DNA microarraying on compact disc surfaces. Application to the analysis of single nucleotide polymorphisms in Plum pox virus
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Topology of coated surfaces
Physical measurements were carried out to determine the homogeneity of the avidin film. As is shown in the Fig 1SI, the thickness of the film was close to 240 nm and 91 nm for PC and PMMA, respectively, which indicates a homogeneous immobilization of avidin on both supports. The thickness of avidin film was measured using Therma-Wave Opti-Probe 5220 (Fremont, CA). Contact angle measurements were done using OCA 20 Video-Based Contact Angle Meter from DataPhysics Instruments, GmbH (Filderstadt, Germany) on different parts of the surfaces to test the coating uniformity. The coefficient of variation of measurements taken (n=12) was 2.8 % and 3.3% for PC and PMMA, respectively, what indicated a homogeneous protein immobilization along the surfaces.

Fig. 1SI. Topology of surfaces after coating with avidin at 100 mg/L. (A) PC and (B) PMMA. A coated area of 50-mm2 was scanned.

Influence of coating and printing buffer on hybridization performance
Avidin coating (100 mg/L) and 0.01 µM probe A were used along with the experiments to optimize the protein adsorption. As is shown in Fig. 2SI, the effect of pH on avidin adsorption clearly denoted that electrostatic forces are involved on immobilization process. As a rule, the best coating conditions for PC and PMMA were PBS at pH close to isoelectric point (pI 10.5) independent of the printing buffers used. Regarding to the influence of printing buffer on probe binding, the best results were obtained using PB at pH 9.0 in both supports. In terms of fluorescence intensity the best buffer coating/printing combination was PBS, pH
According to literature, avidin-biotin binding is favored at pH close to 9.0 what it is in good agreement with the obtained results. With regard to avidin-biotin binding efficiency, neither higher nor lower phosphate concentrations (2 and 100 mM) improve the results obtained with PBS, pH 10.5.

**Fig. 2SI Influence of coating and printing buffer on fluorescence.** (A) PC (B) PMMA. (I: CB, pH 9.6; II-VII: PBS at pH 4.0 (II); pH 6.0 (III); pH 7.5 (IV); pH 9.6 (V); pH 10.5 (VI); pH 12.0 (VII)