In Situ DNA Synthesis on Glass Substrate for Microarray Fabrication Using Self-Focusing Acoustic Transducer

Jae Wan Kwon, Member, IEEE, Sanat Kamal-Bahl, Student Member, IEEE, and Eun Sok Kim, Senior Member, IEEE

Abstract—This paper presents a droplet-ejection-based technique for synthesizing deoxyribonucleic acid (DNA) sequences on different substrates, such as glass, plastic, or silicon. Any DNA sequence can be synthesized by ejecting droplets of DNA bases by a self-focusing acoustic transducer (SFAT) that does not require any nozzles. An SFAT can eject liquid droplets around 3–5 μm in diameter, which is significantly smaller than those ejected by commercial ink jet printers and reduces the amount of reagents needed for the synthesis. An array of SFATs is integrated with microchannels and reservoirs for delivery of DNA bases to the SFATs. Poly-l-lysine-coated glass slide is patterned, and is used as a target substrate for in situ synthesis of multiple T bases. The significant advantage of this scheme over some of the existing commercial solutions is that it can allow geneticists to synthesize any DNA sequence within hours using a computer program at an affordable cost in their own labs. This paper describes the concept and scheme of the on-demand DNA synthesis (with an acoustic ejector integrated with microfluidic components) along with the results of an actual DNA synthesis by an SFAT.

Note to Practitioners—Deoxyribonucleic acid (DNA) microarrays allow geneticists to monitor the interactions among thousands of genes simultaneously in a chip. There are commercial systems for producing DNA microarrays, but none of them give flexibility to synthesize DNA microarrays on-demand in the geneticist’s own lab. Affymetrix’s GeneChip technology produces DNA probe sequences premade at Affymetrix with a set of 4n photomasks for n-mers. Other techniques transfer premade DNA sequences to a substrate (glass, plastic, or silicon) through ink-jet printing or contact dispensing. Agilent and Rosetta use their ink-jet printing technology to produce DNA probe sequences at their factories. The ink-jet print heads used for printing microarrays use either piezoelectric or thermal actuation, and eject liquid droplets through nozzles. Thus, the smallest droplet size ejected from these devices depends on the size of the nozzle. The small nozzles are difficult to construct with good uniformity and also tend to get clogged. Hence, nozzle-less ejection is desired when the droplet size needs to be minimized without clogging problems at all.

A focused acoustic beam produced by a piezoelectric film has been shown to eject liquid droplets [3]. Focusing of the acoustic waves can be achieved by using a physical lens or through wave interference. A physical lens, though, requires additional fabrication steps and material on top of the transducer fabrication [4], [5]. On the other hand, focusing an acoustic wave through wave interference requires no physical lens, but just a proper electrode patterning for phased acoustic sources. Thus, our transducer, named the self-focusing acoustic-wave transducer (SFAT), is easy to fabricate, and also can easily be made to produce directional ejection [6]. Since the SFAT is fabricated with a microelectromechanical systems (MEMS) processing technique on a silicon substrate, it can also easily be integrated with microfluidic components, such as channels and reservoirs [7].

This paper describes a DNA microarray fabrication technique based on SFATs. The SFAT ejects droplets of DNA bases, drawing the bases from onchip reservoirs through capillary force in the microchannels. The microchannels, reservoirs, and ejectors are fully covered with a thin parylene film. The DNA sequence of length 3-mer composed of T bases has been synthesized with an SFAT, and the synthesis result has been confirmed with fluorescence imaging. The idea presented in
this paper clearly demonstrates the feasibility of a portable and automated DNA synthesis system that allows geneticists to synthesize any DNA sequences in their lab at an affordable cost within a short period of time.

II. SFAT DESIGN

The SFAT consists of a piezoelectric ZnO film sandwiched between two Al electrodes that are patterned into annular rings to form so-called Fresnel half-wave band sources (FHWB) [8], [9]. The acoustic waves generated by the adjacent annular sources arrive at the focal point with a finite delay due to a path-length difference between the annular sources and the focal point. If the path length difference is designed such that the acoustic waves generated by the adjacent sources are delayed by a multiple of the wavelength, then the acoustic waves arrive at the focal point in phase, constructively interfering with each other and intensifying the acoustic pressure. Thus, the focused acoustic beam can eject liquid droplets from the SFAT chamber onto a glass or any other substrate as illustrated in Fig. 1. Although SFAT can eject droplets without any nozzle, the SFAT chamber onto a glass or any other substrate as illustrated in Fig. 1. Although SFAT can eject droplets without any nozzle, we cover the SFAT with parylene film to minimize exposure of the nucleic acid on the wafer. The parylene cover is relatively larger in size (200 μm with a hole (of about 200 μm in diameter) to minimize the acoustic pressure. Thus, the focused acoustic beam can eject liquid droplets from the SFAT chamber through liquid surface tension. The opening in the parylene cover is relatively larger in size (200 μm) than the nozzle sizes (tens of micrometers).

The diameter of each FHWB ring is determined by the acoustic wavelength in liquid and the designed focal length. The width of each electrode ring and the adjacent aperture are chosen to introduce proper phase shifts [8], [9]. In general, a higher peak particle-displacement and narrower acoustic beam diameter can be obtained with a larger number of FHWB sources. However, as the number of FHWB sources increases, the width of the outermost electrode ring becomes narrower, presenting fabrication difficulty and undesired influence by fringing the electric field between adjacent electrodes.

III. FABRICATION OF SFAT

The SFAT device integrated with microchannels and reservoirs is fabricated as described below, following the processing steps illustrated in Fig. 2.

Fig. 1. Cross-sectional view of the SFAT with a cover. The cover forces the liquid level to be maintained at a constant level by automatic liquid delivery while the ejector ejects the liquid out. Also shown at top is a glass substrate that receives the ejected droplets of DNA bases.

Fig. 2. Fabrication steps for an SFAT ejector with embedded reservoir and channels. (a) Pattern silicon nitride on a silicon substrate for SFAT area KOH etching. (b) Pattern silicon nitride for reservoir area. (c) KOH etch and deposit silicon nitride. (d) Pattern channel area and KOH etch with protected convex corner. (e) Deposit Al–ZnO–Al on the other side of the silicon substrate. (f) Deposit pattern parylene on a glass substrate. (g) Bond the SFAT substrate and glass substrate. (h) Remove the glass substrate.

The double-side-polished silicon wafer is first deposited with 0.8-μm-thick SiNx by low-pressure chemical vapor deposition (LPCVD), followed by the patterning of the SiNx (on the wafer backside) and then etching of silicon in KOH. After etching about 100-μm-thick silicon (for the SFAT active area), the SiNx is patterned again to open windows for the reservoir areas. The silicon in the wafer is then etched further in KOH to form the silicon nitride diaphragm for the SFAT. At this point, the reservoir area has about 100-μm-thick silicon remaining as shown in Fig. 2(c).

The ejectors and reservoirs on a chip are connected through microfluidic channels, so that liquid can continuously be delivered from the reservoirs to the ejectors by capillary force in the microchannel. The liquid is initially filled in the reservoir using a syringe. The liquid is then provided continuously from the reservoir to each ejector, and a certain liquid level is maintained in the ejector (though the ejector ejects liquid out), owing to the liquid surface tension pulling the liquid in from the reservoir. The microchannels are fabricated through spray-coating photoresist instead of spin coating. The spray coating is needed to conformally coat photoresist over the deep trench formed in the SFAT and reservoir areas. The wafer is then soft baked for 12 min with its coated side facing down. After UV exposure and photoresist development, the wafer is hard baked, again with its coated side facing down. The exposed silicon is then isotropically etched with XeF2 vapor to form microchannels. Photore-
sist is used to protect the other side of the wafer during the XeF₂ etching.

After forming the channels, a 0.3-μm-thick Al layer is deposited and patterned for the bottom electrode on the wafer front side. Then, 5-μm-thick ZnO is sputter deposited from a ZnO target, followed by a 0.3-μm-thick Al deposition for the top electrode. Fig. 3 shows the SEM image of the reservoir, microchannels, and SFAT device before the parylene cover is placed over them.

Finally, the channels and reservoirs are covered with parylene film, as illustrated in Fig. 2(f) and (g) and described in detail in [10]. For transferring and bonding a 6-μm-thick parylene cover over microfluidic components on a silicon wafer, we first deposit parylene on a glass substrate, and bond the glass substrate and silicon wafer with an epoxy as shown in Fig. 4.

The glass substrate is pretreated with soap water before parylene is deposited over it so that the glass can easily be detached from parylene after the bonding step. The glass slide coated with parylene has alignment marks, which are used for fine aligned bonding with the microfluidic devices. Then the glass is removed by peeling off the parylene layer. The bonded parylene cover is without void.

The channel, reservoir, and ejector areas are not wetted at all by the epoxy (20 μm thick) as shown in Figs. 5 and 6. With the fabricated microfluidic system with a parylene cover, we obtain continuous ejection of liquid droplets from the ejectors, as liquid is pulled into the ejectors from the reservoir through surface tension.

The parylene cover is needed since the reagents for the DNA synthesis evaporate quickly at room temperature. A parylene cover with a circular opening of 200 μm in diameter slows the evaporation greatly: A larger opening allows too fast evaporation of the reagents, while a smaller opening makes the liquid surface tension too high to be overcome for ejection. Fig. 6 shows the completed SFAT with microchannels, reservoirs, and parylene cover with a 200-μm opening.

IV. EXPERIMENTAL SETUP

The fabricated SFAT device is packaged in a dual-in-line package (DIP) as shown in Fig. 7, and the package is placed on a mounting station with high-frequency microstrip as shown in Fig. 8.

A 481-MHz sinusoidal signal (corresponding to the resonance frequency of the piezoelectric film in SFAT) is pulsed for a 20-μs pulsewidth with a high-speed switch, amplified with an RF amplifier to about a 2.6-W instantaneous power level, and the amplified signal passes through impedance matching networks for the SFATs. Each of the matching networks is
Fig. 7. Photo of the SFAT array packaged in a DIP. Electrical contact between the package pins and the device pads are made using indium wire.

Fig. 8. Photo of the packaged SFAT ejectors mounted on a station that holds a glass substrate with a self-aligning hinge.

tailored for each SFAT to ensure maximum power delivery to the SFAT after the SFAT’s impedance is characterized in the package (with liquid in the SFAT chamber) and mounting station with a network analyzer.

A CCD camera with a microscope is placed horizontally to record the water ejection process through a stroboscopically blinking light-emitting diode (LED). Synchronization of the flash illumination with the sinusoidal pulse input is achieved by turning on an LED with another pulse source triggered by the pulse generator that turns on and off (i.e., pulses) the sinusoidal signal. Since the trajectory of the ejected droplets can easily be affected by air flow near the device, an O-ring is placed on top of the device to block sideways airflow. A patterned poly-l-lysine glass slide is placed on top of the O-ring to collect the ejected droplets of the DNA bases. The block diagram and photo of the experimental setup are shown in Figs. 9 and 10, respectively.

To synthesize any sequence of oligonucleotide from four DNA bases, we use standard phosphoramidite chemistry [11] on a poly-l-lysine coated glass slide. For this experiment, we synthesize a T-T-T sequence (along with Cy3 fluorescence tag on a poly-l-lysine-coated glass slide) as shown in Fig. 11. We use a syringe to fill up the reservoir with activated monomer (dT-CE phosphoramidite), which is brought to the SFAT through microchannels (due to capillary force and surface tension), and ejected by the SFAT. A poly-l-lysine-coated glass slide is photolithographically patterned into spots (100-μm diameter) as illustrated in Fig. 11.

Available 3’-end of the ejected bases react and couple with free hydroxyl group on the poly-l-lysine-coated glass substrate tailored for each SFAT to ensure maximum power delivery to the SFAT after the SFAT’s impedance is characterized in the package (with liquid in the SFAT chamber) and mounting station with a network analyzer.

A CCD camera with a microscope is placed horizontally to record the water ejection process through a stroboscopically blinking light-emitting diode (LED). Synchronization of the flash illumination with the sinusoidal pulse input is achieved by turning on an LED with another pulse source triggered by the pulse generator that turns on and off (i.e., pulses) the sinusoidal signal. Since the trajectory of the ejected droplets can easily be affected by air flow near the device, an O-ring is placed on top of the device to block sideways airflow. A patterned poly-l-lysine glass slide is placed on top of the O-ring to collect the ejected droplets of the DNA bases. The block diagram and photo of the experimental setup are shown in Figs. 9 and 10, respectively.

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Available 3’-end of the ejected bases react and couple with free hydroxyl group on the poly-l-lysine-coated glass substrate [11]. Then, the target glass is rotated about a mechanical hinge to perform washing and capping steps needed for the DNA synthesis. The capping step is followed by oxidation step to stabilize the coupling, which is followed by deblocking process (i.e., removing dimethoxytrityl (DMT) protecting group at 5’-end) for incoming oligonucleotides when the target glass, along with the mounting station, returns to the synthesis section at the starting step of the next iterating cycle. The most frequently used oligonucleotide synthesis method is beta-cyanoethyl phosphoramidite chemistry. This oligonucleotide synthesis is a cyclic sequence of reactions, adding one nucleotide to the growing oligonucleotide chain and proceeds in four main steps during each cycle: 1) deblocking; 2) activation/coupling; 3) capping; and 4) oxidation. The glass slide is then rotated back over the SFAT device, using a mechanical hinge on the mounting station to avoid the need for alignment of the glass slide with the SFAT device.

The alignment between each step is facilitated through the mechanical hinge mentioned above. Using the mechanical hinge, we make sure that the glass substrate is always at the same location above the SFAT after the washing, deblocking, and capping treatments that are needed for DNA synthesis. The glass substrate does not have to be removed from the SFAT platform for the treatments. It can only be rotated by 180° through the hinge to be treated with the necessary steps and then be rotated back to the same position.
Repeating the ejection and solidification steps \( n \) times, produces \( n \)-mer DNA probes. Cy3-CE phosphoramidite labeling dye is used at the last step to see (through fluorescence) whether the DNA has been synthesized. Before fluorescence imaging, the glass slide is washed thoroughly with acetonitrile and dried with gas to remove any residues of the dye attached to the glass substrate. The fluorescent dye directly combines with former sequences following the same synthesis protocol. In other words, the synthesized spot is invisible if any single error occurred during the synthesis steps.

V. EXPERIMENTAL RESULTS

Ejected droplets (taken with an optical stroboscopic method) in different time frames are measured to be 10 \( \mu \)m in diameter. The 10-\( \mu \)m-diameter droplets are ejected through an opening of 200 \( \mu \)m in diameter in the parylene cover from the center of the SFAT. The sizes of the droplets (continuously ejected for a minute) are very uniform as summarized in Table I.

Due to the parylene cover that provides the surface tension and capillary force, liquid is continuously brought into the ejector through the narrow channel from the reservoir, while the ejector consumes the liquid by ejecting it out. More than 90\% of the liquid in the reservoir can be delivered into the ejector through the channels, and the automatic liquid supply from the reservoir allows the droplet ejection to continue for more than 1 min.

Though each reservoir for the SFAT can contain any one of the four DNA bases (A, T, C, and G), we choose base T for the proof-of-concept in one of the reservoirs. The base is automatically transported to the SFAT from the reservoir through microchannels, owing to the surface tension provided by the parylene cover. We synthesize a sequence T-T-T on a patterned poly-l-lysine-coated glass slide by ejecting droplets of base T and going through the standard phosphoramidite chemistry described in the previous section. To test whether the DNA sequence is properly synthesized on the glass slide, we do fluorescence microscopy with excitation wavelength of 535 nm (according to the dye that we use).

Fig. 12 shows the images of the glass slide (that have been synthesized with the T-T-T sequence by ejecting T bases by the SFAT) under an optical microscope and ultraviolet (UV) light to observe fluorescence. The fluorescence images provide evidence of the DNA sequence being properly synthesized. We eject base T onto a glass slide, followed by capping and de-blocking steps, before next base T is ejected onto the same spot. Since at each cycle of DNA mer formation, the capping step is performed to disable a synthesis-failed spot at the coupling step, the fluorescence dye (introduced at the very last) binds only if
the required DNA sequence (TTT) has been synthesized. The fluorescent dye is of standard phosphoramidite chemistry, and directly binds to previous DNA under the same synthesis protocol. Thus, the synthesized spot would have shown no fluorescence (i.e., invisible), if any single error has occurred during the synthesis steps.

VI. DISCUSSION

Genotyping is currently very time-consuming and very expensive. Though Affimetrix’s GeneChip and various microarray techniques have allowed a geneticist to do combinatorial DNA sequencing on a chip surface, they do not allow a geneticist to synthesize DNA probe array readily and conveniently from four DNA bases. The GeneChip contains DNA probes premade at the factory with a set of photo masks, while the microarray techniques spot premade DNA sequences on a chip. No convenient technique is available for a geneticist to synthesize DNA probe array on a chip from four DNA bases. Yet, as a geneticist carries out DNA sequencings, often times a need arises to synthesize DNA probes in unique sequences.

Thus, a portable DNA probe-array-synthesis system could be made so that a geneticist can synthesize a 2-D DNA probe array of any desired DNA sequence on a glass chip at the geneticist’s site, starting from four DNA bases. For such a system, the SFAT would be ideal for ejecting DNA bases in any desired direction from a chamber. The SFAT-based ejectors require no heat, no nozzle, and no acoustic lens, and consume very little power and respond to electrical input extremely fast. The arrays of the ejectors are fabricated with MEMS technology, which can also be used in fabricating other microfluidic management components on a silicon chip. Microchannels, chambers, and reservoirs can be integrated with the SFAT ejector array on a single silicon chip to make the synthesis system compact and light as shown in Fig. 13.

The 2-D array of an SFAT ejector can be integrated with microchannels and four reservoirs (for the four DNA bases) on silicon chip to eject micro droplets of the four DNA bases (A, C, T, and G) in any desired sequence. As an example, a silicon chip that contains a $6 \times 6$ SFAT array (for $3 \times 3$ DNA probes) and a microfluidic transportation system for the four DNA bases is illustrated in Fig. 14.

The compact design includes four reservoirs for the bases and also remarkably reduces the number of crosses among the liquid transportation lines. In Fig. 14, each array block consists of $2 \times 2$ SFATs, each of which contains different DNA bases.

To fabricate a DNA probe array, one can start out with a glass chip whose surface is coated with a specific material (for example: Poly-L-lysine) that will bond chemically with any of the four DNA bases with standard phosphoramidite chemistry [11]. The glass chip is placed in the target holder, and is brought over the SFAT-ejector-array chip, which ejects DNA base droplets of optimal size to ink nine spots on the glass chip with the desired DNA bases. In order to deliver enough bases (having a fast evaporating diluent) for sure coupling, the size of the droplet can vary from 2 to 5 $\mu$m to tens of micrometers in diameter, which could be easily decided through optimization steps of the system. Four SFAT ejectors with the four DNA bases cover one spot so that any of the four bases can be ejected to the nine spots. Then, the glass chip is moved to the wash section, and the uncoupled bases (i.e., the bases that are not chemically bonded to the glass chip) are washed away. After the removal of the uncoupled bases through washing with an organic solvent, the glass chip is brought back to the exact same position over the ejector chip, which then shoots out another set of desired DNA bases over the nine spots. This process is repeated until one obtains the desired DNA sequences over the nine spots. If the array has $N \times N$ SFATs, the number of the spots that can be covered will be $N^2 / 4$.

VII. CONCLUSION

This paper describes a DNA microarray fabrication technique based on SFAT ejectors. For proof of concept, a sequence of T-T-T has been synthesized on a glass substrate with an SFAT integrated with reservoirs and microchannels on a single chip. The synthesis is confirmed with fluorescence imaging, and demonstrates the feasibility of a portable DNA synthesis system based on SFAT-integrated microfluidic components. A portable DNA synthesis system will provide a significant benefit to geneticists by allowing them to sequence DNAs at their sites at an affordable cost within a short period of time.

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REFERENCES


**Jae Wan Kwon** (S’96–M’04) received the B.S. degree in electronics engineering from Kyungpook National University, Taegu, Korea, in 1994, and the M.S. and Ph.D. degrees in electrical engineering from the University of Southern California, Los Angeles, in 1997 and 2004, respectively.

Currently, he is an Assistant Professor in the Department of Electrical and Computer Engineering, University of Missouri-Columbia. His research interests include microelectromechanical systems (MEMS), acoustic and piezoelectric transducers, microfluidic systems, microfabrication processing technology, piezoelectric transducers, microfluidic systems, BioMEMS, and nano-bio-technology.

**Sanat Kamal-Bahl** (S’00) received the B.Eng. degree in electronics engineering from V.J.T.I. University, Mumbai, India, in 1996 and the M.S. degree in electrical engineering from the University of Maryland, Baltimore, in 2002. He is currently pursuing the Ph.D. degree in the Department of Electrical Engineering, University of Southern California, Los Angeles.

Currently, he is a Graduate Research Assistant in the MEMs Groups at the University of Southern California. He was a Graduate Research Assistant with the Department of Electrical Engineering, University of Maryland, Baltimore, in the Digital Design and Test Group from 2000 to 2003. He did a summer internship with Motorola, San Diego, CA in 2001. His research interests include MEMS, RF/analog mixed signal, and digital circuit design.

**Eun Sok Kim** (M’91–SM’01) received the B.S. (Hons.), M.S., and Ph.D. degree in electrical engineering from the University of California, Berkeley, in 1982, 1987, and 1990, respectively.

Currently, he is an Associate Professor in the Department of Electrical Engineering, University of Southern California, Los Angeles. His research interests include microelectromechanical systems (MEMS), acoustic and piezoelectric transducers, microfluidic systems, microfabrication processing technology, and materials study. From 1991 to 1999, he was a Faculty Member with the Department of Electrical Engineering, University of Hawaii, Manoa. Previously, he was a co-op student, Design Engineer, and Summer–Student Engineer with IBM Research Laboratory, San Jose, CA; NCR Corp., San Diego, CA; and Xicor, Inc., Milpitas, CA, respectively.

Dr. Kim is on the editorial board for the *Journal of Micromechanics and Microengineering*. He was awarded a Research Initiation Award (FY 91–93) and a Faculty Early Career Development (CAREER) Award (FY 95–99) by the National Science Foundation. He received the Outstanding EE Faculty of the Year Award (voted by UH IEEE student chapter) in 1996.