Selective surface treatment of micro printing pin and its performance

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Biological microarray construction relies on the sequential deposition of liquid samples, typically by contact or ink-jet printing. One drawback of contact printing is excessive solution pickup on the pin’s outer surface, resulting in inefficiency. The authors combated this problem with a simple method that treats pin surfaces selectively so the outer surface becomes hydrophobic while the inner surface remains hydrophilic. Silicon-micromachined pins were utilized to evaluate the effect. The results demonstrated elimination of preprinting, greater droplet size consistency (e.g., 42±5 μm vs 63±13 μm), and more spots (~800 vs ~300) printed per loading. Consequently, an average spot diameter, between 30 and 100 μm, can be controlled, depending on the pin design. © 2006 American Institute of Physics. [DOI: 10.1063/1.2337882]

Microarrays are synthesized by two distinct methods today. First, they can be made photolithographically to directly synthesize high density biological arrays on the substrate, a method exemplified by Affymetrix. This method, however, is too costly and time consuming for small-scale productions of custom biological microarray due to the number of photolithographic steps required for production. Second, they can be made with presynthesized oligonucleotides or polymerase chain reaction products by either contact printing, eject droplet printing, or otherwise. Contact printing with robotic microarrays, as pioneered by Brown and Botstein, is widely used because of its flexibility in application. For example, no prior knowledge of genome sequence is required when printing with distinct or unknown genetic materials. Also, tips and robotics are durable and inexpensive—desirable factors when performing multiplexed printing over prolonged periods. A wide variety of contact printing devices have been fabricated to produce deoxyribonucleic acid (DNA) microarrays with high density. In addition to stainless steel, other materials such as tungsten, microfabricated steel, ceramic, and silicon have all shown promise in delivering precise droplet deposition. A common practice for most contact printing includes “preprinting.” Due to the presence of excessive solution clinging to the outer surface, a pin would deposit a number of larger droplets at the beginning of the printing run. This phenomenon persists until the excessive solution is either drained out during initial printings (usually 10–20 spots) or evaporated to the environment. Preprinting not only slows the printing time but also wastes valuable samples.

Our goal is to minimize the excessive pickup of sample solution on the outer surface of the printing pin. We approach our goal by strategically defining the wettability of the surfaces during pin fabrication. The problem with the outer surface of the pin is that it should possess a water-repelling nature, low interaction with the biological molecules, chemical resistance, as well as its durability when subject to successive washing and drying. In this study, we used hexamethyldisilazane (HMDS)
and octadecyl trichlorosilane (OTS) (water contact angle \( \sim 112^\circ \)) as appropriate hydrophobic materials. Finally, the photoresist was dissolved. This dissolving step did not affect the surface wettability. In this letter, the efficacy of this method was evaluated using custom silicon-micromachined pins, whose manufacturing method and printing performance were reported elsewhere.\(^{12,13}\) Designed to be mounted on most commercial arrayers, the silicon pins are 525 \( \mu \text{m} \) thick, 1400 \( \mu \text{m} \) wide, and 45 mm long. Note that this selective treatment can be applied to most other printing pins as well, including commercial ones.

Liquid loading experiments were performed to test the effect of selective hydrophobic treatment. A coated pin and an uncoated pin were both dipped into a 4 \( \mu \text{l} \) hemispherical 3XSSC droplet for 10 s and withdrawn from the droplet. The untreated pin was coated with excessive solution on its outer surface [Fig. 2(a)]. On the other hand, the treated pin was free of the excessive liquid [Fig. 2(b)]. Since the amount of liquid loading is now determined only by the volume of the capillary within the pin and not affected by how deep the pin is immersed in the sample, a consistent liquid volume is loaded regardless of the sample volume remaining in the capillary. The average spot volume was found to be \( \sim 2 \text{ pl} \). The robustness of the silicon pins and its hydrophilic nature and geometry, tended to have more accumulation of excessive solution in the beginning of the run than stainless-steel pins and required approximately 30 spots run before consistent spots were reached. In comparison, treated silicon pins (both by HMDS and OTS) require little or no preprint, since the first spot printed was already small. Consistent spots of approximately \( 50 \mu \text{m} \) nominal diameter were achieved.

To test under typical robotic printing condition and evaluate cross contamination, another set of printing was performed with a treated silicon pin using salmon sperm DNA on polylsine slides (contact angle 65\(^\circ\) with 3XSSC). The pins were loaded onto an Affymetrix 417 robotic arrayer. Four hundred spots were printed in one run with an average spot size of 38 \( \mu \text{m} \). The two color fluorescence hybridization tests, shown in Fig. 4, were performed using complementary oligonucleotides. The two oligonucleotides, M13R and M13F, with the same concentration were mixed with ratios of 2.0, 2.1, 1:1, 1:2, and 0:2 and printed on slide. They are then hybridized with complementary oligonucleotides with Cy3 and Cy5 attached. (b) Intensity profile along a line segment. This demonstrates the spot size consistency from run to run and no sample contamination during printing.

![FIG. 2. (Color online) Liquid loading from a sample droplet. Selective hydrophobic coating of printing pin results in better liquid loading efficiency. (a) Untreated pin picks up additional liquids at its exterior surface. (b) Treated pin shows no excess liquid pickup.](image)

![FIG. 4. (Color online) Reproducibility of spots with OTS-treated pins evaluated by two color fluorescence hybridization. (a) The two oligonucleotides, M13R and M13F, with same concentration, are mixed with ratios of 2.0, 2.1, 1:1, 1:2, and 0:2 and printed on slide. They are then hybridized with complementary oligonucleotides with Cy3 and Cy5 attached. (b) Intensity profile along a line segment. This demonstrates the spot size consistency from run to run and no sample contamination during printing.](image)
TABLE I. Overall performance of selectively treated silicon pin. In comparison with the untreated pins, the treated pins eliminate the need for preprinting (i.e., solution saving), print smaller spots at a higher precision, and print more spots per run. The data are collected using salmon sperm DNA with 3XSSC solution.

<table>
<thead>
<tr>
<th></th>
<th>Preprint spots</th>
<th>Average spot size (µm)</th>
<th>Standard deviation (µm)</th>
<th>Total spots printed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless-steel pin (commercial)</td>
<td>10–20</td>
<td>100</td>
<td>±14</td>
<td>150</td>
</tr>
<tr>
<td>Si pin</td>
<td>50</td>
<td>63</td>
<td>±13</td>
<td>300</td>
</tr>
<tr>
<td>Si pin selectively treated with HMDS</td>
<td>Not required</td>
<td>48</td>
<td>±8</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Si pin selectively treated with OTS</td>
<td>Not required</td>
<td>42</td>
<td>±5</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

In summary, we presented a selective surface treatment to control the surface wettability of a contact printing device and evaluated its performance using silicon-micromachined pins. By tailoring the surface energy of different regions of the printing pin, we have eliminated the need for preprinting in contact printing and improved the overall consistency of the spot volumes deposited onto glass surfaces. Table I summarized the results. The selective surface treatment resulted in (1) solution saving, (2) shorter printing time, and (3) higher quality spots, which are important attributes for the creation of high throughput, larger scale microarrays.

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