Design and construction of a micromilled fluidic device as part of a DNA biosensor

R J Townsend[1]*, **N R Harris**[2], **D Wenn**[2], **D Brennan**[2], and **N J Grabham**[2]
[1]School of Engineering Sciences, University of Southampton, Southampton, UK
[2]School of Electronics and Computer Science, University of Southampton, Southampton, UK

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Abstract: Under the Optonanogen project (EU contract IST-2001-37239), a novel biosensor has been developed, which incorporates a disposable acrylic polymethylmethacrylate (PMMA) fluidic header. This header is designed to deliver a sample to a series of chemically primed cantilevers where hybridization of target DNA sequences and resulting deflection of the cantilevers is detected optically. Two different microfluidic headers are described, which are designed to incorporate the cantilever chip and which demonstrate a novel approach to microfluidic header assembly, integration with macroscale fluidics, fluidic handling, and priming strategies. The first header facilitates the delivery of a single fluid sample to all cantilevers, whereas the second permits discrete delivery of samples to isolated cantilevers, despite all cantilevers being contained on a single chip. This second, multi-path header therefore allows simultaneous analysis of multiple samples, or multiple parallel tests on a single sample. This paper describes these headers and for the multi-path device details the design changes incorporated to ensure effective isolation of the sample including a novel valve to improve priming of the microfluidic circuit.

Keywords: bio-sensor, cantilevers, priming, passive valve, microfluidics

1 INTRODUCTION

The topic of sensitive biosensors and biochips is a growth area, and one of the most promising approaches are cantilever based sensors [**1**]. Within the Optonanogen project [**2**], a novel biosensor has been developed, based on an array of microcantilevers etched into a silicon chip [**3**]. To form the sensor, nucleic acid target probes are immobilized on one side of each of the 20 cantilevers by ink-jet printing. Upon injection of a biological sample over the cantilevers, hybridization of DNA contained within the sample (and complementary to the immobilized nucleic acids) generates surface stresses on the cantilever and causes bending. The cantilever deflection is then detected optically using, as a source, an array of vertical cavity surface-emitting laser (VCSEL) with integrated microlenses, and as a detector, a custom array photodetector chip. The sensor was initially designed to detect human gene mutations including single nucleotide polymorphisms, particularly those associated with increased risk of breast cancer. The current paper concentrates on the fluidic design of the header, its ease of use and the ability to deliver the test sample to the cantilevers and has led to a patent application (GB 0624482.6 – filed 7/12/06).

In the context of this project, the microfluidic headers are intended to be disposable items to prevent contamination between test samples. It is, therefore, necessary to have them as separate components which are easily assembled or plugged into the sensor instrument, reliably sealing with a manifold within the instrument. For convenience and ease of use, the priming of the headers should be straightforward, and indeed is an important aspect in terms of the function of the cantilevers. Where multiple samples are to be processed simultaneously, the header must incorporate multiple channels and a method to maintain isolation between the separate samples

Corresponding author: School of Engineering Sciences, University of Southampton, Highfield, Southampton SO17 1BJ, UK. email: r.j.townsend@soton.ac.uk

is required. Other requirements include a good optical path through the header to the cantilevers and the rapid fabrication of the headers during the development phase.

2 FLUIDIC DESIGN

In this section two header designs are described and their purpose, in terms of sample delivery, is described.

2.1 Common path header

The initial stage of the header design involved the fabrication of a header to deliver a single sample to the sensor chip (and cantilevers). This version allows demonstration of the principle of detection and requires individual cantilevers to be immobilized individually prior to final assembly. The cantilevers themselves are formed over a common window through the silicon chip. The assembled header is pictured in Fig. 1 together with its manifold. This design uses a single inlet to feed a sample to the main fluid channel that expands to the width of the chip, directs flow across the top of all 20 cantilevers, and then is guided to an outlet. An additional port is included beneath the chip, which allows the region around the chip and the window below the cantilevers to be primed in order to remove all air bubbles. External valves are used to open or close the priming port and therefore alter the direction of flow over, or through the chip. The common path header allows a single solution to be passed to all of the cantilevers, therefore, by immobilizing a different target probe onto each cantilever, a range of parallel measurements can be performed simultaneously on the sample.

2.2 Multi-path header

It is desirable to provide a discrete, isolated flow path to each cantilever in the array. This would allow multiple samples to be used. Also, the immobilization process itself could be carried out *in situ* and monitored in real time. Therefore, an alternative, multi-path header has been designed, fabricated, and tested. In order to achieve the discrete flow paths, the cantilevers must themselves be isolated from each other on the silicon chip. For this purpose, modified arrays of cantilevers with individual windows were produced by Centro Nacional de Microelectrónica in Barcelona. The cantilever chip is shown in Fig. 2 where it can be seen that each cantilever is isolated from its neighbours by a section of

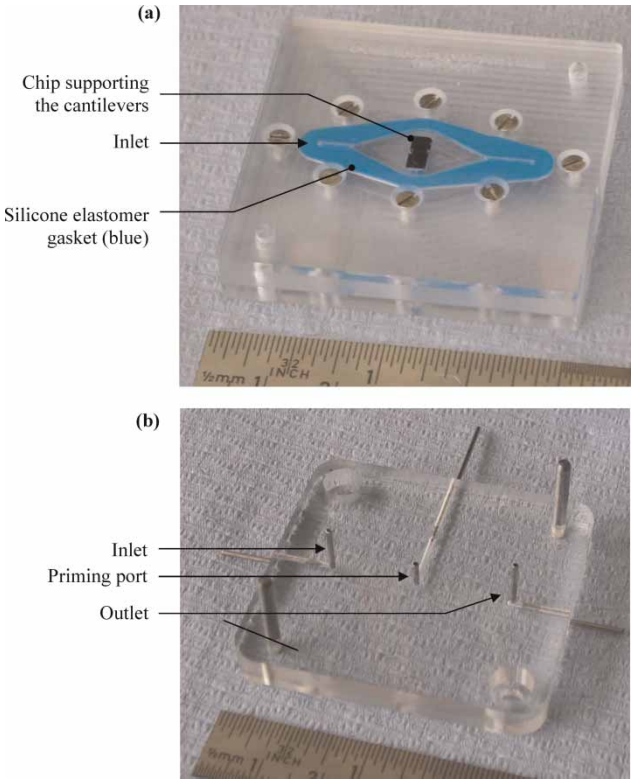

Fig. 1 Acrylic construction of (a) single channel fluid header and (b) inlet/outlet manifold

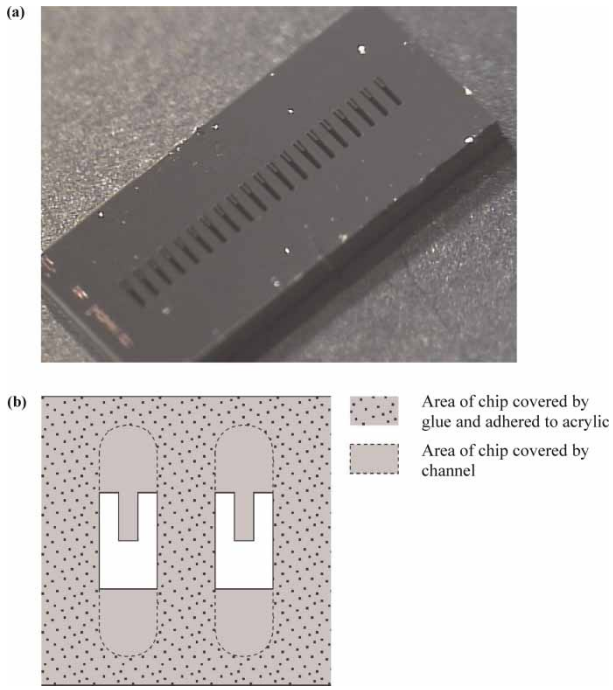

Fig. 2 Photograph of chip and cantilevers (a) and schematic of chip looking down over two cantilevers showing location of channel and adhesive (b)

silicon wall preserved during the etch process. The cantilevers have a pitch of 250 μm and typically measure 200 μm long, 40 μm wide, and approximately 0.3 μm thick. The assembled multi-path header and manifold are pictured in Fig. 3 where 20 discrete inlet channels can be discerned, each leading to one of the cantilevers. Downstream of the cantilever array, the fluidic channels are successively merged in order to obtain just a single outlet channel. The movement of the sample solution within the chip region is shown schematically in Fig. 4, which also illustrates the priming port located beneath the chip which permits fluid to pass down through the chip.

Despite their apparent fragility, the cantilevers are able to bend significantly without breaking even under the relatively high flowrates during priming using water and similar viscosity solutions. Damage to the cantilevers is possible if they are allowed to come into contact with another surface, but with the configuration of the fluidic header presented the tip of the cantilever is unable to touch any other surface when deflected downwards. Reversal of the flow could potentially cause the cantilever to bend upwards and contact the top of the polymethylmethacrylate (PMMA) flow channels, although reversal of the flow is deemed unnecessary for priming and operation of the headers.

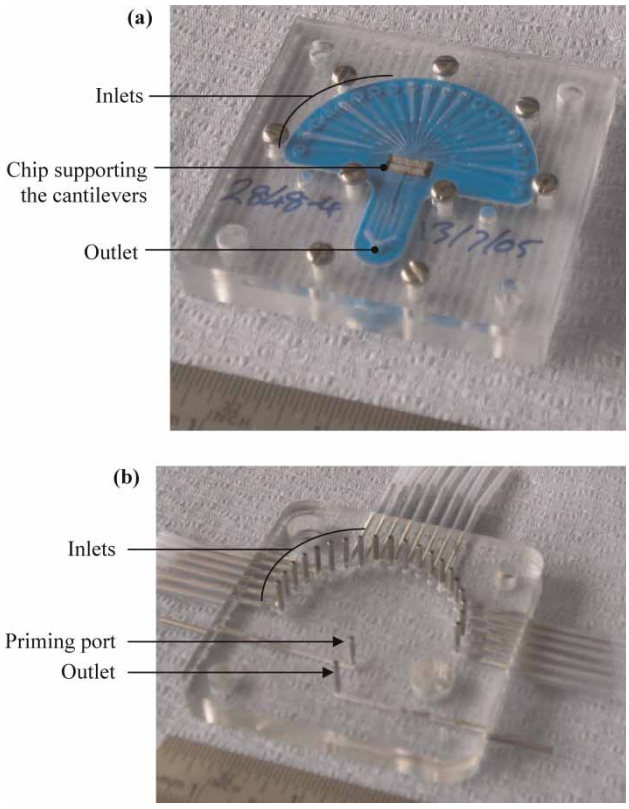

Fig. 3 Schematic of cross-section through microchannel and chip showing fluid path over cantilever and priming port

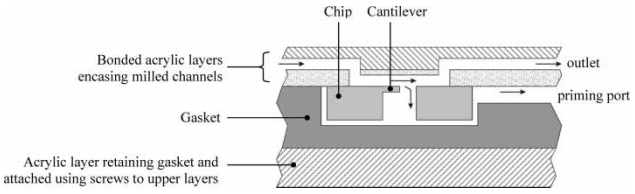

Fig. 4 Acrylic construction of (a) 20 channel fluid header and (b) inlet/outlet manifold

3 FABRICATION AND ASSEMBLY

3.1 General

The microfluidic headers are fabricated from acrylic (PMMA) which is mechanically milled using a Datron micromill, comfortably achieving channel dimensions down to 150 μm wide and 100 μm deep ±10 μm. Direct milling of components permits rapid turn-around of new test devices and design modifications are handled by simple adjustment of the CAM macro. This means that a new device can be redesigned, machined, and assembled in a matter of days.

Fluid channels are milled into the surface of acrylic layers and by bonding multiple acrylic layers together the channels can be capped. Channels can also be sealed and capped through the use of polydimethyl siloxane (PDMS) gaskets, fabricated with the aid of moulds which again are machined using the micro mill.

3.2 Assembly of common path header

The common path header requires a relatively straightforward design, particularly as only a single sample is intended to pass over the cantilevers. Three acrylic layers are machined using the micromill to encase various parts of the fluidic circuit. The top interface encases a gasket which forms the main fluid channel, guiding the fluid over the top of the cantilevers where the cantilevers point downstream, and also serves to seal against the inlet and outlet manifold tubes. Using UV curing adhesive, the chip is held in place within the same cavity as the main channel. The bottom interface contains a second gasket which again couples to the manifold and forms the priming port. The bottom acrylic layer also acts as a base onto which the other layers are screwed, and gaskets compressed to form reliable seals.

3.3 Assembly of multi-path header

For the multi-path header in the region of the cantilevers, the required dimensions are too small to allow gaskets to be used for sealing the individual channels and novel methods are required. In this device, the channels which interface to the cantilevers are machined in a 1 mm thick acrylic layer and are left exposed to allow the later mounting of the cantilever chip. These channels are 150 μm wide with a 100 μm gap, and do not extend beyond the footprint of the chip. Rather, at each end of the channels, a small via hole is machined through the acrylic in order to connect to the secondary feeding channels which are produced in a separate 1 mm acrylic layer. These secondary channels must be sealed and this is achieved by thermally bonding the two acrylic layers. This is achieved as follows.

After thoroughly cleaning the milled components in an ultrasonic bath, the bonding process first involves vapour coating or spinning (methyl methacrylate (MMA), unpolymerized acrylic) onto all acrylic components to be bonded. Simple nylon dowels are used to align these layers which are then placed within a clamp where an even pressure is applied to the stacked acrylic. Within the clamp, the external faces of the acrylic components are placed in contact with glass plates which ensure that the surfaces remain optically clear. The assembled clamp is then placed within an oven at 80 °C for approximately 60 h, after which time the MMA has polymerized and cross-linked to the layers above and below it, resulting in an optically transparent bond between the layers. Relatively complex multi-layered fluidic circuits can be fabricated in this way, in much the same way that fluidic devices can be fabricated in silicon through both etch-fabrication with the use of patterned sacrificial masking layers, or direct laser surface micromachining followed by assembly using wafer bonding techniques such as anodic bonding [**4**, **5**].

After the two acrylic layers have been thermally bonded, the cantilever chip is mounted over the exposed channels. This is achieved using a purpose-built alignment jig that consists of an enclosed x-y-θ stage, micromanipulator, CCD camera, and UV light source. The chip is held fractionally above the channels and imaged by the camera through the acrylic layers. This allows the alignment of the cantilevers to the channels to be achieved. Once this has been done, the chip is moved into contact with the acrylic and UV-curing adhesive is dispensed around the edges of the chip. The adhesive wicks under the chip and along the walls between the cantilevers and channels by capillary action. This is shown schematically in Fig. 2(b), which indicates the regions of the chip surface to which the adhesive wicks. The UV light source is then switched on to cure the adhesive. This ensures that each channel is isolated, as well as providing a good mechanical bond at the edge of the chip.

3.4 Gasket sealing

Elsewhere in the headers such as underneath the chip and the interface with the manifold, fluid sealing is aided by the use of silicone elastomer (PDMS) gaskets. These gaskets are formed in-house using custom moulds in brass or acrylic which are again fabricated using the micromill. In the case of the multi-path header for which a single gasket is formed, the bonded acrylic component encasing the fluid channels then screws down to an acrylic base which clamps over the gasket and forms the disposable part of the device. This means that the header can also be disassembled making it easy to change various components, such as the gasket, which is a useful feature during the development stage.

3.5 Manifold design

For both types of header, the header assembly plugs onto an acrylic manifold which is intended to be a permanent feature within the sensor instrument. This manifold consists of stainless steel tubes (gauge no.19) inserted into an acrylic block using an interference fit and then annealed to improve the seal around the tubes and prevent stress-cracking of the acrylic. Vertically placed tubes couple with holes in the disposable header and retained gasket, and horizontally placed tubes couple with tubing from the macroscale fluidic system. Figure 5 illustrates schematically how these parts plug together. When the header is pushed down onto the manifold, the tubes pass into holes formed into the gasket. Due to interference between the steel tubing and gasket, the tubes deform the gasket material which creates a tight seal around each tube. Alignment pins ensure that the tubes are accurately aligned with the header and gasket holes; any misalignment will prevent the formation of an effective seal around the tubing. To prevent damage to the gasket material, the tubes must be deburred or filed slightly and

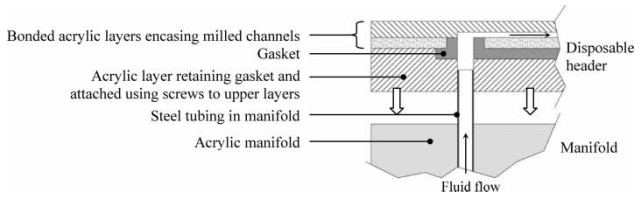

Fig. 5 Schematic of interface between disposable header and manifold

means that the header can be repeatedly removed and reconnected with the manifold with the gasket able to continuously form a reliable seal. No vertical clamping pressure is required on the assembly as the gasket seal is made around the sides of the tube making the connection to the manifold extremely simple and convenient. To give a clearer representation of the multi-path header assembly, Fig. 6 shows an exploded view of the multi-path header components and manifold.

3.6 Injection moulding

Injection moulding of the acrylic components has also been investigated as a low cost alternative for high volume production and also to improve the optical quality of the surfaces within the channel area. Preliminary work shows that the microchannels which pass the samples over the cantilevers can be formed in this manner. Figure 7 shows a microscope image of a small section of the channels which display smooth, straight side walls and where the acrylic has successfully flowed around the mould.

4 FLUIDIC OPERATION

4.1 Priming of header

Reliable priming of the cantilevers without a visual check is challenging, even when using, for example, an IPA solution (50:50 isopropyl alcohol to de-ionized water), due mainly to the cantilever geometry and stagnant region beneath the cantilevers. Systems that are difficult to prime do not necessary lend themselves to automation, and although priming with the aid of CO_2 may be a consideration, it was felt good practice to first improve the geometric design. Concentrating on the 20 channel multi-path header, features in the original header design which encouraged the trapping of bubbles included small steps in the milled channels leading to the chip. To address this, dead-volumes, steps, and cross-sectional changes within the channels were removed where possible, which significantly removed the likelihood of a bubble becoming trapped.

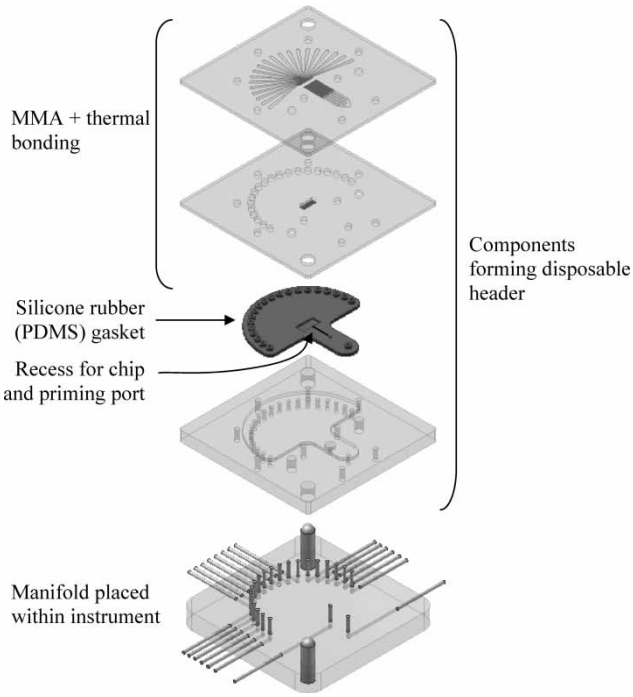

Fig. 6 Exploded view of multi-path header components

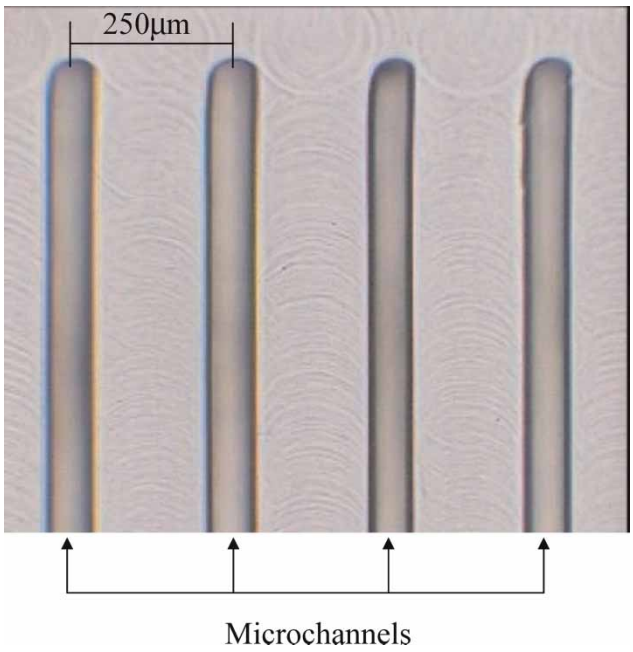

Fig. 7 Microscope image of injection moulded acrylic into which microfluidic channels are formed. Pitch of 250 μm corresponds to that of the cantilevers

4.2 Chip region and isolation of samples

Bubbles trapped around the chip will disturb the movement of the sample across the cantilevers and not only affect their exposure to the sample, but also disable and deflect cantilevers due to surface tension effects. It is, therefore, essential that the chip is reliably primed so that the intended deflection of the cantilevers as a result of hybridization (of the order of 10 nm) can be accurately detected. There is limited scope to modify the geometric design of the fluidic system in the vicinity of the cantilever chip and therefore the straightforward priming of this region. The chip and cantilever geometry is formed by dry etching processes, which inherently forms

angular geometry, and is important for the mechanical properties of the cantilevers. The port located beneath the chip in both header designs aims to address priming of the chip by permitting a high flowrate of solution to pass down through the chip. However, in the multi-path header, the void beneath the chip that the priming port forms has been observed to allow diffusion between neighbouring samples when operating at low flowrates around 1 μl/s and may thus cause cross-contamination of neighbouring cantilevers. An alternative PDMS gasket was fabricated in which the gasket material is pressed up against the under side of the chip, thus closing off the window below each cantilever and rendering the priming port redundant. Flowing fluorescein simultaneously through every other channel showed that isolation of the samples was maintained between adjacent channels and cantilevers, as shown in Fig. 8 where the fluorescein is injected into alternate channels but no fluorescein is seen to contaminate the neighbouring channels due to the effective seal beneath the chip. Using this gasket, priming of the chip region could be achieved, although it was necessary to monitor the chip during the priming process, increase or pulse the flowrate until all bubbles were removed and using valves at the inlets to address and prime individual channels where necessary.

4.3 Fluidic actuated valve

In order to automate priming of the chip while maintaining isolation of adjacent samples during measurement, a novel valve was designed and tested. This passive valve is formed within the multi-path header gasket and is designed to allow fluid to pass down through the chip to the common priming port under high flowrate conditions, thus removing any bubbles beneath the cantilevers, while sealing to the back of the cantilever chip under low flowrate, measurement conditions. The principle of its operation is shown schematically in Fig. 9 where it can be seen that during high flowrate conditions, increased fluid back-pressure, generated from the flow resistance of the downstream channels, forces a diaphragm within the PDMS gasket to deflect, thus opening the route to the priming port. This priming port has a low resistance to flow allowing a relatively high flowrate to pass. A significant volume still passes through the smaller channels downstream of the chip used primarily during operation, which is actually desirable in order to remove any remaining bubbles in the normal outlet channels. However, when the flowrate is reduced, the diaphragm experiences a lower pressure and acts like a spring such that it returns to a position in contact with the chip. In this case, the spring force pushing the diaphragm up is greater than the fluid pressure pushing it down. This allows the diaphragm to maintain isolation between adjacent channels under normal low flowrate operational conditions.

The valve has been designed with the aid of the finite-element (FE) package, ANSYS, to investigate the effect of geometric modifications and pressure acting on the diaphragm surface. Fluid pressures were estimated by calculating the pressure drop expected between the chip and outlet port. A gasket has been tested which demonstrates successful operation of the valve, with the results described by the graph in Fig. 10. It shows that during low flowrates, all flow passes though the operational outlet port

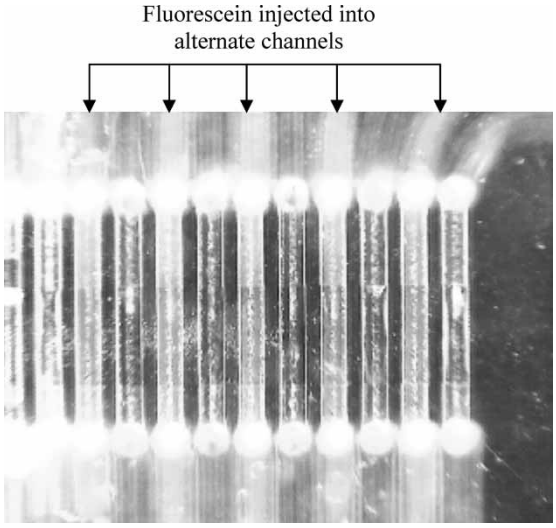

Fig. 8 Movement of fluorescein through alternate channels of multi-path header and over chip, showing no leakage of fluorescein to neighbouring channels

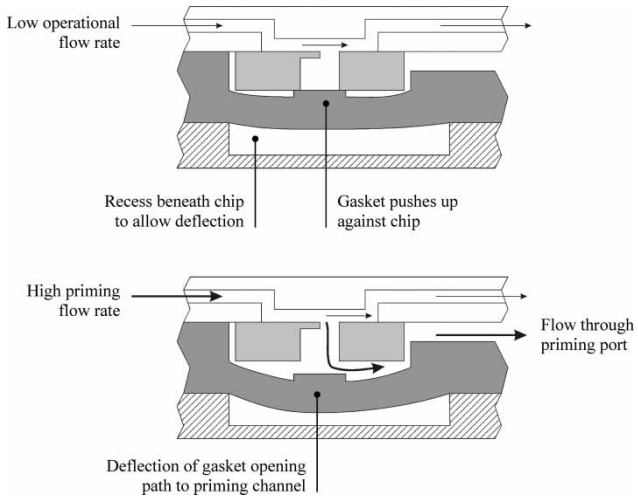

Fig. 9 Schematic showing operating principle of priming valve

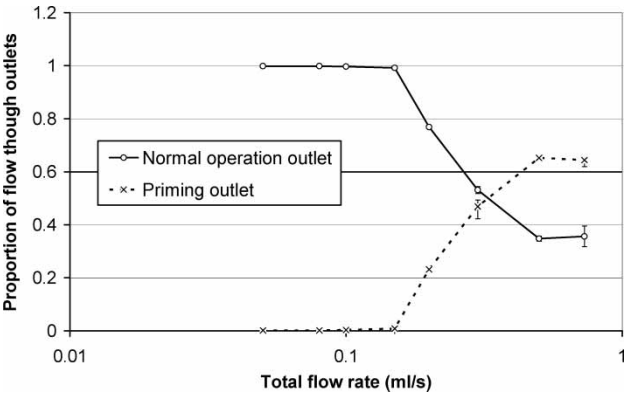

Fig. 10 Graph showing action of valve influencing the proportions of flow passing through outlet port and priming port over a range of flowrates

and thus over the cantilevers. At higher flowrates ∼0.5 ml/s, the valve opens and flow is seen through both the outlet and priming ports. Direct observation of the chip area under the microscope showed that bubbles were reliably and suddenly removed using a burst of high priming flowrate. This technique provides a means of reliably and quickly priming all 20 channels.

5 CONCLUSIONS

Two disposable micromilled fluidic header designs for incorporation into a DNA biosensor have been realized and refined to limit the dead volume, ease priming, and conveniently interface with a manifold within the sensor instrument. The technique of directly machining PMMA has been successfully combined with thermal bonding to create a quick, cheap and relatively simple method of producing quite complex microfluidic structures. A system to allow repeated assembly onto and removal of the consumable from the fluidic manifold has also been demonstrated. The multi-path header also has the ability to process up to 20 samples concurrently, thus reducing fabrication costs, and biological sample processing time and complexity. To aid priming of this complex system, a novel design of a passive valve has been designed using FE modelling, and its real-world performance successfully demonstrated. This allows simple and immediate priming of the cantilever chip and fluid channels while maintaining isolation between multiple channels and samples during operation. The combination of all these features has resulted in a practical sample delivery system for a multi-channel sensor and therefore demonstrates a methodology that has potential in other fluidic systems.

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