Quantification of prostate cancer biomarkers using multiplexed IHC on prostate TMAs

Modern high-throughput technologies are uncovering hundreds of potential cancer genes that may have diagnostic, prognostic and therapeutic significance. To validate the relevancy of these genes, their protein expression is analyzed in tissue samples from cancer patients in either whole tissue sections or tissue microarrays (TMAs). Traditionally, analysis of protein expression has relied on manual evaluation of staining intensity which can be time and labor intensive, subject to inter- and intra-observer variability and can fail to identify subtle differences in biomarker expression. It is also unable to resolve chromogen mixtures if the target biomarkers spatially overlap.

In this study, researchers validated the reliability of the Vectra® Slide Analysis System for quantitating prostate cancer (PCa) and PCa progression biomarkers using multiplexed immunohistochemistry (IHC) on prostate TMAs. TMA sections were double or triple stained with different combinations of antibodies against the biomarkers AMACR, E-cadherin, AR and ERG, counterstained with hematoxylin and scanned using the Vectra System. To analyze biomarker expression, researchers first used Nuance® software to build a spectral library for the 4 chromogens, based on their unique spectral characteristics. This library was then used to unmix the signals in order to eliminate cross talk and achieve more accurate quantitation (Figure 1). Following spectral unmixing, the inForm® Advanced Image Analysis software was used to segment tissue and subcellular compartments using its unique pattern-recognition-based image analysis (Figure 2). The outcome of segmentation was assessed and validated by a pathologist.

Biomarker signals were quantified within the compartments of interest using inForm software and statistical correlation analyses were performed on the results. The authors found a close agreement between the triple (E-cadherin/AR/ERG) and double (E-cadherin/AR and E-cadherin/ERG) immunostaining assays used for quantification of the spatially overlapping biomarkers AR and ERG ($r = 0.897$ and $0.613$ respectively). The expression levels of the non-overlapping biomarkers AMACR and E-cadherin, measured using double immunostaining assays, were found to be consistent with previously published data from other groups.

These results validate the reliability of the Vectra System (with Nuance/inForm software) for quantifying non-overlapping biomarkers and for studying biomarker colocalization using chromogenic multiplexed IHC.

Figure 2 (above): Tissue and Cell Segmentation using inForm Software. A) User-defined training regions for epithelium (red) and non-epithelium (green). B) Segmented epithelial (red) and non-epithelial (green) compartments. C) Composite image of tissue and cell segmentation of prostate epithelium. D-F) Nuclear, membrane and cytoplasmic compartments respectively. In panels E and F the background colour has been removed for illustrative purposes.

Figure 1 (left): Spectral unmixing using Nuance. A) Composite image of PCa tissue stained with multiplexed IHC assay. B-E) Unmixed signals for AR (DAB), ERG (Warp Red), E-cadherin (Vina Green) and Hematoxylin counterstain respectively.