DNA SEQUENCING BY LIGATION ON SURFACE-BOUND BEADS IN A MICROCHANNEL ENVIRONMENT

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DNA sequencing advancements in cost and throughput are under intensive development to enable widespread discovery of genomics information. Some groups have focused on conventional dideoxy sequencing, making improvements that include performing separations in microchannels [1], while others have shifted to using cyclic array sequencing technologies [2]. Our group has previously published a cyclic array technique, sequencing by ligation, using DNA templates tethered to immobilized, 1 μm diameter, polystyrene beads [3]. In this work we report on the extension of this technique to a microchannel chip for the open-source Polonator instrument, with resulting 12× reduction in reagent usage-a dominant cost.

The microchannel chip consists of an array of 32 addressable channels etched in silicon that is then bonded to borofloat glass. The channels are addressable through individual ports to permit multiplexing of individual bead arrays. Subsequently sealing these ports with polyimide tape allows a single system inlet and outlet to deliver common reagents as required for cyclic array sequencing. Beads are bound in monolayers on the channel's glass surface by selectively passivating and silanizing the silicon and glass, respectively. Specifically, passivation is achieved through C_4F_8 fluoropolymer deposition prior to anodic bonding, and silanization is performed with aminopropyltriethoxysilane that enables bead binding through NHS-ester crosslinking. When mounted on a custom vacuum-chuck with integrated peltier thermal control, the Polonator's scanning confocal epi- fluorescence microscope and reagent handling system allows DNA sequencing from an area containing almost 4×10^9 DNA-loaded beads. This flexible, addressable array of surface-modified microchannels offers substantial reagent reduction for bead-based cyclic array sequencing, bringing us one step closer to realizing the vision for a $1,000 sequenced human genome.

The channel array layout and microfabrication process are shown respectively in Figures 1 and 2. Each channel is 2 mm wide with 160 mm^2 active, silanized area, that is typically arrayed with 60×10^6 beads. Figure 3 shows a portion of a channel surface as fabricated using this process and design. The silicon channel is 8.5 μm deep with a 200 nm C_4F_8 passivation treatment that inhibits subsequent silanization. This passivation layer has been measured to be 5% more autofluorescent than bare silicon at 550 nm (for Cy3 fluorescent dye), which is tolerable. Furthermore, our experiments show that this surface modification mitigates bead binding by more than 18× relative to bare SiO_2 and 2× relative to bare silicon.

The complete microchannel chip is shown in Figure 4 with its associated thermally-controlled vacuum chuck. The chip can be accurately and repeatable located to within 25 μm laterally with kinematic registration features on the chuck's thermally-conductive copper surface. A pair of nanoport fittings (Upchurch Scientific) on the underside (*not shown*) enable interfacing to the Polonator's reagent handling system.

DNA sequencing results on surface-bound beads in the microchannel environment are shown in Figure 5. This false colored image is a composite of separate fluorescent images corresponding to each nucleotide base. This prototype channel's dimensions vary slightly from the channels in Figures 1-4 to show bead confinement in the channel.

Word Count: 492

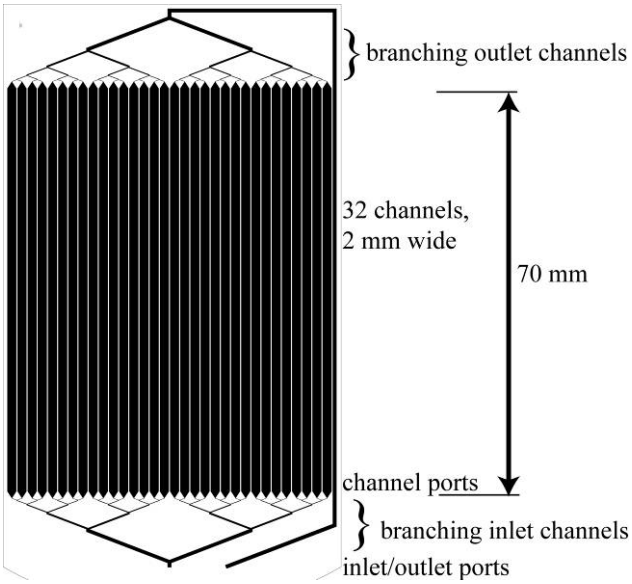

Figure 1: Mask design for microchannel chip, as patterned onto 150 mm diameter silicon wafers.

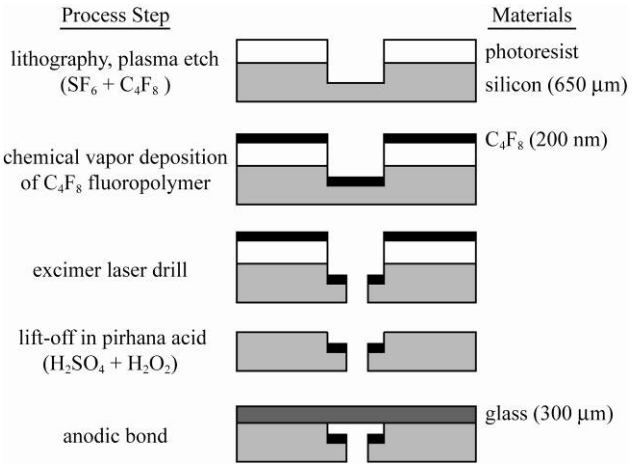

Figure 2: Fabrication process.

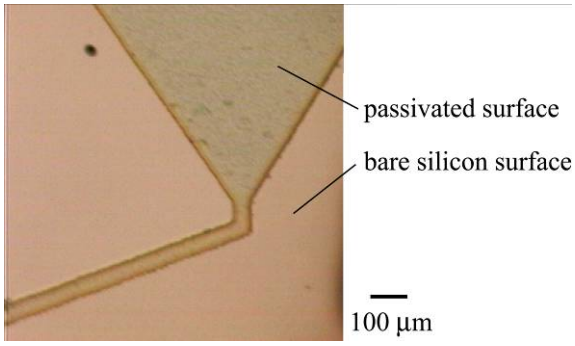

Figure 3. Photograph of channel area with surface passivation. The 200 nm thick passivation layer enables selective silanization for bead binding in a monolayer.

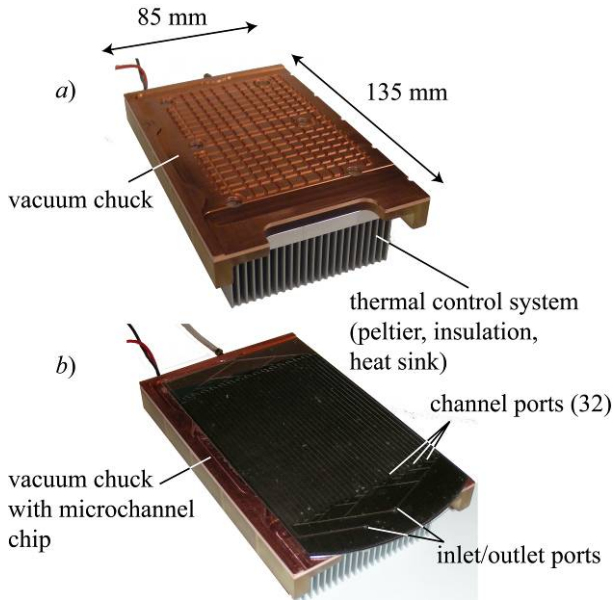

Figure 4. a) Vacuum chuck b) with microchannel chip. The assembly constrains the chip accurately and repeatably for scanning fluorescence microscopy and controls its temperature between 4-60 °C uniformly.

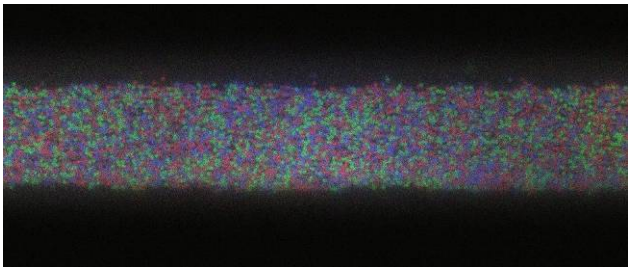

Figure 5. DNA sequencing by ligation demonstrated in 50 µm wide channel. The surface bound beads have unique tethered DNA templates that can be queried using biochemical protocols previously published [3]. Reagent volumes for this experiment were sub-microliter.

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