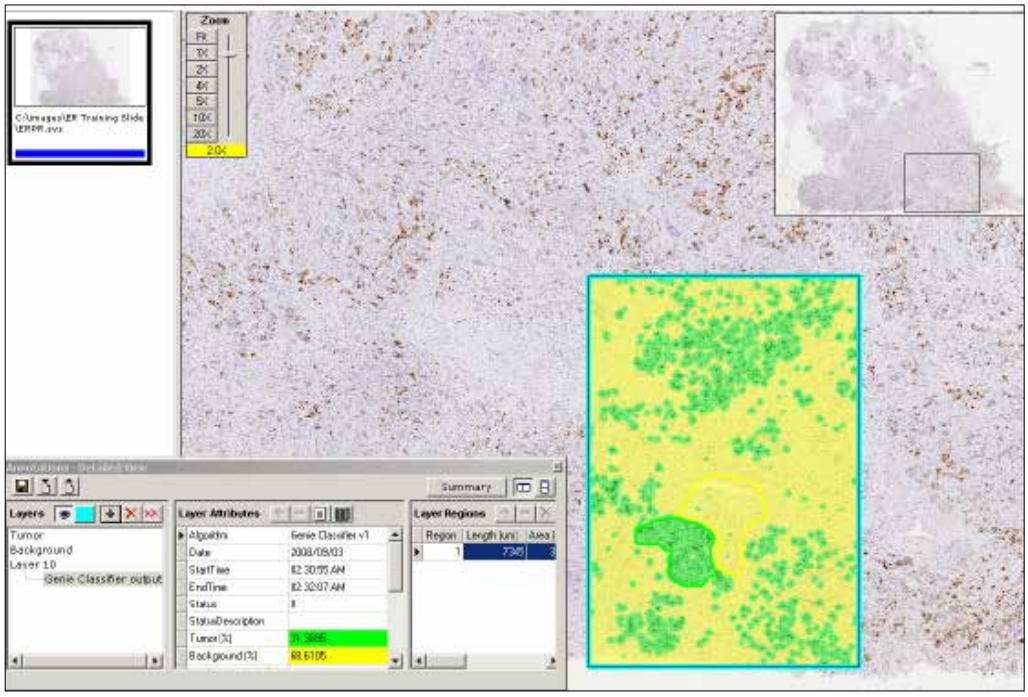

Figure 3: *Aperio GENIE has been run across a region of interest within a digital slide. The Tumor has been identified in the region. The results data include the amount of Tumor present and a markup image that can then be used as a pre-classifier for another quantitative algorithm.*

Use of Aperio GENIE Histopathology

Aperio GENIE has been most widely used in digital analysis of tumor pathology, predominantly in conjunction with other algorithms that allow quantitative analysis of IHC staining, such as the Aperio Nuclear and Membrane Algorithms. A study by Rizzardi *et al.*, (2012), assessed the ability of Aperio GENIE and another IHC staining algorithm to identify and quantify the expression of the

tumor biomarker S1001a in 225 ovarian tissue microarray (TMA) samples. A comparison of the IHC staining data obtained from manual annotations by a pathologist and by Aperio GENIE showed strong agreement, indicating that the algorithm was able to efficiently classify carcinomatous areas within IHC slide images. A reduction in workflow for the pathologist was also observed in the same study with 10-fold less pathologist time required using Aperio GENIE versus visual examination of each spot on TMA, a finding similar to one observed in a previous study by Ong *et al.*, (2010).

Several studies have assessed the usefulness of Aperio GENIE in combination with other image analysis tools in analyzing the expression of different biomarkers in breast tumors.

A study by Lloyd *et al.*, (2010) used Aperio GENIE and the Aperio Membrane and Nuclear quantification tools to identify tumor regions within breast cancer tissue sections, immunohistochemically stained for HER-2 and ER. The study found a close agreement in scoring for both biomarkers, with 23/23 of HER-2 positive cases and 10/10 ER positive cases falling within the scoring range established by the pathologist's visual assessment of the samples, and only minor variance between the algorithm and pathologist's scoring.

A study by Laurinavicius *et al.*, (2012) used Aperio GENIE and quantitative algorithms to assess the expression of a broad number of biomarkers, including ER, PR, HER2, Ki67 and p53 in 103 ductal breast carcinoma samples. Laurinavicius, *et al.*, 2012 found Aperio GENIE in combination with quantitative algorithms analysis to be an efficient tool for analysis of multiple IHC biomarkers, clarifying complex interdependencies in ductal breast carcinoma IHC profiles. The approach of using Aperio GENIE and quantitative analysis to analyze a wide range of potential biomarkers was also used in a study by Rizzardi *et al.*, (2014) who looked at the expression of CCND1, HMMR, IGF1 in prostate carcinoma samples.

Outside of identifying tumor areas for subsequent analysis of IHC staining, Aperio GENIE has been used to identify and study particular structures and regions within tumors. Smith *et al.*, (2013) used Aperio GENIE to stratify human lung tumor xenografts established in nude and severe combined immunodeficient mice into separate tumor, stroma, and necrotic regions and to determine the percentage of each compartment per tumor. This information, along with biomarker expression data, was used to classify certain tumors according to the stromal architecture and predict the tumor response to therapeutic agents.

Klapczynski *et al.*, (2012) used Aperio GENIE to quantify histological changes in rat renal failure models. The algorithm was used to assess a number of histological features associated with renal failure, such as glomerular expansion, tubular dilation, and interstitial inflammation. The study observed that image analysis using Aperio GENIE provided a robust analysis of multiple endpoints in commonly used rat models of renal failure, and offered certain advantages over manual visual assessments, such as better reliability and consistency of classification.

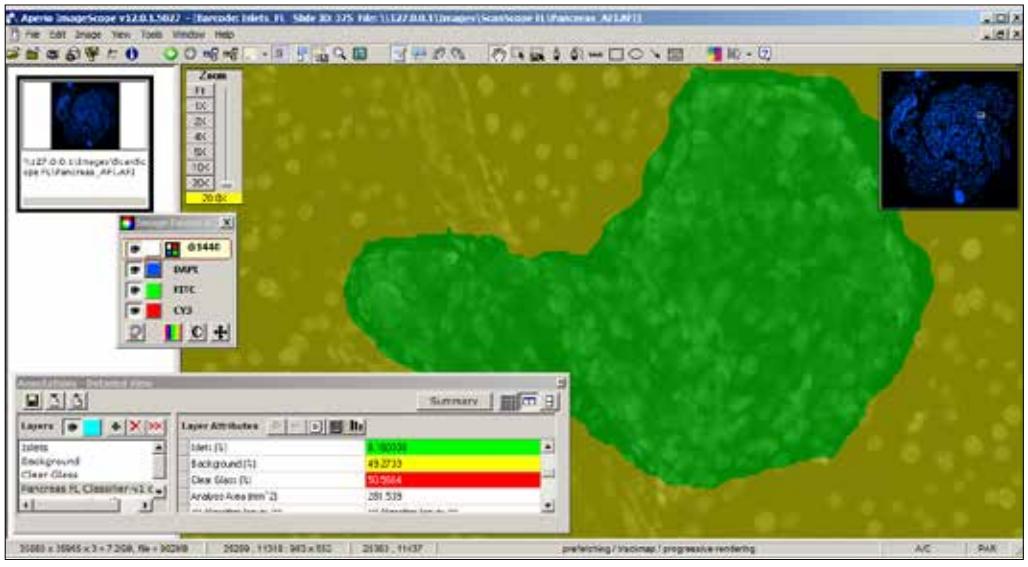

Figure 4: *Illustrates the application of Aperio GENIE in immunofluorescently stained pancreatic tissue.*

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In neurological pathology, Aperio GENIE has been used to identify neurofibrillary tangles and neuritic Aβ plaques that are characteristic of Alzheimer's disease with comparable efficiency and significantly reduced time compared to manual counting (Neltner *et al.*, 2012). Aperio GENIE can be used on fluorescent images to identify immunofluorescent labeled structures such as blood vessels, or to distinguish stroma from tumor in a sample stained with a fluorescent dye.

Conclusion

The rapidly expanding area of digital pathology offers many potential benefits over traditional histopathological methods and provides a solution to some of the key issues associated with manual assessment of tissue samples including tumor heterogeneity with staining variability and inter-observer scoring variability (Diaz *et al.*, 2004). The Aperio GENIE algorithm is a key tool in image analysis, allowing for both a significant reduction in a pathologist's workload by automatically recognizing morphological ROIs, as well as quantification of structures and specific regions within whole slide images.

The flexibility of Aperio GENIE enables it to be utilized across a broad range of tissues, as proven in both pharmaceutical and academic studies, such as breast, prostate, lung, brain and ovarian tissues, among others. Furthermore, the intuitive training classification workflow facilitates usage in both human and animal studies, ensuring that the analysis can be readily adapted to identify ROIs across a wide range of tissue morphologies. By employing Aperio GENIE, users can accurately identify and quantify discrete populations within heterogeneous tissue samples, either as a precursor to quantitative image analysis, or to improve accuracy and reproducibility in identifying ROIs.

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