

Si fabrication technologies for biomedical applications:
 Double stranded DNA separation
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This paper will discuss the challenges faced in adopting the standard CMOS processes for developing a Lab-on-a-Chip system (LoC). The LoC system will be used, amongst other applications, for DNA separation. The relevance of shrinking the size of the different component using Si technologies and its impact on the processing will be reported in this work. The functionality of the device is tested by separating double stranded DNA fragments of variable length (10-400 base pairs) in a micropillar filter.

We recently presented a (LoC) system targeting fast, sensitive and highly specific SNP detection [1]. A number of components, mixers, microreactors, micropillar filters and detectors are fabricated using CMOS processing, while micropumps and microvalves are fabricated in non Si based technologies. This paper will focus on the fabrication challenges for the full LoC and will present the characterization of only one of its components, a micropillar filter. The filter acts as the column in a high performance liquid chromatography (HPLC) system. Micro-fabricated Si columns offer significant advantages over conventional ones, made of close, randomly packed beads. The pillar dimension and spacing can be made very small in the sub microns range, thus increasing the surface to volume ratio, an important characteristics of HPLC columns. The fact that pillar forms an ordered array results in improved separation performance (resolution is higher and less dependant on fluid velocity). The miniaturization of the columns allow using a small sample quantity for testing and a reduced pressure for operating the system. Si fabrication also offers economy of scale for future commercial applications.

This paper will report on the main challenge faced during Si fabrication: etch high aspect ratio very fine structures along with low aspect ratio coarse structures. The difference in critical dimensions for fine and coarse features is higher than two order of magnitude in our current design. The coarse structures like microchannels, mixers and microreactor cavities are etched to a depth of 250 μ m or more as to accommodate higher volume in a smaller footprint. The fine structures are etched to a depth of 30-50 μ m, and require very accurate control of the vertical profile. To achieve this multiple silicon deep reactive ion etching steps were implemented. However, this means that the structures defined during one etch step must be protected while performing subsequent etches. This is particularly challenging because, if the fine etch is done first, protecting the pillars with resist or oxide is not a suitable solutions as we reported earlier[2]. Here we describe a novel fabrication process adopted from standard CMOS technology which overcomes this issue. The process starts with defining the filter structures in an oxide, followed by depositing a protection nitride layer. The thickness of nitride is a critical parameter, it has to be tightly controlled when the CD of the pillars becomes small (in sub-micron range). This is followed by patterning and etching of the coarse structures. The nitride is then used as

a mask during a thermal oxidation step, allowing thermal oxide growth only in the coarse etched structures. After removal of nitride, the fine structures are etched and one of the etched pillars with a depth of 25 μ m and a CD of 1 μ m shown in figure 1. The final surface condition of silicon pillars is a very critical parameters for the proper functioning of the device. Different cleaning process and surface condition for optimal separation will be reported. The last step in processing are anodic bonding of a Pyrex wafer to the device wafer followed by opening of the access holes from the silicon backside, which are used for the external fluidics connections. A photograph of the final chip is shown in figure 2, where various components are also indicated.

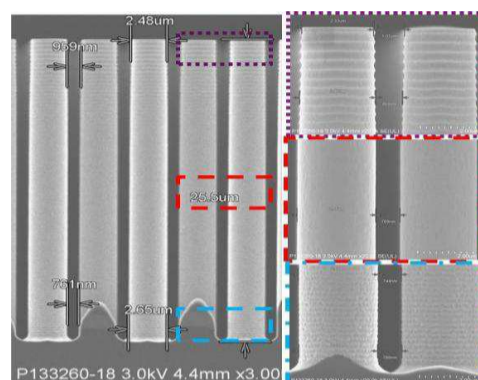


Figure 1: SEM image of Micropillar filters

The fabricated micropillars have been used to perform Ion pair reverse phase (IP-RP) chromatography experiments. IP-RP is a variant of HPLC which has excellent resolution and capacity to separate single and double stranded nucleotides in large size range[3]. In experiments a mobile phase continuously flows in the micropillar column, where at a certain moment a small volume of DNA sample is injected. The injected sample is transported downstream through the micropillar filter by the flow of the mobile phase and the DNA fragments of different length are spatially separated due to their different affinities towards the micropillars. We will show that a short chromatography chip, only 2cm long, is sufficient to separate dsDNA fragments, ranging in length from 10 to 400bp. The separations were highly reproducible and clear correlations between retention and DNA size as well as between UV absorbance signal and sample concentration were found.

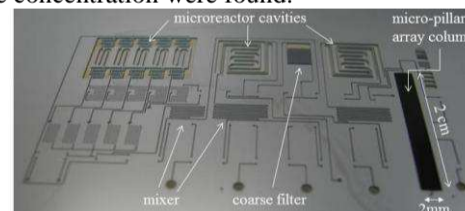


Figure 2: Optical image of the final device.

1. B. Majeed, et al, "Silicon based system for single-nucleotide-polymorphism detection: chip fabrication and thermal characterization of polymerase chain reaction microchamber", JJAP, Vol. 51 (4), pp.04DL01, (2012)
2. B. Majeed, et al, "Silicon microfilter device fabrication and characterization for diverse microfluidics applications", ECTC, Orlando, FL, USA, pp.1834-1838, May. 2011
3. L. Zhang et al, "Elution behavior of short dsDNA strands in silicon micropillar array columns in ion pair reversed-phase chromatography mode," Electrophoresis, vol. 33, no. 21, pp. 3205–3212, Nov. 2012