# Steady Flow Micro-Reactor Module for Pipelined DNA Computations

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**Abstract.** Microflow reactors provide a means of implementing DNA Computing as a whole, not just individual steps. Contrary to surface based DNA Chips [1], microflow reactors with active components in closed flow systems can be used to integrate complete DNA computations [2]. Microreactors allow complicated flow topologies to be realized which can implement a dataflow-like architecture for the processing of DNA. A technologically feasible scalable approach with many reaction chambers however requires constant hydrodynamic flows. In this work, the experimental construction of a basic constant flow module for DNA processing in such a context is addressed. Limited diffusional exchange in parallel flows is used to establish spatio-temporal segregation of reaction conditions which can be crossed by magnetic beads without barriers. As previously outlined [2], linked up with an optical programming technology, this will enable DNA selection to be programmed and complex population selection to be performed. The basic first experimental step in the realization of this program is described here: the establishment of a stable hydrodynamic flow pattern which is scalable to many reactors in parallel and the demonstration of a scalable and synchronous clocking of magnetic bead-based processing. First results with fluorescently-labeled DNA transfer will also be presented at the conference. The way in which this module may be integrated to solve the maximal clique problem has been proposed elsewhere [2].

## 1 Introduction

Integrating DNA computing to the point where complex computations can be performed routinely is a major conceptual and technological challenge. Whereas early experiments with DNA processing involved manual processing steps [3], the scalability of DNA computing to significant problem sizes requires new progress in biotechnological integration. Suitably matched architectures for DNA processing must be developed which take advantage of the integration potential of such technology. Two major trends in such integration may be discerned: DNA Chip technology (as in [1]) on the one hand and microflow systems (as employed in $\mu$TAS [4] involving fluid flow in sealed channel networks on the other. In previous work [5], we have described the application of microflow reactors to spatially

structured molecular evolution experiments with DNA and RNA. Completely passive flow systems find application in maintaining the long-term evolution of isothermal amplification systems in 0-, 1- and 2-dimensions [6]. Self-assembling rotors made from magnetic beads may be employed to actively mix solutions in the well-mixed (0-dimensional) case [7]. The scale of practical active valves and mixing modules in microsystems is usually in the range of several hundred micrometers, and there is a problem in integrating the separate control of these elements. There is a need for simple active DNA processing elements with common synchronous control.

Recently, a concept for such an application of steady flow microflow reactors to DNA Computing has been proposed [2]. This differs from the general alternative proposal for using microflow reactors to enhance the scalability of DNA strand routing [8]. It is based on practical experience with large microflow reactor networks, rather than their ideal behavior. A single type of active module, the strand transfer module, was proposed to implement a hybridization based strand separation step in DNA computing and a scheme for optically programming a network of such modules via photochemistry was introduced. Experimental progress with the development of an optical programming technology for such modules will be reported elsewhere.

The basic function of the module can be seen from Fig. 1. Selected DNA strands are to be transferred from one channel to another using two different buffer solutions (e.g. at different pH) and magnetic beads with attached oligonucleotides. Matching DNA templates bind to the beads on the left in continuous flow and are transferred to the right solution by a magnet which synchronously clocks all module chambers. The release of DNA into the second solution can be achieved by denaturants such as formamide or NaOH. Mixing of the two solutions is limited by the laminar flow. DNA is released in the denaturing solution on the right and leaves via the right hand channel, being neutralized before delivery to a downstream module.

The realization and coupling of such modules presents a number of practical difficulties. In general, connecting many modules with alternating buffer solutions requires channel crossings. The beads must be restrained physically, since a single magnetic control of the entire array is required for good scalability. (The alternative of using inhomogeneous magnetic fields to trap beads at specific locations as employed in [] is not clearly scalable.) Furthermore, the hydrodynamic flows must be setup to preserve the fluid-fluid boundary in each module. The remainder of this paper is structured as follows. In Sect. 2, we present two microreactor designs and their construction for the strand transfer module. The second design proved advantageous for the hydrodynamic stability as investigated in Sect. 3, by fluorescent labeling, and should prove useful in improving specificity of strand transfer. The behavior of magnetic beads in the microflow reactors under laminar flow is described in Sect. 4. The paper concludes with a brief discussion of the results and implications for the strand transfer module. Further results with fluorescently labeled DNA binding and strand transfer should be available at the conference.

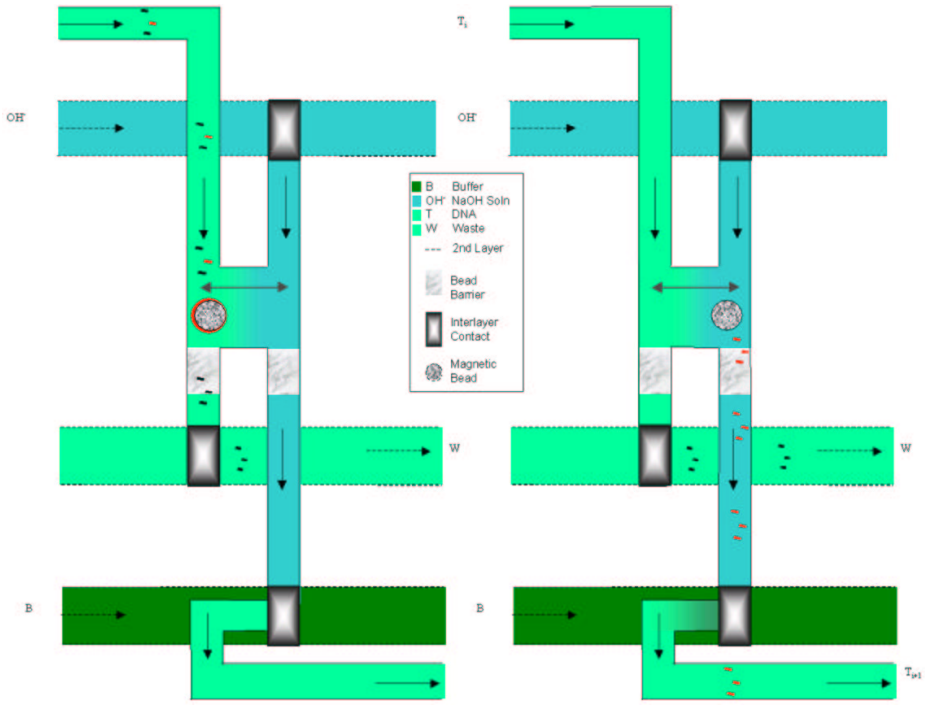


**Fig. 1.** Alternating states of strand transfer module as proposed in [2]. Selected (red) and non-selected (black) DNA flow in the left solution past beads (only one shown) in a hybridizing buffer. Non binding DNA leaves through the left channel. Upon bead transfer (right hand diagram), bound DNA dissociates from the bead in the non-hybridizing buffer and leaves through the right hand channel before being neutralized (below) for further processing.

## 2 Reactor Design and Construction

The design implementation of the basic module involved the electronic specification of four photomasks (designed via Mentor Graphics Boardstation Software and produced by GeSIM, Großerkmannsdorf, Germany) which were then transferred to 100 Si wafers (400 $\mu$m thick) via a twin depth etching and double sided structuring of the wafers using TMH and KOH [6]. Channel widths varied between 50 and 200 $\mu$m and depths of 50- 100 $\mu$m were employed. At certain locations, the structures were etched right through to make connections for channel crossovers on the reverse side. Planar barriers of only 10 $\mu$m depth were employed to restrain the passage of 15-30 $\mu$m diameter beads and no problem with blocking in filtered buffer solutions were observed. After microstructuring, the Si wafer was anodically bonded to two 500 $\mu$ thick pyrex (borosilicate) wafers with thermal expansion coefficients matched to Si. Ultrasonically drilled holes in these pyrex wafers allowed the connection of capillary tubing using UV-hardening glues

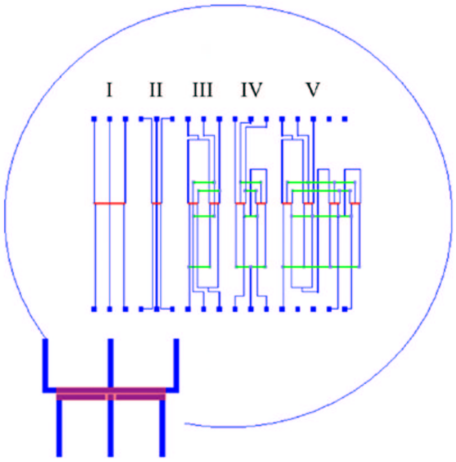


**Fig. 2.** Mask overly design for five microflow reactors on a single Si wafer. At the bottom left is an inset showing the detail of the bead barrier. Flow is from top to bottom, and the bead barrier is shown in purple. Microfluidic inputs are in the top row and outputs in the bottom row (3.5mm spacing). Some horizontal channels are on the reverse side of the wafer to avoid contact at channel crossovers. Reactors I and II differ in the width of the bead transfer module. Reactors III contains two parallel strand transfer modules and reactor IV contains two serial transfer modules. Reactor V has a combination of two parallel and two serial modules.

as described previously, to connect the channels of the microreactor with external fluids.

An initial design of the microreactor, as shown schematically in Fig. 1, suffered from insufficient hydrodynamic stability (see Sect. 3) and so a further design iteration was performed in which a central channel (washing channel) for a neutral buffer solution was introduced. Because scalability of the reactor module was a key issue, we included some coupled modules already in this initial design phase. The mask overlay design of five single and coupled modules is shown in Fig. 2.

A photograph of a section of the completed reactor is shown in Fig. 3. The channel connections on the reverse side of the reactor are not visible. The bead barriers are too fine (25 $\mu$m) and shallow (10 $\mu$m) to be visible. The connection technology is clearly visible with 400 $\mu$m diameter polyethylene capillary tubing. The structures are significantly larger than is necessary for operation, reflecting the early stage of the development.

## 3 Hydrodynamic Test of Reactor

Fluorescent dyes provide a convenient method of distinguishing between two different solutions in microreactors. In order to test the hydrodynamics stability

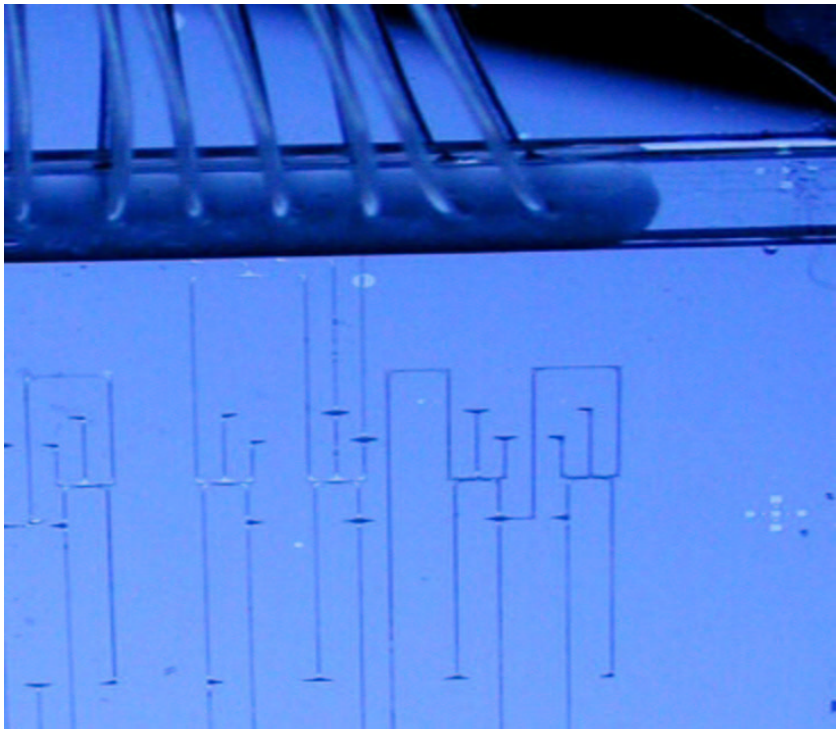

**Fig. 3.** Image of part of 5 microflow reactor wafer, as specified by the masks of Fig. 2. The connections to capillary tubing is shown above. The spacing between tubes is 3.5mm. A plexiglas stabilizer is glued with a UV-hardening glue to the outer glass wafer after polyethylene capillaries are inserted through drilled holes in the plexiglas (800 $\mu$m) above the holes in the pyrex (300 $\mu$ ultrasound drilled). On the right are markers for mask alignment. The top of the rightmost microreactor V and part of IV are shown. The darker bars above and below the bead barriers (central) involve etching through to channels on the reverse side of the Si wafer (not shown). The back side is also sealed by a pyrex wafer, this time without connections to external tubing.

of the flow in the reactor we have pumped a rhodamine 6G solution by a precision syringe pump at different flow rates via the right channel. In the left picture one can see the diffusion of the rhodamine 6G solution when the pumping speed is zero. In the right hand picture the pumping speed is 4 $\mu$l/min. As one can see on Fig. 4, there is a clear separation between the flow into the right channel and the flow into the left channel. A small amount of dye is divided in the central channel (introduced for washing and stability). This stable flow can be achieved by simultaneously pumping both solutions at equal rates (by volume), and does not require an individual regulation. This is important for the integration to multiple modules. A test of the parallel reactor module (II in Fig. 2) showed similar results (data not shown).

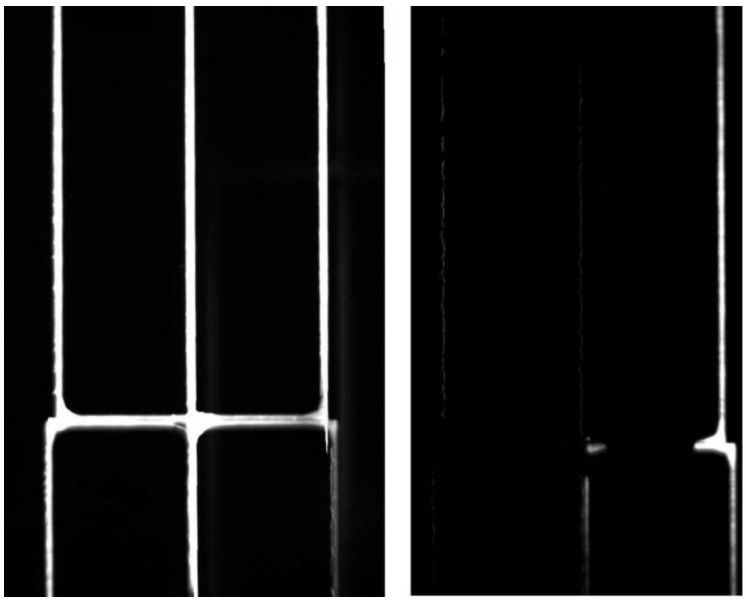

**Fig. 4.** Fluorescence image of flow in strand transfer module. Flow is from bottom to top and the dye enters on the right channel. Left image: zero flow rate, no separation of solutions. Right image: finite flow rate (see text), no dye in left channel.

## 4 Active Switching of Magnetic Beads

In addition to the hydrodynamic stability of the different buffer solutions, the strand transfer module has to allow the reliable restraint and switching of magnetic beads between the two solutions. We incorporated latex paramagnetic beads with a diameter of 30 $\mu$m (obtained from Micromod Ltd) into the microreactor. The beads were injected using a syringe pump (as for fluid pumping above), and restrained to the desired microreactor by the bead barrier (a ledge 10 $\mu$m in depth). In contrast with individual module manipulation of beads, the bead restraint is designed to allow the parallel processing of beads in multiple modules in the microreactor. Bead delivery was also successful in the presence of channel crossovers.

The switching behavior of the beads is shown in Fig. 5. A strong magnetic field gradient was employed (SrCo, Maurer Magnetic, Grningen, Switzerland) was employed. This was particularly important for smaller beads (data not shown). For this experiment, the original microreactor design, with 200 $\mu$m channels and no third input for stability was employed. Similar behavior is recorded in the revised microreactor design (see Sect. 2).

Magnetic beads as small as 8 $\mu$m can be used with bead barriers down to 5 $\mu$m. Below this size, clogging of the barriers becomes a significant problem.

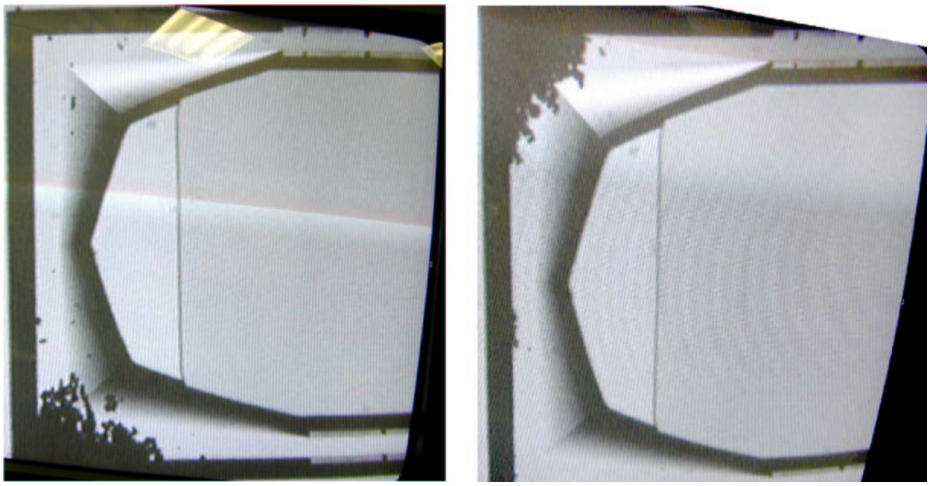

**Fig. 5.** Switching of magnetic beads between two buffer solutions in microflow module. Flow is from the right and the bead barrier is on the left of each picture. The left hand picture shows the situation with the magnet below, the right hand image the same for the magnet above. Single beads may be discerned. Under-etching of the silicon channel structures with the anisotropic etch method can be seen. This assists the mobility of the beads.

## 5 Conclusion

The above results demonstrate the first step in the experimental realization of strand transfer modules for DNA Computing. Attention has been paid to the true scalability of the concept, and the design is based on practical experience with microreactor design. It should be possible to integrate thousands of such modules on a single Si wafer. A concept for DNA Computing based on such a module has been proposed previously. While the authors are aware of concerns over errors in bead based separation of DNA, the microflow reactor framework provides many opportunities for kinetic control which may prove beneficial here. Initial results in this direction have been presented recently [9]. The next stage of experimental results involving DNA bound to beads and selective transfer should be evaluated at the conference.

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## References

[1] Q Liu, L Wang, A G Frutos, A E Condon, R M Corn, and L M Smith. DNA computing on surfaces. *Nature*, 403:175–9, 2000.

[2] J S McCaskill. Optically programmable DNA computing in microflow reactors. Preprint available on request, based on talk delivered at Leiden CNC in 1998.
[3] L M Adleman. Molecular computation of solutions to combinatorial problems. *Science*, 266:1021–1024, 1994.
[4] B H Weigl and P Yager. Microfluidic diffusion-based separation and detection. *Science*, 283:346–7, 1999.
[5] J S McCaskill. Spatially resolved *in vitro* molecular ecology. *Biophys. Chem.*, 66:145–158, 1997.
[6] K Schmidt, P Foerster, A Bochmann, and J S McCaskill. A microflow reactor for two dimensional investigations of in vitro amplification systems. *In 1st Int. Conf. Microreaction Tech. (Dechema e.V. Frankfurt)*, 1997.
[7] K Schmidt and J S McCaskill. Schaltbarer dynamischer Mikromischer mit minimalen Totvolumen. *PCT/EP98/03942*, 1998.
[8] A Gehani and J Reif. Micro flow bio-molecular computation. *Biosystems*, 52:197–216, 1999.
[9] Z H Fan, S Mangru, R Granzow, P Heaney, W Ho, Q Dong, and R Kumar. Dynamic DNA hybridization on a chip using paramagnetic beads. *Anal. Chem.*, 71:4851–4859, 1999.