

Replication of a DNA Microarray

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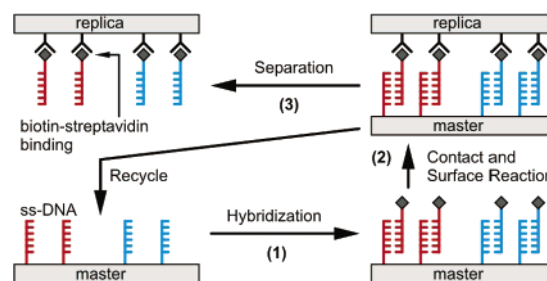
Here we report a method for efficient replication of DNA microarrays. As shown in Scheme 1, the approach comprises three steps. First, a master DNA microarray consisting of single-stranded DNA (ssDNA) elements is exposed to a solution containing the biotin-functionalized complement of each array element. Following hybridization, a replica surface modified with streptavidin is brought into contact with the master. This results in linking of the biotin-functionalized complement with the replica surface. Next, the replica is separated from the master, and some or all of the complementary strands are transferred to the replica surface. The resulting complementary DNA microarray now contains position-coded sequences that mirror the information contained on the master DNA microarray. Finally, ssDNA on the master can be rehybridized and the procedure repeated to produce additional replicas. This concept was previously used by Gaub and co-workers,¹ who used it to construct a programmable force sensor.^{1,2}

Two general methods are in use for fabricating DNA microarrays: in situ synthesis and ex situ spotting.^{3,4} In situ synthesis integrates photolithography and solid-phase oligonucleotide (oligo) synthesis.³ This method has the advantages of small feature size ($\sim 8 \mu\text{m}$ /array element) and design flexibility⁵ but is limited to oligos having a maximum length of 60 base pairs (bps).³⁻⁵ The second approach for fabricating microarrays is ex situ spotting of presynthesized oligos.⁴ Spotting is normally implemented by printing with DNA-coated pins or microfabricated nozzles.^{6,7} It can be used to prepare array elements having >60 bps, but serial printing is still required because the number of pins or nozzles is usually smaller than the array size.⁸ In addition, spot sizes are large ($75\text{--}500 \mu\text{m}$) compared to in situ fabrication methods.⁴ Other ex situ methods for patterning presynthesized oligos include microfluidic delivery,⁹ microcontact printing,⁸ and dip-pen nanolithography.¹⁰

The data in Figure 1 provides experimental verification for the viability of the process illustrated in Scheme 1. The master was prepared by reacting a 5'-amine-modified oligo (38-mer) with a glass slide coated with an *N*-hydroxysuccinimide-functionalized polymer.¹¹ Attachment of the oligo to the slide was confirmed by observing the fluorescence resulting from hybridization of the complement modified at the 3' end with fluorescein and at the 5' end with biotin (Figure 1A). When the slide was exposed to noncomplementary fluorescein-labeled ssDNA, no fluorescence was observed (data not shown).

Next, the replica surface was prepared by reacting maleimide-modified streptavidin with thiol-modified poly(dimethylsiloxane) (PDMS)¹² (detailed information about the experimental procedures are provided in the Supporting Information). The replica was then brought into contact with the master for 10 min at a pressure of 1.4 N/cm^2 in a pH 7.0 solution containing 60 mM sodium citrate and 600 mM NaCl at $22 \pm 2 \text{ }^\circ\text{C}$. To facilitate contact between the master and the replica it was necessary to provide a means to drain the solution away from the interface, and therefore $10 \mu\text{m}$ -deep recessed trenches were fabricated in the replica surface.² These

Scheme 1



trenches are visible as a dark grid pattern ($200 \mu\text{m}$ on center and $30 \mu\text{m}$ wide) in Figure 1B.

The replica was separated from the master, and then the fluorescence intensity from both surfaces was measured and compared to those of the master prior to transfer (Figure 1A). Following contact of the two surfaces, DNA transfer from the master (Figure 1C) to the replica (Figure 1B) is observed as a decrease in fluorescence on the master surface and a corresponding increase in fluorescence on the replica surface. Figure 1D shows fluorescence intensity profiles (obtained by averaging all the line profiles between the dashed white lines) in the other three frames of Figure 1. The pulse-shaped features in these profiles arise from the presence of the recessed trenches in the replica. Two facts can be deduced from the profiles. First, there is conservation of the

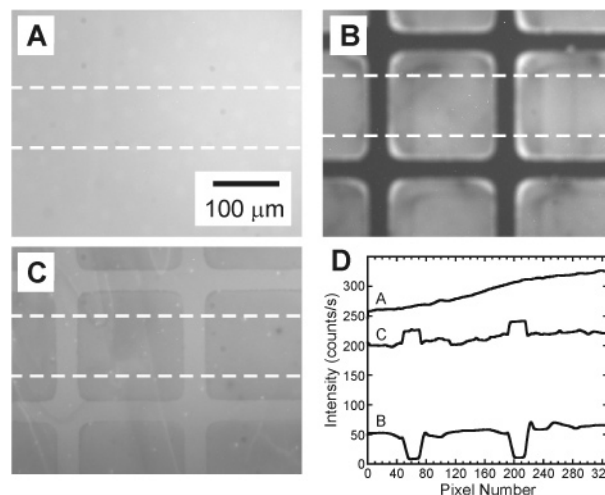


Figure 1. Fluorescence micrographs showing the transfer of fluorescein-labeled DNA strands from a master slide to a replica surface (PDMS): (A) the master slide modified with probe DNA hybridized to the complement (modified with fluorescein and biotin) before transfer, (B) the PDMS replica after transfer, (C) the master slide after transfer, and (D) averaged intensity profiles from frames A, B, and C. Each profile was obtained by averaging all line profiles between the two white dashed lines and dividing the result by the integration time. The image integration time was 10 s for frames A and C and 30 s for frame B. The gray scale for all three images is from 0 to 4100 counts. The transfer involves conformal contact and subsequent separation of the master and replica (Scheme 1).

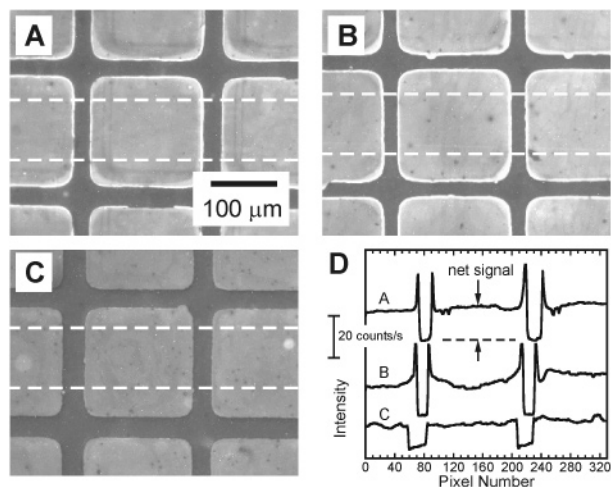


Figure 2. Fluorescence micrographs demonstrating the durability of the replication process. Nonfluorescent ssDNA (38 mers) was transferred from the master to three replicas. Next, each replica was hybridized to the fluorescein-labeled complement. Fluorescence micrographs of the first, second, and third replicas prepared from a single master are shown in frames A, B, and C. For all micrographs, the integration time was 60 s, and the gray scale is from 0 to 4100 counts. Frame D shows averaged fluorescence intensity profiles from frames A, B, and C. Each profile was obtained by averaging all line profiles between the two dashed white lines. The amount of hybridized complement on each replica can be quantified using the net fluorescence intensity determined from an averaged intensity profile (as shown for the intensity profile for frame A) by subtracting the intensity at the center of a trench from the intensity at the center of a square pad. The net fluorescence intensities arising from the first, second, and third replicas are respectively 16, 14, and 11 counts/s.

fluorescence signal: the sum of the fluorescence intensities for profiles B and C roughly equals the fluorescence intensity on the master prior to transfer (profile A). Second, only $\sim 20\%$ of the DNA originally present on the master is transferred to the replica surface; that is, the fluorescence intensity from the master is four times higher than that from the replica after transfer. The latter observation is a consequence of the three-dimensional polymeric structure of the coating on the master: only the topmost layer of DNA is transferred to the replica. Importantly, control experiments indicate that the biotin–streptavidin interaction is essential for the transfer of the complementary DNA strands (Supporting Information, Figure S1), and therefore we conclude that the effects of nonspecific transfer are below the detection limit of the measurement system. Note, too, that proper separation of the master and replica requires that the force necessary to separate biotin and streptavidin be larger than the force required to separate the DNA duplex into its component strands.²

Figure 2 provides information about the durability of the replication process. In this experiment, nonfluorescent ssDNA was transferred to each of three replica surfaces from the same master using the procedure shown in Scheme 1. The resulting three replicas (Figure 2A is a replica surface after the first transfer, and frames B and C of Figure 2 correspond to the second and third replications, respectively) were then imaged after hybridization with fluorescently labeled, complementary ssDNA. This experiment demonstrates three important points. First, it shows that multiple replicas can be prepared from a single master. Second, it confirms that the replicas are functional; that is, they are able to specifically recognize and

bind their complement. Control experiments (Supporting Information, Figure S2) indicate that no hybridization occurs when the replicas are exposed to fluorescently labeled, noncomplementary ssDNA. Third, the amount of DNA transferred decreases with each successive array replication. Specifically, the net fluorescence intensities arising from the first, second, and third replicas are respectively 16, 14, and 11 counts/s. These values were determined from the averaged intensity profiles shown in Figure 2D by subtracting the intensity at the center of a trench from the intensity at the center of a square pad. These values are smaller than the intensity measured from fluorescently labeled ssDNA immediately after transfer from the master (~ 50 counts/s, Figure 1B), which may suggest that some of the ssDNA transferred to the replicas is not fully active toward hybridization. Additional experimentation is needed to clarify this point.

Several issues important to the development of a practical replication technology have not been addressed in this preliminary report. First, the master surface displays only a single DNA sequence, and the spot size is large (~ 1.5 mm). Replication using master microarrays modified with many different sequences still remains to be demonstrated. Second, the replica spot size (~ 170 μm , frames A–C in Figure 2) is large compared to that of a commercial DNA chip (8 μm). However, we believe this can be reduced by a factor of 10 or so. Additional experiments presently underway in our lab will address these two important issues.

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Supporting Information Available: Information about the chemicals and materials used in this study and about the control experiments described in the text are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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