

Supporting Information

Effective Enhancement of Fluorescence Detection Efficiency in Protein Microarray Assays:

**Application of a Highly Fluorinated Organosilane as the Blocking Agent on the Background Surface by
a Facile Vapor-Phase Deposition Process**

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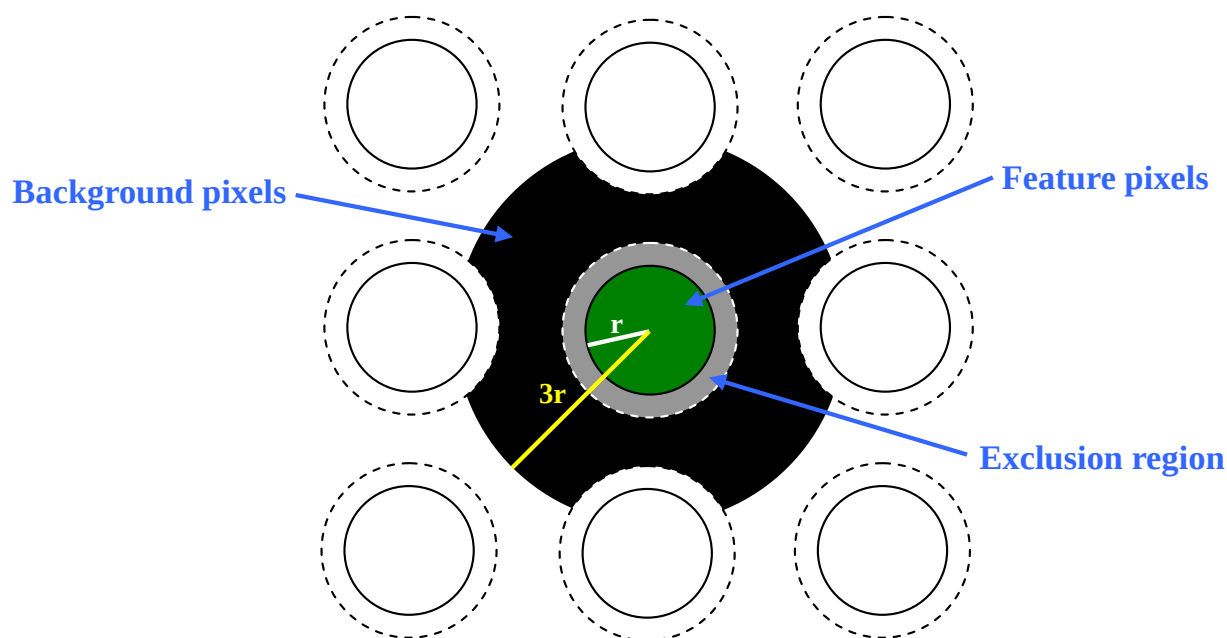


Figure S-1

All the signal and background intensities were obtained by a GenePix 4000B microarray scanner and analyzed by the software of GenePix Pro 3.0. As a result, we can directly get the signal and background intensities from the software. To obtain the fluorescence intensity for the spot located in the center of a 3x3 array in Figure S-1, the collected data will be processed in such a way that:

(1) The software provides a function to “auto-select” these 9 spot areas, which are defined as “Feature pixels” of the 9 spots. The median intensity of the pixels in the center area of the feature pixels represents the signal intensity of the center spot.

(2) Two pixels extended from the boundary of the feature pixels, where is defined as “Exclusion region”, are not calculated for either the feature pixels or background pixels.

(3) The pixels extended three-fold the radius from the center spot that exclude the pixels of all exclusion regions and feature pixels are defined as “Background pixels”. The median intensity of these pixels in the area of the background pixels represents the background or noise intensities of the center spot.

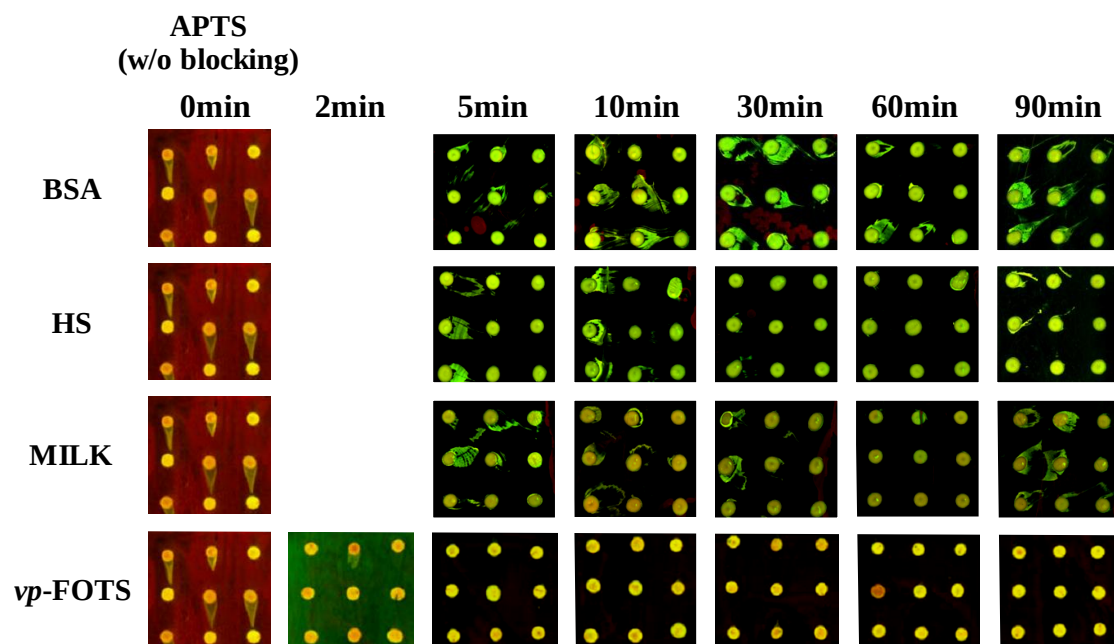


Figure S-2

Figure S-2. Images of the immunoassay results after conventional wet and vapor phase FOTS blocking processes with different blocking time.

Table S-1. Summary of all the average data of intensities, binding efficiencies (E_b), signal-to-noise ratios (S/N ratio), and Cy5 signal RSD in the (a) direct assay and (b) indirect ELISA. The data with first antibody concentration of 500 and 1000 $\mu\text{g/mL}$ are almost saturated, and those with first antibody concentration of 0 (without antibody) and 10 $\mu\text{g/mL}$ are barely differentiable; therefore, we combine them together respectively.

Table S-1

(a) Direct Assay								
Experimental conditions*			Intensity		Performance		Cy5 signal RSD	
			Signal (Cy5 / Cy3)	Noise (Cy5 / Cy3)	E _b	S/N ratio	Intra (%)	Inter (%)
1st antibody Cy3 concentration (μg/mL)	500 or 1000	1. Blank (n=2)	704 / 519	475 / 43	0.05	1.5	10	11
		2. APTS_w/o blocking (n=3)	13040 / 7263	4144 / 562	1	3.1	13	25
		3. HS (n=3)	7722 / 5054	359 / 68	0.59	21.5	13	24
		4. MILK (n=3)	15474 / 5357	196 / 49	1.19	78.9	10	29
		5. BSA (n=3)	11391 / 8611	2973 / 3121	0.87	3.8	11	12
		6. vp -FOTS (n=3)	44303 / 42496	182 / 78	3.40	243.4	6	9
	250	7. Blank (n=2)	735 / 577	203 / 39	0.09	3.6	24	10
		8. APTS_w/o blocking (n=3)	8637 / 6810	3045 / 306	1	2.8	15	10
		9. HS (n=3)	5122 / 4873	417 / 62	0.59	12.3	12	23
		10. MILK (n=3)	12742 / 4984	197 / 49	1.48	64.7	8	32
		11. BSA (n=3)	6908 / 6688	721 / 250	0.80	9.6	14	16
		12. vp -FOTS (n=3)	25088 / 36431	178 / 76	2.9	140.9	8	27
	100	13. Blank (n=2)	548 / 443	160 / 40	0.12	3.4	11	11
		14. APTS_w/o blocking (n=3)	4747 / 3313	2693 / 405	1	1.8	17	45
		15. HS (n=3)	2778 / 2589	368 / 57	0.59	7.5	9	17
		16. MILK (n=3)	6672 / 2541	198 / 46	1.42	33.7	15	55
		17. BSA (n=3)	3946 / 3129	567 / 82	0.83	7.0	18	8
		18. vp -FOTS (n=3)	4643 / 19497	142 / 69	0.98	32.7	15	9
	30	19. Blank (n=2)	262 / 592	129 / 50	0.09	2.0	31	12
		20. APTS_w/o blocking (n=3)	2965 / 983	2573 / 325	1	1.2	18	53
		21. HS (n=3)	774 / 1453	315 / 55	0.26	2.5	14	27
		22. MILK (n=3)	1186 / 749	207 / 50	0.40	5.7	13	14
		23. BSA (n=3)	1920 / 1081	531 / 76	0.65	3.6	12	57
		24. vp -FOTS (n=3)	452 / 2761	159 / 55	0.15	2.8	16	30
	0 or 10	25. Blank (n=2)	-	105 / 40	-	-	11	4
		26. APTS_w/o blocking (n=3)	-	2427 / 73	-	-	13	53
		27. HS (n=3)	-	236 / 51	-	-	16	11
		28. MILK (n=3)	-	147 / 47	-	-	13	11
		29. BSA (n=3)	-	385 / 50	-	-	11	35
		30. vp -FOTS (n=3)	-	144 / 53	-	-	14	30

(b) Indirect ELISA								
Experimental conditions*			Intensity		Performance		Cy5 signal RSD	
			Signal (Cy5 / Cy3)	Noise (Cy5 / Cy3)	E _b	S/N ratio	Intra (%)	Inter (%)
		1. Blank (n=3)	134 / 361	118 / 107	0.1	1.1	28	10
		2. APTS_w/o blocking (n=4)	1452 / 2439	831 / 1724	1	1.7	20	26
		3. BSA (n=3)	3937 / 4286	642 / 422	2.7	6.1	23	18
		4. vp -FOTS (n=4)	13127 / 13580	192 / 128	9.0	68.4	20	21

*Each experimental condition contains 5 spots and 16 spots inter the slide for the direct assay and indirect ELISA, respectively. Besides, n in each parenthesis represents the number of replicates.