INTRODUCTION

In this application note, we demonstrate a novel label free method for direct virus sensing. The Nano eNabler™ system is used to create ultraminiaturized antibody arrays with spot sizes (10-15 µm) in discrete locations. These spots occupy 1/100th to 1/1000th the surface area of a conventional microarray (D=100 µm) spot [1]. The ultraminiaturized spatial scale made possible by the Nano eNabler™ system creates an opportunity for direct readout by surface profiling using the principles of atomic force microscopy (AFM) along with more conventional methods. Viruses remain bioactive and amenable to culture and PCR.

DATA SUMMARY

The printing substrate was on 4 x 4 mm silicon chip treated with chromium and gold and then modified to facilitate antibody binding. In Figure 1 ultramicroarrays of capture antibody against group B coxsackievirus were constructed using the Nano eNabler™ system, a novel molecular printing system. Pure virus samples were placed on the chip with incubation, and wash followed by AFM analysis [2]. The AFM images in Figure 1 show signal height changes in the presence of captured virus. Data in Figure 2 demonstrates the clear specificity of detection for six unique group B viruses along with virus enumeration capabilities.

CONCLUSIONS

Key advantages of the AFM approach include label-free detection, operation in biological liquids, retention of sample biological activity for subsequent analysis (e.g., infection of cell cultures) and extremely accurate height measurement as a corroborating diagnostic tool. The latter capability is useful because many pathogens, such as viruses, have well defined heights with little variation from particle to particle. With respect to these advantages, we have successfully eluted coxsackievirus particles from an imaged ViriChip™ and subsequently infected green monkey kidney cells (data not shown). We have also demonstrated successful RT-PCR from ViriChip™ captured virus samples isolated from coffee, sludge, sputum, serum and urine as published in Analytical Biochemistry 330: 350-352 (2004).

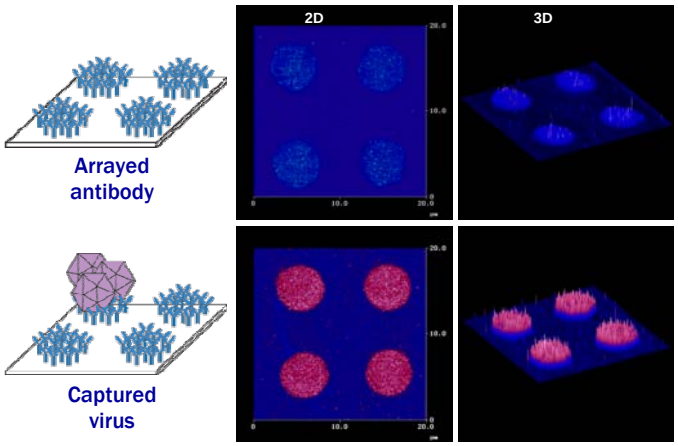

Figure 1. Group B Coxsackievirus capture and detection on antibody spots with AFM. The left panel shows a graphic depiction of negative (top) and positive (bottom) whole virus capture process with 2D and 3D AFM views for each on the right.

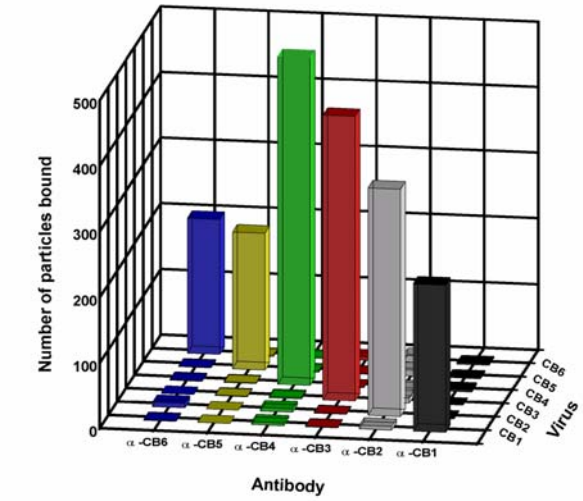

Figure 2. Specificity of group B Coxsackievirus capture on antibody coated chips (non-arrayed). The 6 × 6 matrix of chips was analyzed and the number of virus particles bound to each 25 µm² field was obtained. The mean virus particle count was calculated and plotted. A display scale was chosen to optimize graphic representation of relative particle counts for all samples, and resulted in truncation of the CB4 data (~2500 particles) at the 500-particle maximum for the graph.

REFERENCES

[1] Lynch, M., Mosher, C., Huff, J., Nettikadan, S., Johnson, J., and E. Henderson. Functional protein nanoarrays for biomarker profiling. *Proteomics* **4**: 1695-1702, 2004.

[2] Nettikadan, S., Johnson, J., Vengasandra, S.G., Muys, J. and E. Henderson. ViriChip™: a solid phase assay for detection and identification of viruses by atomic force microscopy. *Nanotechnology* **15**: 383-389, 2004.