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PAPER

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Electronic Supplementary Information (ESI) for:

Fabrication Three-dimensional Hydrogel Oligonucleotide Microarrays to Detect Single Nucleotide Polymorphisms

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1. Additional Experiment Section

1.1. 2D oligonucleotide microarray fabrication

The probe DNA oligomers (Pm) was dissolved in 15 μ L of spotting buffer (3 $\times$ SSC, 1.5 M betaine, 0.005% (w/v) SDS) with desired concentration and spotted on the commercial 2D slides by a SmartArrayer 48 system (Capitalbio Ltd., Beijing, China) under non-contact spraying mode. After an overnight incubation under 80% humidity at 30 $^{\circ}$ C and further incubation for 15 min at 100 $^{\circ}$ C, the slides were rinsed with 30 mL of washing buffer (1 $\times$ SSC, 0.01% (w/v) SDS; 3 times) and then incubated in 30 mL of blocking buffer (pH 7.4, 50 mM PB, 0.15 M NaCl supplemented with 0.1 M ethanolamine) at 30 $^{\circ}$ C for 1 h to de-active remaining free aldehyde groups.

1.2. Hybridizing and labeling the 2D oligonucleotide microarrays

After blocking step, the 2D oligonucleotide microarrays were hybridized with Target DNA under desired concentration in 25 μ L hybridization buffer (4 $\times$ SSC, 0.1% (w/v) SDS) for 25 min at 55 $^{\circ}$ C. Then, the microarrays were subjected to a series of rinsing steps: (1) 30 mL of hybridization buffer (3 times); (2) 30 mL of washing buffer (3 times); and (3) 30 mL of water, respectively. The microarrays were dried by centrifugation (480 g for 1 min). Subsequently, the microarrays were labeled by Pf (100 nM in hybridization buffer), washed, and dried as previously described, respectively. Finally, the microarrays were detected by conventional laser confocal fluorescence microarray scanner (Luxscan-10K/A, Capitalbio Ltd., China).

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2. Additional Figures

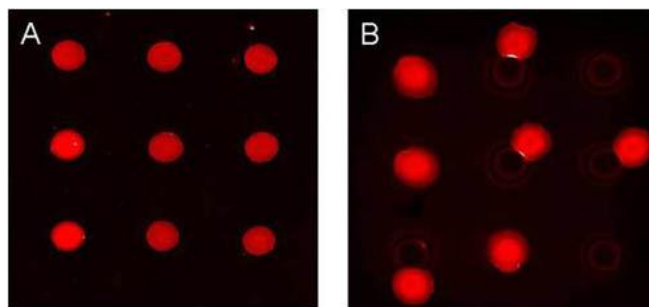


Fig. S1 Fluorescence images of 3D oligonucleotide microarray on PEG modified glass slide (A) and glass slide without PEG modification (B), respectively.

After hybridized with target DNA and labeled by Pf, the 3D oligonucleotide microarray on PEG modified glass slide shows good spot feature. The experimental result indicates that PEG crosslinkers improves the stability of the microgels on the slide.

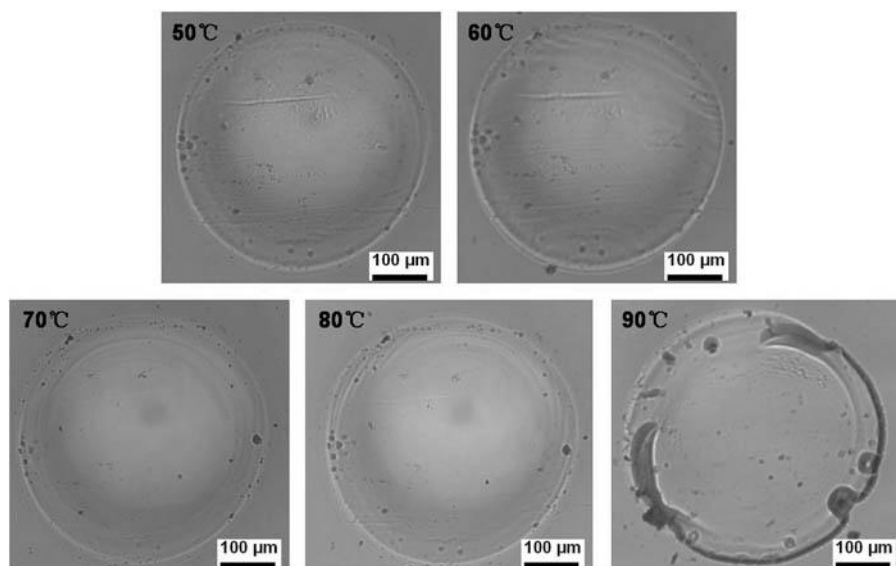


Fig. S2 Microscopic images of polyacrylamide hydrogel spots in hybridization buffer at various temperatures.

The morphologies of polyacrylamide hydrogel spots remain unchanged when the temperature of the buffer is lower than 80 °C. The experimental result indicates that the 3D slides have good thermal stability.

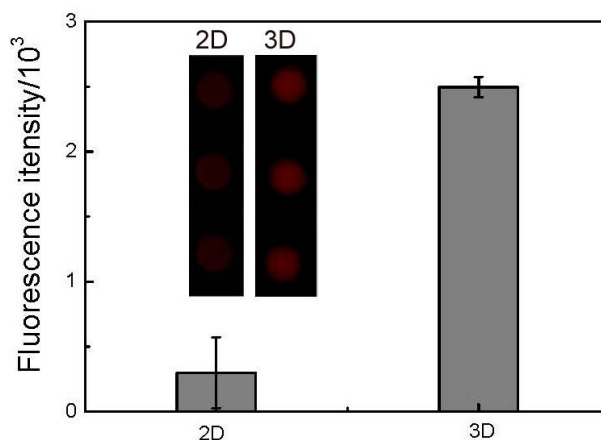


Fig. S3 Fluorescence images and the corresponding analysis for detecting Tm by 2D or 3D oligonucleotide microarray, respectively. The concentration of Pm is 5 μ M probe solution, the concentration of Tm is 10 nM and the concentration of Pf is 100 nM, respectively.

5 The fluorescence intensity of 3D oligonucleotide microarray is about 8 times higher than that of 2D oligonucleotide microarray.

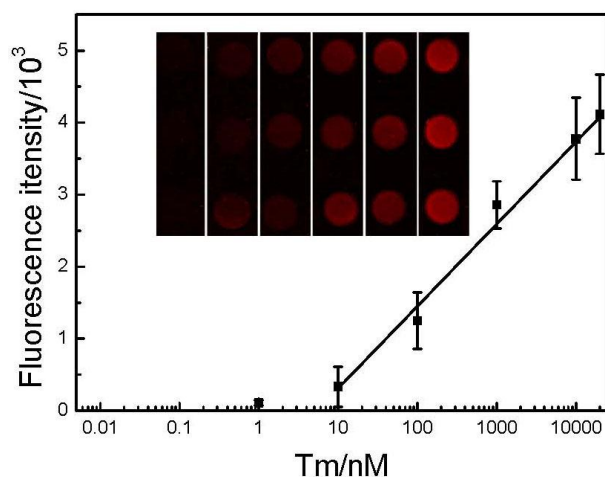


Fig. S4 Fluorescence images and the corresponding plots of the fluorescence intensities of 2D microarrays as a function of logarithm of Tm concentrations. The concentration of Pm is 5 μ M in probe solution, and the concentration of Pf is 100 nM, respectively. The detection limit of 2D microarray-based assay is 10 nM.

3. Full Format of References

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