Application of Genie™ Pattern Recognition Software for the Automated Analysis of Immunohistochemical Markers in Preclinical Xenograft Models

Marie Cumberbatch, Neil Gray, Sarah Loddick, Chris Womack. Molecular Pathology, Oncology TS, AstraZeneca, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK.

INTRODUCTION

Automated analysis of xenograft tumour samples currently involves manual annotation of digital slides followed by application of an appropriate image analysis algorithm to the regions of interest. However, some projects comprise large numbers of slides leading to a lengthy manual annotation process. Genie™ (Aperio Technologies Ltd.) is a pattern recognition software tool which can be trained to identify automatically specific structures or cell types within a digitally acquired specimen thus removing the need for manual annotation. Genie™ classifiers developed for the automated mark-up of specimens can then be combined with a tuned image analysis algorithm for the fully automated analysis of digital slides.

The purpose of these studies was to examine the utility of Genie™ for:

- the automated mark-up of tumour versus necrosis and subsequent analysis of pAKT473 in breast (BT474) and prostate (PC3) xenograft models.
- the fully automated quantitation of androgen receptor expression in tumour (stroma excluded) in a transgenic model of prostatic cancer, the rat Dunning model, where manual annotation would be impractical due to the complex morphology of these tumours.

METHOD

BT474 and PC3 xenograft tumours

Two PD studies comprising 35 BT474 tumours (**Figure 1**) and 25 PC3 tumours (**Figure 2**) were stained by immunohistochemistry (IHC) for pAKT473. For each study, digital images were acquired into Spectrum (Aperio technologies Ltd) and all tumours annotated manually for the identification of viable tumour areas versus necrotic areas which were excluded using the exclusion pen tool. In addition, a Genie classifier for each xenograft model was developed for the automatic recognition of tumour versus necrosis. A Colour Deconvolution algorithm set to intensity mark-up for colour channel 3 (DAB) was tuned to determine percentage weak/medium/strong positive and total positivity for pAKT473 in manually and Genie™ annotated tumours.

Rat Dunning model

Tumours (Oncodesign, Dijon France) were stained by IHC for androgen receptor and acquired digitally into Spectrum. A Genie classifier was generated for the separate recognition of tumour and stroma and was applied subsequently for the automated analysis of androgen receptor expression in tumour only (**Figure 3**).

pAKT473 expression in BT474 xenograft tumours: manual versus automated annotation of tumour and necrosis

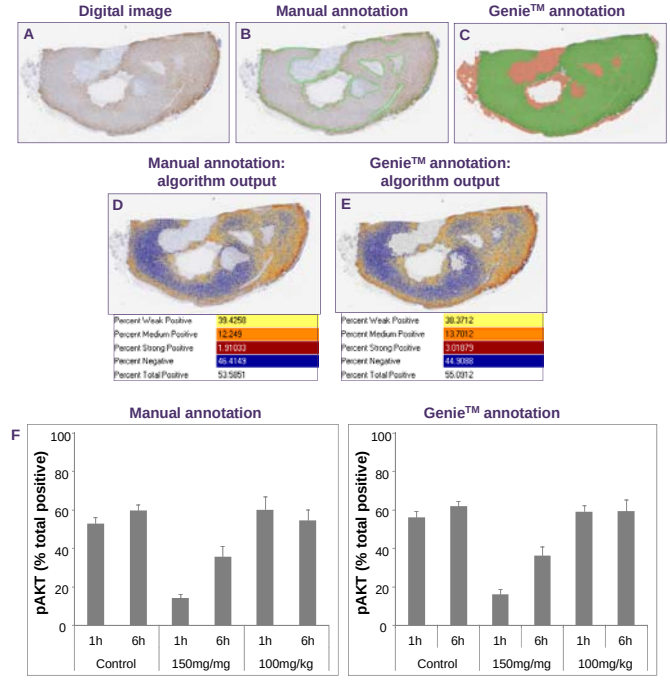

Figure 1. (**A**) representative digital image; (**B**) example of manual mark-up; (**C**) example of Genie™ mark-up for tumour (■) and necrosis (■); (**D**) image analysis output for pAKT expression following manual mark-up; (**E**) image analysis output for pAKT expression following Genie™ mark-up; (**F**) comparison of image analysis output for pAKT expression across study (n=35 tumours) for manual versus Genie annotation.

pAKT473 expression in PC3 xenograft tumours: manual versus automated annotation of tumour and necrosis

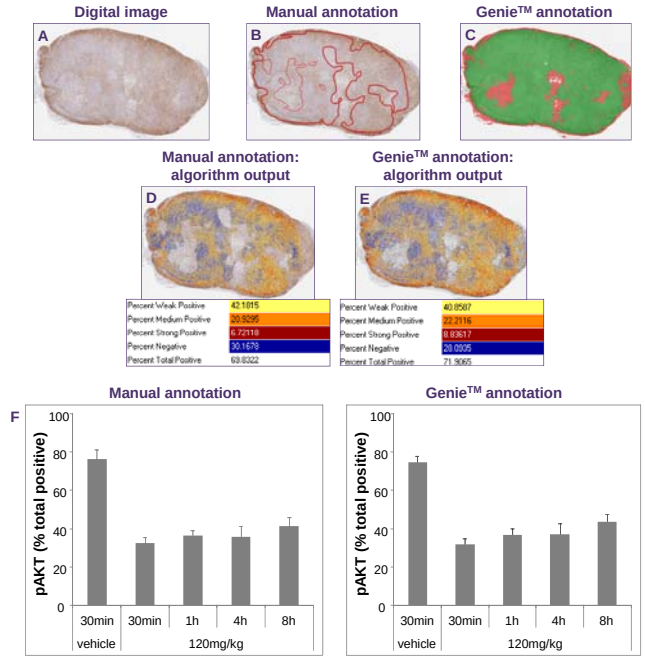

Figure 2. (**A**) representative digital image; (**B**) example of manual mark-up; (**C**) example of Genie™ mark-up for tumour (■) and necrosis (■); (**D**) image analysis output for pAKT expression following manual mark-up; (**E**) image analysis output for pAKT expression following Genie™ mark-up; (**F**) comparison of image analysis output for pAKT expression across study (n=25 tumours) for manual versus Genie annotation.

Androgen receptor expression in Dunning tumours: exclusion of stroma using Genie™

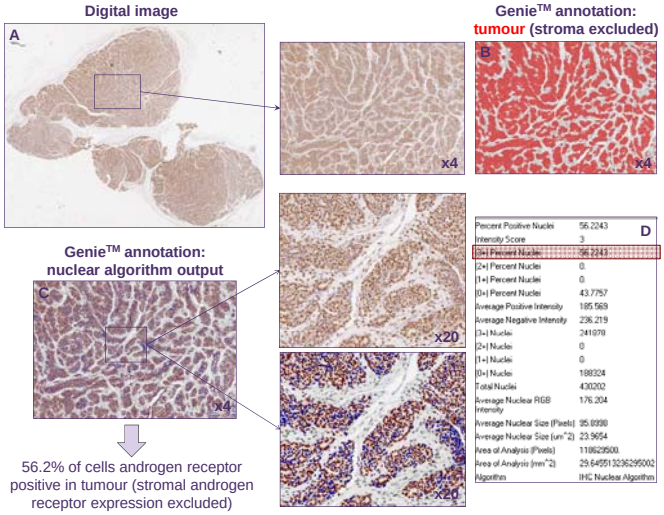

Figure 3. (**A**) representative digital image; (**B**) example of Genie™ mark-up for tumour (■), stroma excluded (■); (**C**) image analysis output for nuclear androgen receptor expression for tumour only (● positive nuclei, ● negative nuclei); (**D**) nuclear algorithm output for androgen receptor expression for tumour only.

CONCLUSION

- A high degree of concordance was observed between manual and Genie™ mark-up for tumour versus necrosis in breast (BT474) and prostate (PC3) xenograft models such that the image analysis output for manual versus Genie™ mark-up delivered results for pAKT expression that were in complete agreement (**Figures 1 & 2**).
- The classifier developed for structure recognition in the Dunning model of prostate cancer separated tumour from stroma with a reasonable degree of accuracy sufficient for the useful quantitation of nuclear androgen receptor expression in tumour (with stroma excluded) that would be impractical to attempt by manual annotation (**Figure 3**).
- Overall, these data suggest that Genie will have utility in the automated annotation, quantitative analysis and localisation of IHC markers in routine xenograft models. Application of Genie in place of manual annotation of specimens will result in more rapid delivery of study data with increased accuracy and reduced subjectivity.

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