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## Reproducibility of image analysis in virtual microscopy.

D McCleary<sup>1</sup>, J Diamond<sup>1</sup>, D Crookes<sup>1</sup>, H Grabsch<sup>2</sup>,  
P Hamilton<sup>1</sup>

*<sup>1</sup>Queen's University Belfast, Belfast, United Kingdom,*

*<sup>2</sup>University of Leeds, Leeds, United Kingdom*

Virtual microscopy is revolutionising pathology by producing diagnostic quality images of an entire slide. This provides enormous opportunities for automated analysis, particularly tissue microarray (TMA) analysis. However quantitative analysis of TMA virtual slides must produce consistent intra/interscanner results.

This study aims to assess the reproducibility of morphologic, densitometric and texture data on different slide scanners. A single TMA showing samples of gastric lesions and immunohistochemically stained for Cyclin B1 was scanned 5 times on two Aperio CS Scanscopes. The same ten tissue cores were selected from each scan and a series of geometric, densitometric and textural measurements made to compare reproducibility on sequential scans on different instruments.

Measurements showed that intra-machine scans produce consistent densitometric results with a coefficient of variation of 0.039 (machine 1) and 0.013 (machine 2) for mean grey level of positively stained tissue. A comparison of tissue cores scanned on different instruments shows subtle differences in visual appearance of the images. This was confirmed by quantitative evaluation of immunomarker labelling: e.g. a 4% difference was observed between the number of positively stained pixels between machines on a single core and a 32.4% difference in total mean density in positively stained areas.

Consistent results are essential for the quantitative evaluation of TMAs. This study has shown that sequential scans from a single instrument are reproducible. However, there are obvious differences in the quantitative evaluation of TMAs from different instruments and it may be necessary to develop suitable internal control slides to standardise results.