

# AFM DISCRIMINATES BETWEEN NORMAL UROTHELIAL CELLS AND BLADDER TUMOUR CELLS

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## INTRODUCTION

**Atomic Force Microscopy (AFM)** has a huge potential for exciting new applications in medicine. It has become a versatile, powerful, and indispensable technique for studying at the nanoscale, the morphological, dynamic, and biomechanical (e.g. adhesive, elastic) properties of living cells, biomolecules, and tissues under physiological conditions.

**Bladder cancer** is the most common genito-urinary malignancy with transitional cell carcinoma (TCC) comprising nearly 90% of primary bladder tumours. The majority of patients present with superficial bladder tumours, 20-40% either already have or develop invasive disease. Invasive TCC is usually a lethal disease requiring aggressive therapy. Reference to recent literature reveals a clear need to develop new diagnostic methods to differentiate normal from tumour cells with high sensitivity and specificity.

This poster presents some initial results on the use of AFM in discriminating between living normal human urothelial cells (SV-HUC) and bladder tumour cells (MGH-U1) on the basis of their ultrastructure and nanomechanical properties.

## MATERIALS AND METHODS

AFM was used in both imaging and force spectroscopy *application modes*:

**Imaging mode:** It permitted both *qualitative* (topography and cell morphology) and *quantitative* (cell dimensional) information to be obtained by analysing the images. It was performed in *contact operational mode*.

**Force spectroscopy (FS) mode:** The interaction force,  $F$ , between an AFM tip and the adhesive surface was measured from the AFM cantilever deflection,  $x$ , using Hooke's law, and a force-distance ( $F-d$ ) FS curve recorded. Analyses of the FS curves gave information about the adhesive force and elasticity of the sample.

**AFM Imaging and AFM-FS experiments** were performed on:

**Bladder Cells:** Malignant human bladder carcinoma cells (MGH-U1) and normal human bladder epithelial cells (SV-HUC) used for these studies were maintained in culture as described previously (Masters, J.R.W., & Heppburn, P.J., 1986, 'Human bladder cancer in vitro drug sensitivities: Range and stability in long-term culture', *British Journal of Cancer*, 54, 131-135; Christian, B.J., Loretz, L.J., Oberley, T.D., & Reznikoff, C.A., 1987, 'Characterisation of Human Uroepithelial Cells Immortalised in Vitro by Simian Virus 40', *Cancer Research*, 47, 6066 - 6073).

**AFM Section Analysis:** AFM images (Fig. 1) were analysed by section analyses to determine the dimensions (e.g. length, width), and peak-to-valley heights of the samples (Fig. 2).

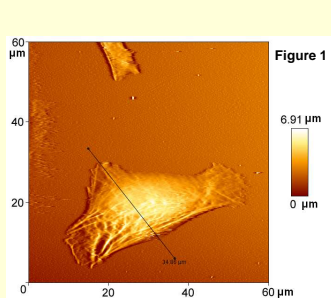


Figure 1

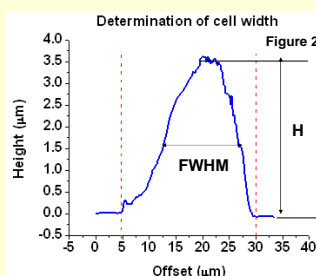


Figure 2

In AFM images, the widths of samples get overestimated due to the effects of tip deconvolution. The widths were therefore estimated using the Stemmer & Engel method (*Ultramicroscopy*, 1990, 34, 129).

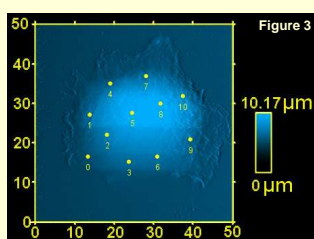


Figure 3

**AFM-FS:** FS curves were recorded on different positions on the sample surface (Fig. 3). The tip was withdrawn from the surface (Fig. 4) at a constant speed of 5 μm/sec. In each experiment, the maximum adhesive force of the sample to the tip ( $F_{max}$ ), the energy of adhesion ( $W_{adh}$ ), and the Young's modulus ( $E$ ) were measured (Fig. 5).

The AFM-FS on bladder cells were carried out in culture medium at room temperature.

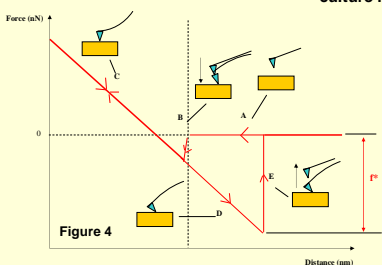


Figure 4

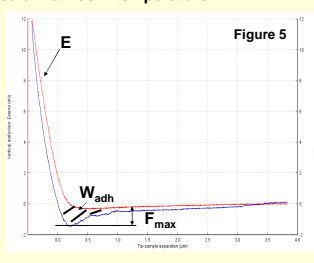


Figure 5

## RESULTS

### AFM Imaging – Ultrastructural properties

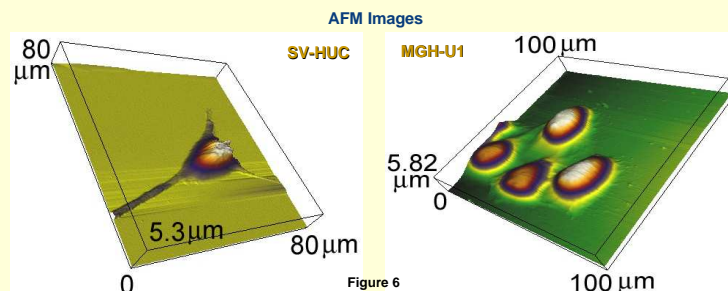
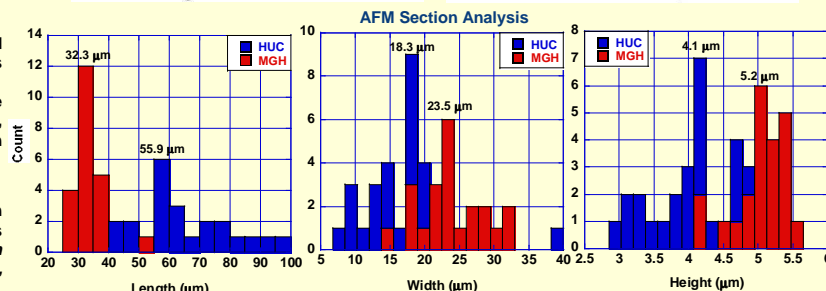


Figure 6



Morphology of **MGH-U1** (rounder in shape) and **SV-HUC** (stretched out) cells is clearly different (Fig. 6, top). In addition, the histograms (Fig. 6, bottom) constructed for 25 cells of each type show that **MGH-U1** cells are smaller in lengths, larger in widths, and thicker in heights as compared to **SV-HUC** cells.

### AFM-FS – Nanomechanical properties

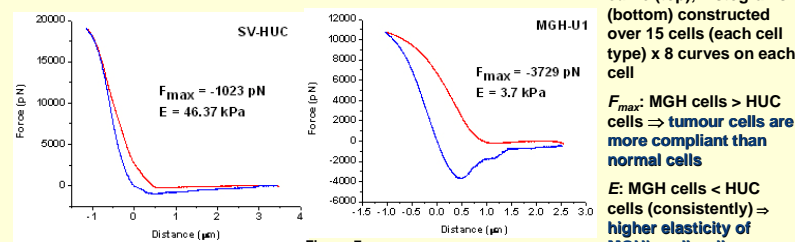
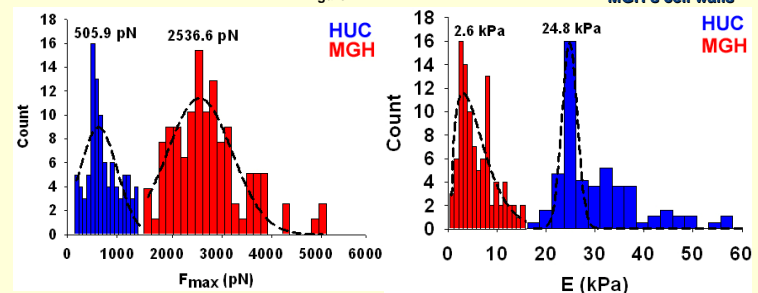


Figure 7

Fig. 7: A typical  $F-d$  curve (top), histograms (bottom) constructed over 15 cells (each cell type) x 8 curves on each cell

$F_{max}$ : MGH cells > HUC cells  $\Rightarrow$  tumour cells are more compliant than normal cells

$E$ : MGH cells < HUC cells (consistently)  $\Rightarrow$  higher elasticity of MGH's cell walls



## CONCLUSIONS

The results clearly demonstrate that AFM provides a nanotool to analyse and discriminate between normal and malignant bladder cells, thus revealing its diagnostic potential for bladder cancer. We aim to use confocal Raman spectroscopy with modulation capability complementarily with the AFM studies to look for differences in the Raman spectra – the so called biochemical signatures of these cells and correlate our data from the two approaches (AFM and Raman) to extend the diagnostic capabilities for achieving high sensitivity and specificity.

## ACKNOWLEDGEMENTS

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