

Automated selection and analysis of tumor regions in breast sections stained with nuclear IHC biomarkers

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Introduction

Advances in genomic and proteomic technology have generated many candidate biomarkers for human disease. This is particularly true in the field of oncology where biomarkers are being developed to monitor tumor progression, guide treatment decisions and predict outcomes (reviewed by Ludwig & Weinstein, 2005). For breast cancer, a number of biomarkers are analyzed routinely by immunohistochemistry (IHC) in the clinic, including HER2, ER, PR, p53 and Ki67. This list is much longer in the clinical research setting and continues to expand. As the numbers of biomarkers increase, so too does the workload of the clinical investigator. The challenge is to find methods to increase throughput and reduce the hands-on time required for IHC biomarker analysis to meet the demands of this workload.

Approach

One of the challenges of automating IHC image analysis of tumor sections is the ability to analyze only the tumor cells, removing non-tumor and non-viable components, such as stroma, lymphocytes, and necrotic regions, from the analysis. Traditional analysis techniques require considerable hands-on time by the pathologist or investigator to manually identify tumor regions for analysis.

In this application note, we demonstrate the use of Genie™, Aperio's new histology pattern recognition tool, to automate the separation of tumor cells from non-tumor components in IHC-stained breast sections. This classifier is then used to select only the tumor cells for analysis of nuclear staining on breast sections probed with biomarkers, ER, PR, and Ki67.

Methods & Results

Breast tissue sections probed with antibodies against nuclear biomarkers, PR, ER and Ki67, detected with diaminobenzidine (DAB) and counterstained with hematoxylin were used for this project. Slides were scanned using an Aperio ScanScope® CS instrument at 20x. Slides were analyzed using Genie and the Nuclear v9 analysis tools as described herein.

A Genie Project and a Genie Training Set named 'breast IHC' was created in Spectrum Plus. For the 'breast IHC' training set, three tissue classes were defined: tumor, stroma/non-tumor, and background (white space). ER, PR and Ki67 stained breast tissue slides were added to the training set. These slides were opened in ImageScope viewing software and annotated according to the tissue classes defined in the training set: tumor (blue annotations), stroma/non-tumor cells (green annotations) and background (yellow annotations) (Figure 1A). Once the training slides were annotated, a montage of the annotations found in the training

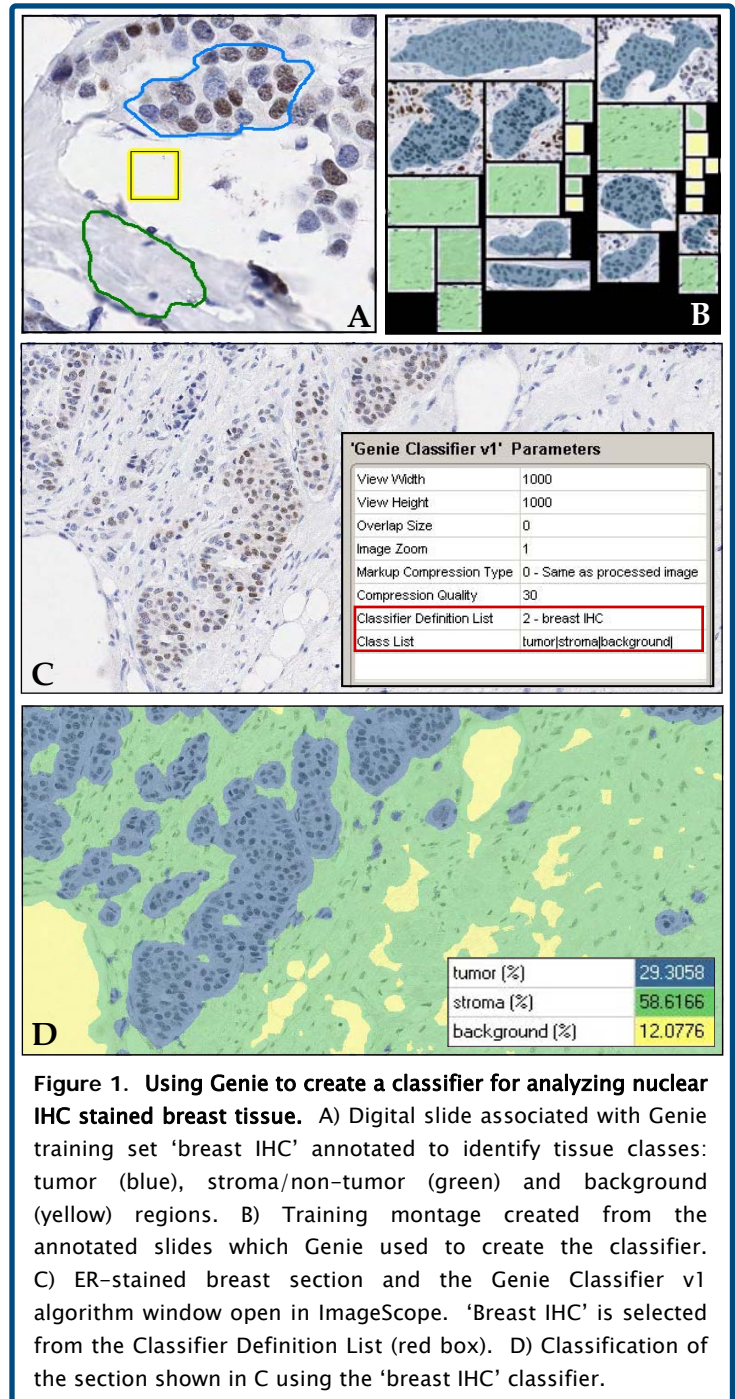


Figure 1. Using Genie to create a classifier for analyzing nuclear IHC stained breast tissue. A) Digital slide associated with Genie training set 'breast IHC' annotated to identify tissue classes: tumor (blue), stroma/non-tumor (green) and background (yellow) regions. B) Training montage created from the annotated slides which Genie used to create the classifier. C) ER-stained breast section and the Genie Classifier v1 algorithm window open in ImageScope. 'Breast IHC' is selected from the Classifier Definition List (red box). D) Classification of the section shown in C using the 'breast IHC' classifier.

slides was created (Figure 1B). The montage was submitted to Genie to create the Genie Classifier, 'breast IHC.'

Next, we evaluated the 'breast IHC' classifier that was created using the Genie Classifier algorithm within ImageScope. The Genie Classifier v1 parameter window was opened in ImageScope and the 'breast IHC' classifier was selected from the Classifier Definition List (Figure 1C). A mark-up image was created from the analysis

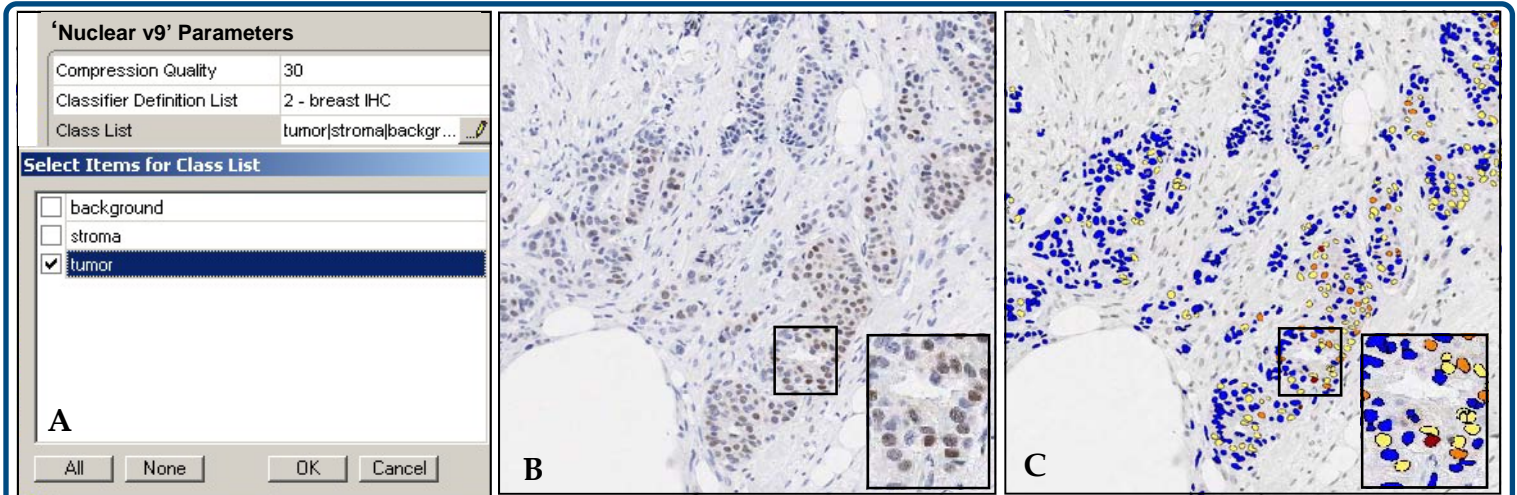


Figure 2. Using a Genie Classifier to select tumor regions for analysis with the Nuclear v9 tool. A) Within the Nuclear v9 parameter window, the 'breast IHC' classifier was chosen from the Classifier Definition List. Under the class list, only the tumor class was selected for analysis. B and C) ER-stained section in which only the tumor cells are analyzed using Nuclear v9 with the 'breast IHC' classifier.

and the percentage of the analysis area associated with each class is detailed in the annotation window as shown (Figure 1D).

For this project, we wished to analyze the staining of nuclear biomarkers within the tumor cells, excluding non-tumor components and background. Without Genie, we would need to manually select multiple tumor cell-only regions and run nuclear analysis on these regions. With Genie, we can automate the selection of tumor cell-only regions, reducing the time required for analysis. To accomplish this, the Nuclear v9 parameter window was opened in ImageScope and the 'breast IHC' classifier was selected from the Classifier Definitions List. Under Class List, only the tumor class was selected (Figure 2A). Representative intensity mark-up results for nuclear analysis of ER IHC are shown in Figures 2B and C. Note that the non-tumor components and background regions are "greyed-out" because they have not been included in the analysis.

We then tested whether results obtained with the Genie Classifier 'breast IHC' are comparable to results from manual selection of tumor cell-only regions (TCORs) on slides stained for ER (26 TCORs), PR (20 TCORs), and Ki67 (29 TCORs). The results are summarized in Table 1. For all nuclear stains, the intensity scores were the same and the percentage of positive nuclear were within 0–5.6% between Genie and manual selection.

Discussion

In this application note, we have demonstrated how to use the Aperio Genie tool to create a Classifier that can be used to analyze the composition of breast tissue sections according to a defined class list and select only tumor regions for analysis by other image analysis tools, such as Nuclear v9. While the focus of this note was breast tissue, it is possible to create Genie classifiers for other tissues and tumor types as needed. The ability to automate the selection of tumor sections greatly increases the throughput potential for cancer biomarker studies, removes the tedium associated with manual annotation of tumor regions, and reduces the hands-on time required for the investigator to analyze slides.

A) ER	29 TCORs	Genie
% Positive Nuclei	20.8	19.8
Intensity Score	+1	+1
B) Ki67	26 TCORs	Genie
% Positive Nuclei	47.9	42.3
Intensity Score	+3	+3
C) PR	20 TCORs	Genie
% Positive Nuclei	0.0	0.0
Intensity Score	0	0

Table 1. Comparison of nuclear stain analysis for three IHC biomarkers, ER, PR and Ki67. Results are compared when tumor cell only regions (TCORs) were selected manually (left column) to results obtained when tumor cells were selected using the 'breast IHC' Genie classifier as shown in Figure 2.

References

Ludwig, JA & Weinstein, JN. *Nat. Rev. Cancer*. 2005;5(11):845–56.

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