Development of an Integrated Polymer Microfluidic Stack

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Abstract. Microfluidic is a field of considerable interest. While significant research has been carried out to develop microfluidic components, very little has been done to integrate the components into a complete working system. We present a flexible modular system platform that addresses the requirements of a complete microfluidic system. A microfluidic stack system is demonstrated with the layers of the stack being modular for specific functions. The stack and accompanying infrastructure provides an attractive platform for users to transition their design concepts into a working microfluidic system quickly with very little effort. The concept is demonstrated by using the system to carry out a chemiluminescence experiment. Details regarding the fabrication, assembly and experimental methods are presented.

1. Introduction

MEMS technology related to microfluidics is the focus of immense interest because of the existing and foreseen applications in a variety of fields, the most prominent ones being life sciences and medicine. The ability to run and observe biological reactions on the micro and nano scale results not only in significant time and financial benefits, but also opens doors for fundamental scientific discoveries fueled by the ability to interact with nature on a comparable size scale.

There are standard technologies used to fabricate microfluidic structures and microfluidics chips in glass and polymer. While these chips become commercially available today, very little has been done to combine stand-alone microfluidics chips with other functional units to allow performing more complex analytical tasks. Even fewer examples can be found of devices that integrate the complete spectrum of sample manipulation, detection and analysis technologies. The ability to interact with and interrogate biology using electronic, magnetic, optic and chemical methods is necessary for a full fledged microfluidic system with broad capabilities.

We present a novel system platform that encompasses the complete requirements of a microfluidic system. This system allows using a combination of microfluidic chips required for complex analytical tasks in a short time. Normally neglected auxiliary services are an integral part of the overall system and provide a user-friendly interface. Thus, the described system provides an attractive platform for users to transition their ideas into a working microfluidic system quickly with very little effort. The system is based on a modular design concept with standard interfaces between each functional module. Each module is a discrete fluidic chip for dedicated tasks such as sample preparation, mixing, analysis, etc. Hence any new microfluidic design can be incorporated into the system for a specific function as long as some fundamental design rules are adhered to. In the scope of this paper, the
concept was demonstrated with a chemiluminiscence experiment on bio-functionalized silicon chips that were integrated into the microfluidic system.

2. Concept of a Microfluidic Stacked System
Commercially available microfluidic analysis systems produced by companies such as iSTAT®, Micronics®, ThinXXS®, Agilent®, and Fluidigm® have set an example of the preferred architecture for a cost effective system. The expensive functional components of the system including the fluid pumping controls and electronic analysis form a permanent non-disposable part of the system, while a disposable chip is the actual microfabricated component which is considered a consumable item. This architecture is an optimal solution for keeping a low cost while simultaneously maintaining flexibility. Disposable microfluidic chips are also advantageous as they avoid the possibility of cross contamination since a new chip is used for every analysis.

A similar design philosophy was adopted for our microfluidic system. The presented system is divided into 3 components – Modular Microfluidic Chip (MMC), Interconnect and Clamping Block (ICB) and Supplies and Instrument Platform (SIP). The MMC is a disposable microfluidic chip and plugs into the permanent platform consisting of the ICB and SIP. The SIP module enables all the fluid pumping and control functions and the ICB module serves the purpose of interconnecting and clamping the disposable microfluidics chip. The microfluidics chip (MMC) itself is modular in design with multiple functional layers stacked to form the complete chip (Fig 1a). The overall geometric format of this chip is identical to that of a microscope slide, thus maintaining compatibility with existing lab equipment. This stacked configuration was chosen as it provides the most compact solution possible and it also has minimal fluidic dead volumes. This approach promises to be the most modular solution as different standard functional layers can be integrated into the stack. New designs can also be easily integrated, provided some basic design rules are adhered to.

The advantages of a micro scale device are rapidly overshadowed if the interaction between the micro and macro world is cumbersome. Sample, reagent and buffers have to transition from the macro world into the micro analysis chip. To make this task simple, the stacked microfluidics chip has one layer dedicated to connecting to the outside world (Fig 1b). This layer has a standard format irrespective of the other layers and it has interface connections for liquids and electrical/electronics. It also forms the mechanical basis for alignment of the subsequent layers.

3. Methods
3.1. Fabrication of Microfluidic Chips via Hot Embossing
Cost is a prime factor that dictates the level of success of a product or technology is capable of achieving. In the world of microfluidics, polymer replication is the most promising technology for
mass production of microfluidics chips. Molding technologies used for fabricating macro scale polymer components such as injection molding and hot embossing have been adapted with considerable success for fabrication of polymer microfluidics chips. We use hot embossing to fabricate a variety of microfluidics chips (Fig 3) since the mold design and fabrication process for an embossing process is easier compared to injection molding, thus making hot embossing an ideal manufacturing process for rapid prototyping and small scale production. Hot embossing has been used to fabricate both single sided and double-sided parts (Fig 3). The Jenoptik HEX02 embossing machine used for embossing is capable of precise alignment for double sided embossing. Parts fabricated by hot embossing exhibit good overall dimensional control and low internal stresses. Molded parts were measured to evaluate the dimensional variation due to the hot embossing process. On average, a dimensional variation of 353 microns was observed over a distance of 70.4 mm on the interconnect layer chip.

Hot embossing typically results in a thin residual layer of polymer at the bottom of the part. This layer had to be removed in order to open the ports for fluidic interconnections. Flycutting was used to remove this residual layer. Interconnection and sealing between the layers of the chip was achieved by a combination of methods that included spin coated layers of PDMS, adhesive backed clear tape and transfer adhesive.

Fig 3: Microfluidic chips produced by hot embossing

3.2. Stack Alignment Methodology
As multiple layers are stacked to form the complete microfluidics chip, precise alignment of the layers is necessary to ensure that all the fluidic interconnects align as they are supposed to. Keeping with the spirit of versatility of the design philosophy, different layers of the chip with various designs should align with sufficient accuracy to form a working device. Post molding, some dimensional variation of the geometry is always observed. These variations have a strong dependence on design geometry, hence the different layers of the modular chip stack exhibit different dimensional changes with respect to the original design. In order to account for this, an unconventional alignment method called elastic averaging was chosen.

Elastic averaging is an alignment technique in which a solid body is aligned to another by over-constraining them using a large number of fairly compliant members. The elastic properties of the material and the constraint structure cause deformations in each individual contact feature to average out over the sum of contact features throughout the solid body. The repeatability and accuracy of an elastically averaged system can be as good as that of a kinematically constrained system. This method also has the advantage that the resultant assembly has higher stiffness and lower localized contact stresses. The repeatability of alignment is inversely proportional to square root of the number of contact points. The accuracy of an elastically averaged interface is on the order square root of n better than the accuracy of the contacting elements where n is simultaneously engaged contactors.
As discussed previously, molded polymer parts have dimensional variation from their original design. The total variation is the combination of errors accumulated from the mold insert fabrication by micromilling process and the embossing process. The alignment members are five V-grooves on the walls of the molded chips. 1mm diameter steel dowel pins attached to a plastic fixture act as the secondary alignment feature. The dowel pins align into the crevices of the V-grooves (Fig 4a). As the dowel pins are fixed into holes in a plastic fixture, there exists some natural compliance in them. Pressure was applied to the opposite edge of the chip stack using an elastic band. The efficiency of passive alignment using this method was verified to be better than 50 microns by repeating the assembly process three times. Fig 5 illustrates the accuracy on one of the alignment attempts. As it was not possible to simultaneously focus on both layers of the chip stack, this image was generated by taking two separate pictures while keeping the setup stationary and only changing the focal plane of the microscope. The images were later overlaid using image processing software.

![Fig 4a: Schematic of passive alignment method](image1)

![Fig 4b: Two stacked chips aligned using elastic averaging with V-Grooves & dowel pins](image2)

![Fig 5: Image of overlaid alignment marks on 2 different layers of modular chip stack. It is observed that the alignment is better than 10 microns in the direction of the force, while in the lesser constrained direction, the alignment is not as accurate (~20 microns)](image3)

3.3. Fluid Supply and Control
Keeping with the design philosophy, the Supplies & Instrument platform (SIP) consists of an independent fluid delivery and control module. Maintaining an independent fluid delivery and control system that can easily plug and play with the rest of the system via a standard interface provides the most flexibility for design changes. Modifications can be made to the module with minimal concerns regarding integration. The fluidic supply and control module interfaces with the rest of the system through standard commercial connectors. A simple vacuum driven system with a push-button user interface has been designed to allow an easy control of fluid flowing through the microfluidic chip. Single use syringes are used as reservoirs and connected with standard Luer-lock connectors to silicone tubing. This unit allows us to test the design of various microfluidic chip configurations at a very low cost. When more precise control or better automation is desired, this module can be swapped out with a more complex, expensive unit.

3.4. Biological Protocol

The demonstrated biological experiment is based on an ongoing project that involves the integration of giant magneto-resistive (GMR) sensors fabricated in silicon, with a microfluidic circuit. The goal of the project is to attach appropriate biology to the silicon surface in order to bind molecules with paramagnetic beads. The GMR sensor generates a signal when bead are bound directly to its surface. In order to optimize the binding chemistry, numerous screening experiments are being run on dummy silicon dies with a surface identical to that of the GMR sensor.

One such screening experiment was carried out using the microfluidic stack and dummy silicon dies. The surface of the silicon dies were covalently coated with protein conjugate using a well defined sequence of aminopropyltriethoxysilane, gluteraldehyde, and strepavidin – horse radish peroxidase (S-HRP) treatment. The dies were then placed within the chambers of the microfluidic stack (Fig 6). A chemiluminescent material (Pico Supersignal, Pierce Biotechnology, Inc.) was flowed through the microfluidic channels to interact with the silicon die surfaces. Light was generated by the interaction between S-HRP and Supersignal. X-ray film was placed directly on top of the stack and exposed for less than 10 seconds. The film was developed and scanned into digital format (Fig 7). Then the mean was calculated for relevant regions with SCION software (public domain from NIH) and results are shown in Fig 7.

Fig 6: Silicon chips assembled in microfluidic slide

Fig 7. Chemiluminescent signal captured on X-ray film. Chips with 1:10 serial dilutions of protein conjugates, Strepavidin – Horse Radish Peroxidase(S-HRP) were placed in different chambers with the far right being the highest concentration of S-HRP.
4. Conclusion

A stacked modular polymer microfluidic system was designed, fabricated and assembled. Layers of the modular chip for interconnection and fluidic testing were fabricated by hot embossing. Biologically activated silicon dies were placed in the fluidic testing layer and chemiluminescence experiments were carried out. The passive alignment accuracy of two assembled layers of the modular chip was shown to be better than 50 microns. Work is in progress to improve alignment accuracies and test multi layered stacks of the modular system. Other biological applications related to genomics, proteomics and whole cell analysis are being explored for integration into the system.

References

[1] Erickson, D. and D. Li, "Integrated microfluidic devices". Analytica Chimica Acta, 2004. 507(1): p. 11.
[2] Andersson, H. and A. van den Berg, "Microfluidic devices for cellomics: a review". Sensors and Actuators B-Chemical, 2003. 92(3): p. 315-325.
[3] Becker, H. and U. Heim, "Polymer hot embossing with silicon master structures". Sensors and Materials, 1999. 11(5): p. 297-304.
[4] Guber, A.E., et al., "Microfluidic lab-on-a-chip systems based on polymers - fabrication and application". Chemical Engineering Journal, 2004. 101(1-3): p. 447-453.
[5] Woolley, A.T. and R.A. Mathies, "Ultra-High-Speed DNA-Sequencing Using Capillary Electrophoresis Chips". Analytical Chemistry, 1995. 67(20): p. 3676-3680.
[6] Balslev, S., et al. Fully integrated optical system for lab-on-a-chip applications. in 17th IEEE International Conference on Micro Electro Mechanical Systems (MEMS): Maastricht MEMS 2004 Technical Digest, Jan 25-29 2004. Maastricht, Netherlands: Institute of Electrical and Electronics Engineers Inc., Piscataway, United States.
[7] Mogensen, K.B., H. Klank, and J.P. Kutter, "Recent developments in detection for microfluidic systems". Electrophoresis, 2004. 25(21-22): p. 3498-3512.
[8] Truckenmuller, R., et al., "Low-cost thermoforming of micro fluidic analysis chips". Journal of Micromechanics and Microengineering, 2002. 12(4): p. 375-379.
[9] Kricka, L.J., et al., "Fabrication of plastic microchips by hot embossing". Lab on a Chip, 2002. 2(1): p. 1-4.
[10] Lee, G.B., et al., "Microfabricated plastic chips by hot embossing methods and their applications for DNA separation and detection". Sensors and Actuators B-Chemical, 2001. 75(1-2): p. 142-148.
[11] Slocum, A.H., Weber,A.C., "Precision passive Mechanical Alignment of wafers". Journal of Micromechanical systems, 2003. 12(6): p. 826-834.
[12] Slocum, A.H., Precision Machine Design. 1992: Prentice Hall: Englewood Cliffs, NJ.
[13] Jones, R.V., "Some uses of elasticity in instrument design". Journal of Scientific instruments, 1962. 39: p. 193-203.