

Analyzing glomerular area and cell density in IHC-stained kidney sections

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Introduction

The primary role of the kidney is to maintain the homeostatic balance of bodily fluids by filtering and secreting metabolites and minerals from the blood and excreting them with water in the form of urine. Toxic substances that are filtered from the blood, such as drugs, drug metabolites, viruses, bacterial toxins, and immune complexes, can accumulate at high levels in the kidneys resulting in tissue damage and loss of function. Glomeruli are the units within the kidney where blood is filtered and, as such, are particularly vulnerable to injury. Glomerular toxicity, injury or disease is confirmed routinely by histopathological examination of kidneys in laboratory animals and renal biopsies from human patients.

Approach

Glomerular disease may result from an inherited defect or as a result of infection, drug toxicity, or complications from diseases that affect the entire body, such as diabetes or lupus. One of the most common manifestations is glomerulonephritis which results in glomerular hypertrophy (swelling) and increased cellularity due to proliferation and inflammatory infiltration. Therefore, the ability to objectively measure glomerular features, such as size, area and cell density, is important for studying natural and drug-induced renal dysfunction in animal models and humans.

In this application note, methods for using Aperio image analysis tools to examine glomeruli are presented. First, we use the Genie™ histology pattern recognition tool to identify IHC-stained glomeruli and calculate glomerular cross-sectional area. Next, we apply the Genie solution to count hematoxylin-stained nuclei within the glomeruli using the Nuclear analysis tool. By combining this analysis with Aperio ScanScope® whole-slide scanning, researchers can quickly and objectively measure glomerular area and cell density in renal cross-sections and needle biopsies.

Methods

Rodent kidney sections were probed with a pan-glomerular antibody detected with diaminobenzidine (DAB) counterstained with hematoxylin as shown in Figure 1A. Slides were scanned at 20x using an Aperio ScanScope® CS instrument and were analyzed in ImageScope™ viewing

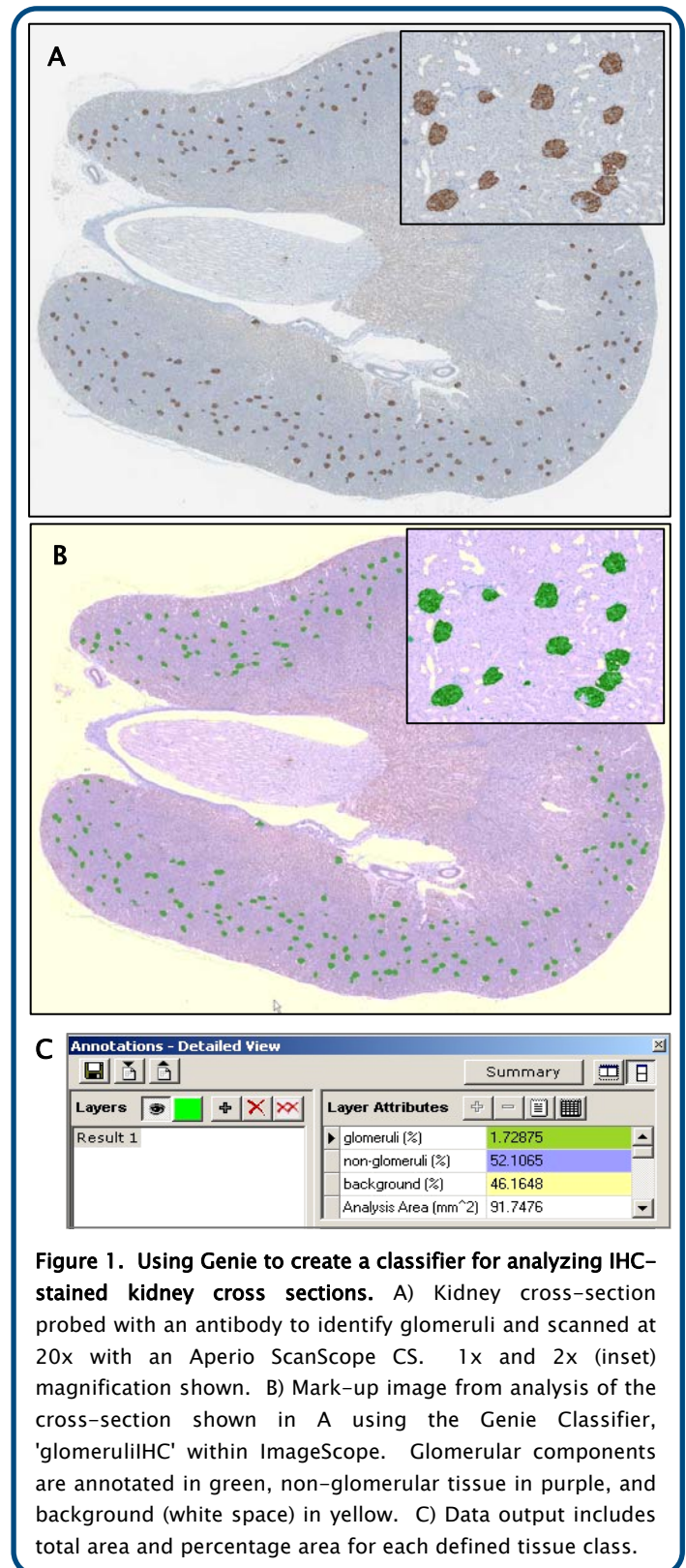
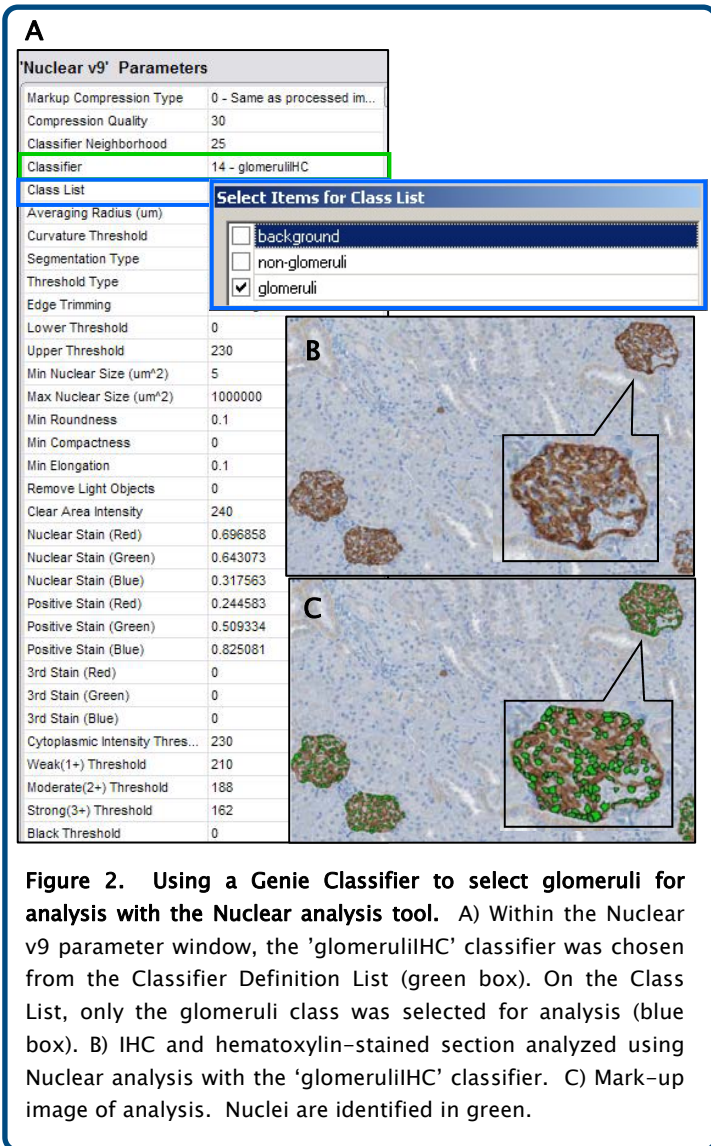


Figure 1. Using Genie to create a classifier for analyzing IHC-stained kidney cross sections. A) Kidney cross-section probed with an antibody to identify glomeruli and scanned at 20x with an Aperio ScanScope CS. 1x and 2x (inset) magnification shown. B) Mark-up image from analysis of the cross-section shown in A using the Genie Classifier, 'glomeruliIHC' within ImageScope. Glomerular components are annotated in green, non-glomerular tissue in purple, and background (white space) in yellow. C) Data output includes total area and percentage area for each defined tissue class.

software using Genie and the Nuclear analysis tool as described herein.



A Genie Project and a Genie Training Set named 'glomeruliIHC' was created in Spectrum Plus. For the 'glomeruliIHC' training set, three tissue classes were defined: glomeruli, non-glomerular tissue and background (white space). An IHC-stained renal cross-section slide was used to train Genie to identify the three classes of tissue defined in the training set: glomeruli (green), non-glomerular tissue (purple) and background (yellow). Once a suitable Genie classifier was created, it was applied to other IHC-stained renal cross-section slides using the Genie Classifier tool within ImageScope. The result of this analysis is displayed as a mark-up image as shown in Figure 1A and 1B. The statistical output data from this analysis is detailed in Figure 1C. Using these data, we calculate that the total cross-sectional area of the kidney is 49.4 mm² and the glomerular cross-sectional area is 1.6 mm² or 3.2% of the kidney.

Next, we wished to count cells within the glomeruli by counting hematoxylin-stained nuclei. Without Genie, we would need to manually select individual glomeruli using the pen tool In ImageScope and run nuclear analysis on each of these regions. With Genie, we can automate the selection of glomeruli, reducing the time required for analysis. To accomplish this, the Nuclear analysis parameter window was opened in ImageScope and the 'glomeruliIHC' classifier was selected from the Classifier Definitions List (Figure 2A). Under Class List, the glomeruli class was selected and non-glomeruli and background were deselected to eliminate these classes from the analysis. Representative analysis regions and intensity mark-up results for Nuclear analysis are shown in Figures 2B and 2C. Note that the non-glomerular components and background regions are "greyed-out" because they have not been included in the analysis. Glomerular cell density is determined by dividing the total number of hematoxylin stained nuclei identified by this analysis by the total glomerular cross-sectional area.

Results and Discussion

In this note, we demonstrate how the Genie histology pattern recognition tool can be applied to automate the measurement of glomerular area and cell density across whole IHC-stained kidney sections. This technique could be likewise applied to analysis of needle biopsies from humans or animal models. Genie could also be used to analyze other aspects of glomerular biology. For example, apoptosis within the glomeruli may be quantified by dual-staining for glomeruli and TUNEL. Proliferation, inflammatory infiltration and other biological processes may be likewise measured.

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