INTRODUCTION

In this application note, we demonstrate a novel method for detecting Prostate Serum Antigen (PSA) from reduced sample volumes. The Nano eNabler™ system is used to create ultraminiaturized antibody arrays with spot sizes from 5-8 µm in diameter occupying 1/100th to 1/1000th the surface area of a conventional microarray (D=100 µm) spot [1]. This PSA level of sensitivity is several orders of magnitude lower than that required for biomedically relevant screening. Furthermore, it requires <1 µl of sample, opening the door to minimally invasive cancer biomarker screening & ultimately single cell diagnostics. Serum PSA and IL-6 (interleukin 6) levels are part of the early detection and prognostic tests for prostate cancer, and provide a good model system for evaluating this detection methodology.

DATA SUMMARY

The printing substrate was a 4 x 4 mm silicon chip coated with gold and then treated with a secondary agent to facilitate protein binding. Ultramicroarrays of capture antibodies (mouse anti-PSA and rat anti-IL-6) were constructed using the Nano eNabler™ system, a novel molecular printing system [2]. After blocking, 1 µl pure protein samples were placed on the chip with incubation, wash and the addition of a fluorophore conjugated detection antibody. The images in Figure 1 show the consistency in printing, and the specificity of the assay. Data in Figure 2 demonstrates a sensitive (low pg/ml) and dynamic detection range.

CONCLUSIONS

These ultramicroarrays demonstrate high specificity and sensitivity with a significant dynamic range of detection. PSA was detected at pg/ml levels translating to attomole sensitivity from 1 µl of sample. The potential benefits of bioassay miniaturization include reduced reagent costs, faster kinetics, higher throughput and improved sensitivity. Reduction in the size of surface immobilized assays creates new opportunities in areas of chemical and biological sensor development. This is particularly relevant in the context of non-amplifiable protein biomarkers that are available only in very small quantities, such as from laser capture microdissected (LCM) materials, neonatal samples and forensics specimens.

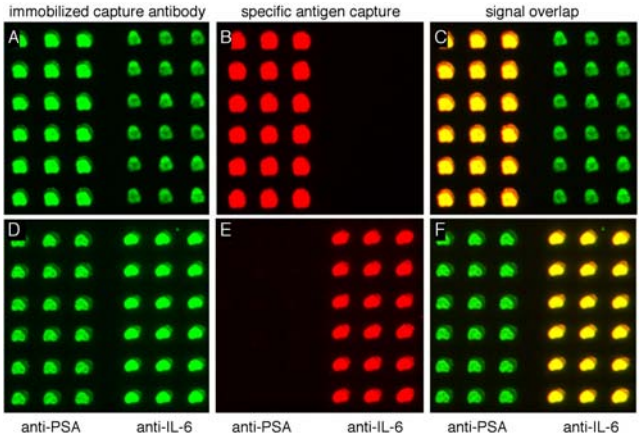

Figure 1. Ultramicroscale immunofluorescent antibody capture assay. Panels A and D show the deposition domains containing capture antibodies for PSA and IL-6, visualized with a fluorophore (green) coupled antibody. Panes B and E show specific capture of PSA and IL-6, visualized with a fluorophore (red) coupled detection antibody. Panels C and F show the overlap of A/B and D/E, to illustrate the one to one correspondence between the capture antibody and the specific signal.

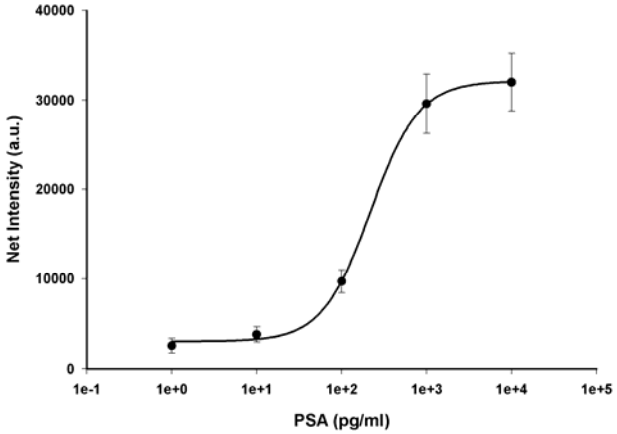

Figure 2. Detection Sensitivity Curve. PSA can be detected down to 1 pg/ml. In this graph the higher limit indicates photosensor saturation and does not represent the upper limit of detection.

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] Henderson, E., Mosher, C., and M. P. Lynch, *US Patent No*. 6,573,369 (2003).