NEW MICROARRAY SLIDES CAPABLE OF ENHANCING FLUORESCENCE

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ABSTRACT

Microarrays are typically analyzed using a biomolecule conjugated to a fluorescent probe. Although this method works well, no signal amplification is possible with this general approach. Plasmonix Inc has taken advantage of a well known phenomenon called Metal Enhanced Fluorescence (MEF) to develop a new microarray slide which incorporates this technology and enhances fluorescent signals by approximately 30-fold. These new slides, called QuantArray slides, are glass slides with a thin layer of metal nanoparticles covered by a silica layer and finally a coat of epoxy silane. The epoxy silane promotes strong adherence of proteins. To demonstrate that QuantaArray slides enhance fluorescence, we coated either QuantArray slides or plain glass slides with the same epoxy silane coating with varying concentrations of biotinylated-BSA. The biotinylated BSA was detected with strepatividin-conjugated DL633 dye and read in a standard slide reader. We found that QuantArray slides could easily detect a spot of 0.006 pg of Bt-BSA, whereas the control slides could barely detect a spot of 0.06 pg of Bt-BSA demonstrating an approximately 30 fold enhancement of the signal. Background staining was negligible. These slides will be useful in proteomics assays requiring an enhanced fluorescent signal.

INTRODUCTION

When a fluorophore is brought in close proximity to a metal nanoparticle, the fluorescence intensity of the fluorophore has been shown to increase dramatically and the fluorescence lifetime of the fluorophore has been shown to decrease, resulting in an increased photostability (1,2). This phenomenon is known as **Metal Enhanced Fluorescence (MEF)**, a term first used by Geddes and Lakowicz in 2002 (1). The resulting increase in signal intensity is the basis for Plasmonix' products which allow our customers to detect levels of material previously undetectable by fluorescence.
Since its discovery, the physical basis for MEF has been well studied and reviewed (2, 3, 4, 5). It is now generally accepted that MEF is caused by the non-radiative coupling of the fluorophore dipole with the electron cloud of the metal (surface plasmons),thereby altering the fluorescence characteristics.

MEF is a near-field phenomenon, meaning that it occurs only when a fluorophore is within 5 – 50 nm of a metal which supports surface plasmons (5).

REFERENCES

1. C. D. Geddes and J.R. Lakowicz, Metal-enhanced fluorescence, *J. Fluoresc*., (2002), 12: 121-129.
2. Metal-Enhanced Fluorescence, ed. C.D. Geddes, John Wiley and Sons, New Jersey, 2010, pp. 625, ISBN: 9780-470-2238-8
3. Aslan, K., Gryczynski, I., Malicka, J., Matveeva, E., Lakowicz, J.R. and Geddes, C.D. (2005). Metal-enhanced fluorescence: an emerging tool in biotechnology, *Current Opinion in Biotechnology*, 16: 55-62.
4. Plasmon-controlled fluorescence towards high-sensitivity optical sensing. Ray K, Howdhury MH, Zhang J, Fu Y, Szmacinski H, Nowaczyk, K, Lakowicz JR. Adv Biochem Eng Biotechnol (2009) 116: 29-72.
5. Advances in surface-enhanced fluoresence. Lakowicz J.R., Geddes C.D., Gryczynski I., Malicka J., Gryczynski Z., Aslan K., Lukomska J., Matveeva E., Zhang J., Badugu R., Huang J. (2004) *J Fluoresc* 14(4): 425 -41.

METHODS

QuantArray-S-ES slides were constructed as follows. Glass slides were plasmon-cleaned and coated with silver nanoparticles using vapor deposition. A layer of SiO2 was deposited on top of the silver, also by vapor deposition. Epoxy Silane was layered on top of the SiO2 using conventional dip methods. Slides were stored at -20°C for no more than 2 months prior to use.

To demonstrate that QuantArray-S-ES slides enhance fluorescence, the following assay was performed both on a QuantArray-S-ES slide and on a glass slide that was epoxy silane coated at the same time as the QuantArray slides. Slides were spotted with various concentrations of biotinylated-BSA in 50 mM sodium phoshphate buffer pH 7.0 containing 5% glycerol using a hand-spotting device from V&P Scientific that spots 6 nL spots. After a 30 min incubation at room temperature, the slides were blocked in 1% BSA and 1 mM EDTA in 10 mM Tris-HCl pH 7.6 for 60 min. After washing, slides were incubated in 1 ug/mL of streptavidin conjugated DyLight 633 for 60 min at RT, then washed and dried. Slides were scanned in a GenePi 4000 (Axon Instruments) scanner.

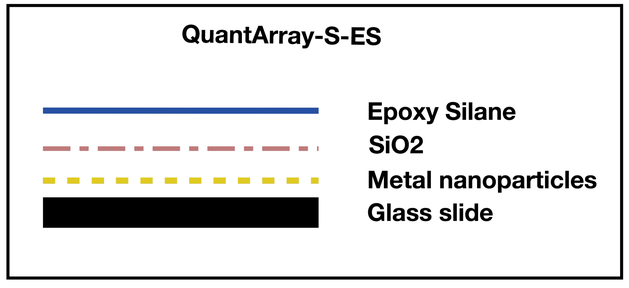

Figure 1. Schematic representation of QuantArray-S-ES slides.

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RESULTS

The figure below shows that QuantArray-S-ES slides are able to detect spots of 0.006 pg of Bt-BSA whereas the control slides were barely able to detect 0.06 pg of Bt-BSA, an approximately 30-fold increase in sensitivity due to the plasmonic metal nanoparticles.

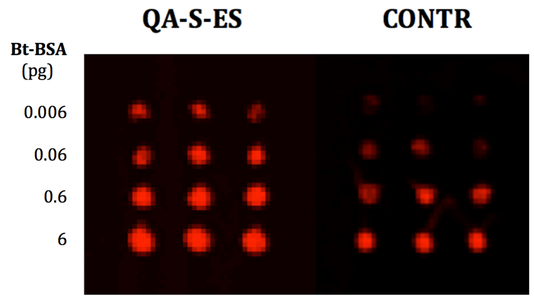
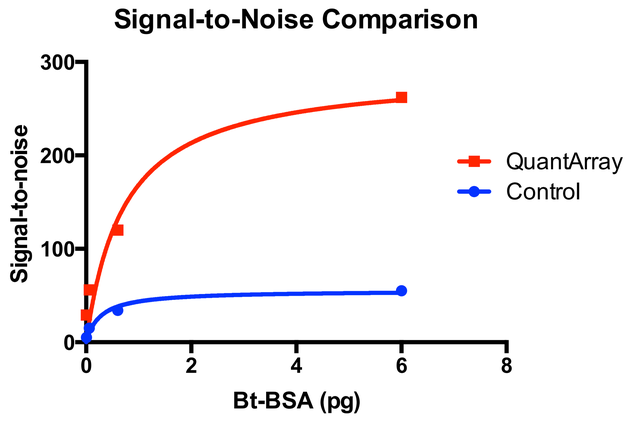


Figure 2. Comparison of QuantArray-S-ES slides with control slides. Both were epoxy silane treated

Figure 3 (below) compares the signal-to-noise at various different Bt-BSA concentrations. The background in the QuantArray slides was approximately twice that of the control slides. At 6 pg of Bt-BSA, the signal-to-noise of the Quantarray slides was 4.8 fold higher that of the control slides.



CONCLUSIONS.

1. QuantArray-S-ES slides are able to detect approximately 30-fold lower protein concentration than control slides with a similar surface chemsitry.
2. The signal-to-noise of the QuantArray slides is 4.8 fold higher than control slides.
3. QuantArray slides therefore may be very useful in microarray studies that need additional sensitivity.