# Fabrication Process for Openable Microfluidic Devices and Externally Actuated Microfluidic Switch

by

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Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements of the degree of

Master of Science in Mechanical Engineering



At the

## MASSACHUSETTS INSTITUTE OF TECHNOLOGY

August 2008 © 2008 Cartas Ayala Marco Aurelio. All rights reserved. The author hereby grants to MIT permission to reproduce and to distribute publicly paper and electronic copies of this thesis document in whole or in part in any medium now known or hereafter created. Author . . . Department of Mechanical Engineering February, 2008 Certified by. . . Daniel J. Ehrlich Director, BioMEMS Lab Whitehead Institute for Biomedical Research **Thesis Supervisor** 1 A. A AI Certified by. . . . . Thesis Supervisor: Todd Thorsen D'Arbeloff Career Development Professor in Engineering Design Accepted by . . . . . . . . . . . . . Lallit Anand **Professor of Mechanical Engineering** Chairman, Committee on Graduate Students

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### Abstract

In this document I discuss the fabrication of metallic, aluminum and aluminum oxide, 3D micro channels, made with standard milling technology, along with two channel closing methods for openable devices: half cured-glued PDMS and Pressure Sensitive Adhesive (PSA) Film. Using the aluminum oxide coated micro channels, along with the half cured-glued PDMS process to close the channels and external fast speed valves for actuation, a microfluidic switch for cell sorting capable of operating at 48 Hz was designed, fabricated and tested. The use of aluminum as a channel substrate provides channel strength and short heat dissipation times, and the use of aluminum oxide enhances light energy absorption, which provides the possibility of further laser actuation. Also, the combination of micro fabrication process and actuation technique makes possible the further scaling and handling of large cells as cardiocytes.

Thesis Supervisor: Daniel J. Ehrlich Title: Director, BioMEMS Lab Whitehead Institute for Biomedical Research

Thesis Supervisor: Todd Thorsen Title: D'Arbeloff Career Development Professor in Engineering Design TO MY FATHER, RODOLFO CARTAS SOSA, MY MOTHER, GRACIELA AYALA VALLEJO, AND MY BROTHERS: RODOLFO, DANIEL AND ALEJANDRO,

Who have helped me go further than what I could have reached alone.

"If the doors of perception were cleansed, everything would appear to man as it is, infinite."

~ William Blake "

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## CHAPTER ONE

## INTRODUCTION



Figure 2 Narcissistic Photo of the Author

#### 1. - Introduction

#### 1.1. - Specific Aims

The main objective of this project is to design and test a process to fabricate openable, reusable, 3D, with high light absorption and heat dissipation, microfluidic devices and test it designing a microfluidic switch for cell sorting with cleaning capacities.

In the literature, to the best of my knowledge, the idea of a fully reusable and openable, and thus cleanable, dynamic microfluidic device has not been explored, and thus remains an open area. In order to produce a fully reusable openable microfluidic device, the microfluidic channels have to be as rigid and hard as possible. Rigidity is desired because makes the dimension of the devices stress independent. Also hard materials are preferred because they tend to be less affected by wear and scratching. Thus, the commonly used soft material fabrication methods can't be used, and an alternative method, e.g. machining, has to be used.

Also, in order to make a fully reusable device, actuation has to be external. Possible external actuation methods for the device are mechanical actuation, e.g. electromechanical valves, an optical actuation, i.e. laser actuation; methods that have quite different characteristics. Mechanical actuation represents a cheaper and highly available alternative, and thus was selected as the actuation method for the device. Since external actuation means that a type of transduction has to be used in order to transfer force to the fluid, the actuation time increases accordingly to the type of external actuation used, being larger for external mechanical actuation. Thus, the cost of selecting a mechanical method is increased actuation time.

As an additional requirement, to make possible the future implementation of a switching method based on bubbles created by light (i.e. laser illumination), the micro channels have to be highly thermally conductive and have capacity to absorb a large fraction of the incident light too.

#### 1.2. – Microfluidics and Cell Sorting

During this century, the so called Century of Biology, it is becoming more and more common the use of small samples: DNA, cells or chemicals, for different reasons: lack of samples, e.g. forensics, high value of the samples, e.g. crystallization, or the possibility of the creation of new methods, e.g. high content screening; and the need for ways to handle small volumes arises. To satisfy this increasing need, the field of Microfluidics has been developed. Microfluidics encompass the analysis of the fluids at the micron scale as well as the micro fabrication techniques required for fabricating devices of such dimensions.

Several kinds of microfluidic devices have been developed so far, from single cell analysis to DNA fast speed detection. One of the most promising applications is the analysis of small cell samples based on their phenotypes. So far, flow cytometry has been used to sort large number of cells based on fluorescence, but this method can only deal with relatively large cell population percentages, around 1% of the sample. Also, flow cytometers are normally only able to analyze size and the intensity of cell fluorescence. Microfluidic devices can provide a more detailed analysis of samples with a lower fraction of interesting cells.

#### **1.3. - Cell Sorter Technology**

Many different techniques have been used for cell sorting; a list of some of the actuation mechanisms is presented in Figure 2. In contrast with the thousands of cells sorted by second by standard flow cytometers, the sorting speed of microdevices is smaller than a couple hundred per second at best. This deficiency is compensated with the possibility of small cost and increased sorter efficiency.

The actuation technique can be of two kinds: internal (or local), and external. Local actuation methods encompass all the methods in which a mechanical element *within* the flowing channels performs the actuation, e.g. PDMS valves actuated by an external pressure source. External methods are those for which the actuation force is provided from outside the device and, the only element that reacts to the application of the force is the fluid itself, e.g. pressure valves or laser-created bubbles.

Most of the local methods, like piezo actuation, are not suitable for our application because these methods use parts that are too sensitive to external forces applied on them (during opening and cleaning). Notable exemptions to this observation are the PDMS valves and the electrode actuated devices; these two methods are considered in the design part along with the external actuation methods

The possible external methods considered were optical actuation and mechanical actuation. Optical actuation, creating bubbles in the fluid with a laser source, can potentially create a device with actuation time of a fraction of a millisecond, which is comparable or better than most internal actuation technologies. The drawback is that an expensive external high power laser source is needed as energy source to create bubbles in the milisecond time range. On the other hand, mechanical actuation is slower, but provides a cheaper mechanism for actuation.

SORTING TECHNOLOGY	GROUP	PAPER	ACTUATION TIME
Electro-osmotic Flow <sup>1</sup>	David C. Duffy, Olivier J A Schueeller, Scott T Brittain and George M Whitesides	Rapid Prototyping of Microfluidic switches in PDMS	
AC Magnetohydrodynamic <sup>z</sup>	Asuncion V. Lemoff an dAbraham P. Lee	An AC Magnetohydrodynamic Microfluidic Switch for Micro Total Analysis System	1 ms
Dielectrophoretic <sup>3</sup>	Lisen Wang and Abraham Philip Lee	Dieletrophoretic Microfluidic Switching for Lab on a Chip Applications	
Electrokinetic/Electro	Petra S. Dittrich and Petra Schwille	An Integrated Microfluidic System for Reaction, High Sensitivity Detection, and Sorting of Fluorescent Cells and Particles	50 ms
Optical/ Laser Trap <sup>5</sup>	K. Grujic, O. G. Helleso, J. P. Hole and J. S. Wilkinson	Sorting of polystyrene microspheres using a Y-branched optical waveguide	
Dielectrophoretic <sup>#</sup>	D. Holmes, M. E. Sandison, N. G. Green and H. Morgan	On-chip high-speed sorting of micron-sized particles for high- throughput analysis	3 ms

Electrowetting <sup>7</sup>	Alan H. Tkaczyk, Dongeun Huh, Joong Hwan Bahng, Yu Chang, Hsien-Hung Wei, Katsuo Kurabayashi, James B. Grotberg,Cahng-Jin Kim, Suichi Takayama	Fluidic Switching of High Speed Air-Liquid Two-Phase Flows Using Electrowetting-On Dielectric	0.2 s
Coriolis Force <sup>8</sup>	latzer, Roland Zengerle and Jens Ducree	Frequency Dependent transversal flow control in centrifugal microfluidics.	18 ms
Acoustic Transducer <sup>9</sup>	H. Jagannathan, G. G. Yaralioglu, A. S. Ergun, and B. T. Khuri-Yakub	An implementation of a Microfluidic Mixer and Switch using Micromachined Acoustic Transducers	
PDMS microvalves <sup>10</sup>	Sung-Yi Yang, Suz-Kai Hsiung, Yung-Ching Hung, Chen-Min Chang, Teh-Lu Liao and Gwo-Bin Lee	A cell counting/sorting system incorporated with a micro fabricated flow cytometer chip	8 ms
Flow Activation <sup>11</sup>	C. C. Chen, S. Zappe, O. Sahin, X. J. Zhang, E. E. Furlong, M. Fish, M. Scott, O. Solgaard	Microfluidic Switch for Embryo and Cell Sorting	0.3 ms
Thermal Bubble	Chih-Ming Cheng and Cheng-Hsien	A capillary System With Thermal -Bubble -Actuated 1xN Microfluidic Switches via Time- Sequence Power Control for Continuous Liquid Handling	1 ms
Light Sorting/Optical Trap <sup>13</sup>	Mark M. Wang, Eugene Tu, Daniel E. Rymond, Joon Mo Yang, Haichuan Zhang, Norbert Hagen, Bob Dees, Elionore M. Mercer, Anita H. Forster, Ilona Kariv, Philippe J. Marchand and William F. Butler	Sorting Particles with Light	2-4 ms

Table 1 Flow Switching Techniques

#### 1.4. - Fabrication Options for Non Permanent Bonded Devices

The main goal for the fabrication process is to provide the capacity of eventually opening and cleaning the micro fabricated devices in order to deal with the recurrent clogging during testing, reducing the number of chips needed for testing and therefore the required yield for the entire process. To achieve openability, non permanent ways of closing the channels are analyzed in the fabrication section.

The used methods can be classified in three categories: physical methods, characterized by the direct application of a force, e.g. pressure; and chemical methods, which use the force between the materials themselves to provide adhesion, e.g. bonding, gluing or adhering; and finally hybrid methods, which use two of the previous together to achieve better results. Vacuum and the direct application of pressure are the common

physical methods used for chucks and momentary holding pieces together and therefore are the logical options for developing an openable microfluidic device. Among the chemical methods, despite not considering the permanent adhesives for obvious reasons, there are many options available. The suitable chemical methods can be classified accordingly to the adhesive type method used into four different categories: thermal adhesive, pressure sensitive adhesive, UV adhesive and multicomponent cured adhesive.





The multicomponent cured adhesive is the most common form of adhesive, it consists of two different substances that upon mixing react and form a rigid layer that provides adhesion between two different materials. Many multicomponent cured adhesives produce a by-product that either evaporates or swells out and must be taken into account when using this kind of adhesive.

Poly-dimethylsyloxane (PDMS) is one the most used multicompound curable adhesives in electronics and microfluidics. PDMS is biocompatible and non fluorescent, characteristics that makes it a good choice for building biological micro devices. PDMS is a perfect example of a polymer formed combining two liquids that release a byproduct which evaporates during curing.

A thermal adhesive consists of a plastic film backed with an adhesive layer. After placed in contact with the surface to bond, the compound film is moderately heated, which activates the adhesive layer; otherwise, the adhesive layer does not adhere effectively to any surface.

A pressure sensitive adhesive, PSA, consists of a layer of plastic film backed with a layer of pressure adhesive. The pressure sensitive layer adheres to surfaces using Van der Waals forces and therefore, requires intimate contact with the other surface to form a strong bond. To achieve this proximity between the surfaces, pressure is applied upon the surfaces have been placed together.

To enhance the adhesion performance, a combination of physical and chemical methods can be used, creating a more reliable result, making the adhesion more uniform and stronger. For instance, thermal adhesive or a Pressure Sensitive Adhesive can be combined with vacuum to enhance uniformity.

The combination of thermal adhesive with vacuum force provides the opportunity to deform a polymer film over the uneven surface, applying pressure where required, before creating a stronger bond during the exposure to a moderate heat source. Similarly, the use of vacuum along with PSA improves sealing by the application of local forces where needed.

## CHAPTER TWO

## DESIGN OF CELL SORTING SWITCH



Figure 4 Final System

### 2. – Design of Cell Sorting Switch

During the course of this thesis, we designed the microfluidic circuit shown in figure 5. Our goal was to design a switch to be used to enrich selected cells out of a continuous microfluidic flow channel. The main design considerations for such a switch are the speed of the device, open plus closing time; the dilution factor, which determinates final concentration achieved; and the switching reliability, which determines the efficiency of enrichment and the purity of the selected subpopulation. We present a brief discussion on the process that led to the selection of the technologies used and follow this with a description of the design process.



Figure 5 Basic AutoCAD Design

#### **2.1 Actuation Selection**

To increase the number of cells processed and decrease the total processing time, the actuation time should be as short as possible. The two components of the total actuation time, both opening plus closing, are the time associated with the direct actuation time and the transduction time, i.e. the time associated with the fluid response. Depending on each actuation technique, one or the other might be the limiting factor. Pressure-Activated PDMS valves have actuation times in the order of 2 milliseconds.<sup>14</sup> Roughly half of this time is required for activation of the pressure source and the remaining time is used for building up pressure next to the PDMS valve. This actuation time is low enough to fabricate a cell sorter, but the fabrication process is not compatible with the requirements of our process.

Electrode actuated devices, which encompass heating and electric field actuation, also have actuation times in the order of few milliseconds15. The electrode methods depend on the creation of a conductive path that would either have to be deposited on the sealing layer or created on the channel surface. If the first option is performed, depositing on the cover layer, careful alignment has to be done each time the device is assembled and a sealing method that does not create a thick insulating layer over the electrodes has to be used. Alignment would compromise bonding yield and reusability of the device, and any electrode pattern is prone to severe degradation over time. Therefore, we decided that the electrode device would be overly complex and likely to be unrealizable.

Due to these considerations, external actuation was preferred over internal actuation. Possible external actuation methods for the device are optical actuation, i.e. laser actuation, and mechanical actuation, e.g. electromechanical valves. Mechanical actuation is a cheaper and highly available alternative, and thus was selected as the actuation method for the device. The cost of using an external mechanical actuation method is a higher actuation time, milliseconds, compared to that which might be possible with an external high power heat source such as a laser.

#### 2.1.1 Mechanical Actuation Selection

The available mechanical actuators for small fluidic applications can be classified in three categories: dispensing valves, pressure source valves, and flow valves, which encompass inline valves and constriction valves.

Dispensing valves are devices that provide a fixed output volume when actuated. Examples of this kind of valves are the valves used for inkjet printers. Despite having a low response time, in the order of milliseconds, the dispensing valves have a serious drawback: the large minimum displaced volume. For micro application valves, the minimum dispensed volume is in the order of a hundred nL. For most applications, a hundred nL is a small volume, just a drop; but not for many microfluidic applications. If a 200-microns-wide by 50-microns-depth rectangular channel is considered, a hundred nL would occupy a 1 centimeter channel, an unreasonable length for any practical device.

Pressure source valves, i.e. valves that control the pressure applied to a fluid reservoir using a second fluid as air, have small enough actuation times, milliseconds too, but they have their drawbacks too. If pressure is applied to the reservoir, the reservoir will reach the supplied pressure once the air in it has the same density of the air at the source (i.e. it has to be filled first). From the dimensional analysis, the filling time is proportional to the area of the reservoir and inversely proportional the cinematic viscosity of the fluid. For air at 1 atmosphere and 20 °C, in order to have actuation times in the order of the milliseconds, a cross section smaller than a millimetre is required, and therefore the reservoir has to be a long piece of tubing. Due to the large size of this tubing, the calculation of any property of the device would be associated with the fraction of tubing filled, and the device properties would be modified as it is operated. Then, if the reservoir moderately sized, it becomes hard to model the dynamic performance of such devices.

Flow valves can operate on a vast number of principles, but essentially they block or enable the fluid flow using an obstruction. This obstruction has a non negligible volume, dead volume (DV), which has a large effect on the response of the valve. The initial actuation depends on the ratio between the DV and the volume that can be filled by the liquid during the valve activation, which can be calculated as the flow just due to the pressure source, F, times the actuation time, T. The ratio of these factors defines three operation modes:

(Case 1:  $F * T \ll DV$ ) During opening, the DV is not filled by the liquid as it unblocks the channel, thus vacuum is generated, and a temporary pressure fall is created. This pressure drop generates a backpressure that moves some of the liquid backwards. The back volume is proportional to the ratio of the pressure differences between the valve zone and the inlet and outlet fluidic circuit ports, and inversely proportional to the ratio of the hydraulic resistances on each side. The hydraulic resistance is the analogue of the electrical resistance, and relates the pressure applied to the output flow; a more detailed description of this concept is given in the appendix.

As the value is closing, the liquid is not fast enough to get out of the volume by itself, thus the value pushes the fluid to the sides. The liquid displaced this way flows to the sides of the value accordingly to the value of the resistances next the value, as shown in figure 6.

(Case 2:  $F^*T \gg DV$ ) In this operation mode the value is slow enough to be felt by the liquid as a strangulation in the flow. The flow has enough time to fill the DV as it is generated, therefore, no backflow is generated, but the system has a long transient. During closing, the fluid has enough time to evacuate the cavity before being pushed by the obstruction, generating no back flow again, but imposing a longer transient with large flow.

(Case 3:  $F^{T}$ -DV) In this mode, a competition between the generation of vacuum and backflow occurs. As a first degree approximation, an average pressure gradient can be defined at each side of the valve as a weighted pressure gradient, and the problem can be treated as a 1D problem. The flow can be modelled as the superposition of two flows product of a flow source and a two pressure sources, which can diagrammatically be represented as shown in figure 6.



Figure 6 Equivalent Flow Circuit Diagram

As before, even external actuation devices designed for microfluidics have large dimensions, and the DV is not the exception. Normally condition 1 holds,  $F * T \ll DV$ . As a consequence, the backflow is proportional to the resistance ratio: R1/R2; where R1 is the fluidic resistance after the valve and R2 the fluidic resistance before the valve. In order to make a reliable circuit independent of the external connections, the resistance before the actuation, mainly due to tubing, has to be small compared to the resistance after the valve, mainly due to the device. Therefore, for any design it is desired that R1/R2<0.01, and as a consequence, the backflow can be greatly minimized.

In order to avoid the possible unsteady state implications of flow valves, a low DV and internal resistance are required. Among the valve options examinated, the Lee Nanoliter Dispensing Valves1 had the smaller dead volume and a low internal resistance and therefore were selected for actuation. The Lee valve also has a good actuation speed, less than 0.5 ms.

#### Lee Valve Limitations

Valve must operate with clean liquid Operation Pressure 3 psi-20 psi



Figure 7 Lee Valve Dispense Volume at Various Pressures, from http://www.theleeco.com

The DV was not disclosed by the Lee Company due to a confidentiality policy, but an estimation of the performance for microfluidics can still be done using the dynamic behavior shown in the previous graph, figure 7. In this graph, the fraction of

<sup>&</sup>lt;sup>1</sup> The Lee Company, <u>http://www.theleeco.com</u>, 2008

the DV that is sent forwards during the actuation of the valve is roughly 5 nL. To obtain the previous graph, the valve outlet was connected to an orifice that increased the total outlet resistance, R1, to 60,000  $L\Omega^2$ ; and the inlet was simply connected to a water reservoir.

For orifices, like the ones used for the valve operation, there is no linear relationship between the flow and the pressure drop, instead the flow increases as the square root of the pressure. Despite this non linear relationship, the resistances from holes and channels can still be compared upon linearization assuming 3 psi of pressure. If a 50-micron-radius channel of length 5 cm is assumed, the total output resistance is increased by a factor of roughly 500, and therefore the fraction of the DV that is sent forward to the device is decreased by roughly the same factor. The net result is that the incoming unwanted flow is reduced to 10 pL, and the additional flow is a mere difference of 1 micron in the flow and therefore can be neglected.

#### 2.2 Valve Actuation and Unsteady Flow

With the selection of the Lee value as a primary actuator, we then designed a micro fluidic configuration for switching of biological cells. Now that the actuation was selected, taking into account the parameters of the value, a basic description of the value actuation can be preformed.

Due to the characteristics discussed above, the Lee valve selected can be considered as a 2 ms on/off pressure source switch. Therefore, the actuation can be modelled as the unsteady pressure driven flow of liquid through a tube. The solution for the circular tube is well known3, and a more general approximation for regular shapes is presented in the Appendix.

Using the derived approximate model, final parameters for the actuation can be obtained. Using the velocity profile, the average velocity can be derived, resulting:

<sup>&</sup>lt;sup>2</sup> LΩ, not Ω, is used for apertures, holes and expansion because the resistance is not linear but quadratic. <sup>3</sup> The solution for this case is presented by Batchelor in his book, 'An Introduction to Fluid Mechanics', although the derivation is just an outline.

$$\overline{V_{Z}} = \frac{-\nabla P^{*} r'^{2}}{\mu} \left( \frac{1}{8} - 4 \sum_{i=0}^{\infty} \frac{e^{-\left(\frac{\chi_{i}}{r'}\right)^{2} \upsilon t}}{\chi_{i}^{4}} \right) \quad (1)$$

As can be seen from equation 1, the time it takes for the system to get to steady state is proportional to the cross area of the channel and inversely proportional to the viscosity of the fluid used, as expected from the non dimensional analysis. As time progress, the number of terms required to describe the flow behaviour decreases, and the relation becomes nearly exponential. For a 250 micron by 75 micron rectangular channel filled with water, the characteristic time is in the order or 5-8 ms, which sets the limit between steady states in microfluidic channels with these dimensions to 5-8 ms.

The total actuation time is two times the actuation time, one for on and one off, plus two times the fluid unsteady states, plus the time the switch is activated. This cycle sets the minimum total actuation time between 14 to 20 milliseconds. Of course, accordingly to the derived model in the Appendix, the device can be driven at higher velocities; but the unsteady state and particular geometry must be considered. A second limiting factor for not driving the device at higher velocities is the pressure needed for doing so. From the model, it can be observed that the pressure gradient supplied has to increase in order to compensate for the lack of time to achieve the desired flow velocity, and thus actuation.

The minimum increment in pressure needed for actuation follows the equation:

 $Q = \ln(1-1/k) \rightarrow k=1/(1-\exp(-Q))$  (2)

Where:

- Q is the fraction of the fluid actuation time and has no units
- k is the lower bound for the minimum number of times the pressure has to be increased

For instance, to make the fluid response time 10 times smaller, reducing the actuation time to 4 ms after considering the valve actuation, a pressure more than 10 times the original value is needed. For instance, if a PDMS device is operated at 3 psi, common for microfluidics, at least 30 psi is needed for 4 ms actuation, resulting in probable debonding.

Additionally, actuation times have to grow in order to maintain cell velocities low if high content/contrast images are also desired. In order to select cells, cells have to move substantially within the time prescribed by the actuation; for an average cell 20-50 microns of travel should suffice. If the minimum actuation times are considered, velocities of several millimetres per second are required.

#### 2.3 Circuit Design

Once the basic formulation for the valve actuation was derived, the device was designed. The full micro-fluidic circuit was designed based on the steady states we discussed above.

#### Models

The following fluidic circuits were considered for valve actuation.





**Figure 8 Equivalent Resistance Circuit A** 

In this design, the valve is placed just above the channel junction. During normal flow, the valve is closed and the flow is divided in two streams: collection and waste. In order to minimize the volume of the liquid collected, and therefore, increase the final sample density, the collection lane has to have a larger resistance than that of the waste lane. In the diagram, the maximum flow ratio is set to one tenth, but this number is still high, and a lower resistance is preferred. When the switch is activated, the valve opens cancelling partially the effect of the flow division. In order to completely cancel the effect of the flow division, the sample lane resistance needs to be larger than the collection lane resistance. Also, since the flow ratio of the hydraulic resistances, the lower the focusing step is needed. The larger the flow ratio of the hydraulic resistance from the valve inlet to the atmospheric pressure is still high, and thus limits the achievable velocity for any pressure supplied.





Figure 9 Equivalent Resistance Circuit B

In this design the valve is attached to the side of the collection lane. The valve is attached directly to the junction to minimize the path and thus the resistance in the valve lane. During normal operation, the flow is divided in a waste and collection lane as before. When the valve is activated, most of the flow is switched to the lane leading to the valve, and upon deactivation, the flow that stayed within rch flows to the collection lane. In order to increase the final velocity of the actuation lane, the resistance rch and rv have to be minimized. The main drawback of this design is that during frequent activation cycles, the flow in rch that may contain the desired samples will flow to the

valve, being lost and possibly clogging the valve. For solving this problem, an additional large resistance can be added between the valve inlet and the pressure source, acting as a washing lane. Once again, a pre-flow-focusing step is required, and now, the smaller that rv is compared to r1, the lower the requirement for flow focusing.

#### **Design** C

Design C is a hybrid between design A and B. It has actuation in the waste lane as well as in the collection lane. The basic actuation of this device is the same as the sum of A and B. As for its main drawback, it inherited the failure for frequent cycles from mode B, and a waste lane has to be incorporated. The high resistance due to the presence of r1 is mitigated by the combined effect of the two valves.



Figure 10 Equivalent Resistance Circuit C

Despite its higher complexity, the design can overcome more easily the time constraint, and therefore was selected. Also, this design can be further simplified eliminating rch and connecting the valve lanes junction to a Y junction, but higher attention to the geometry and the unsteady state must be paid. After the fabrication process was selected, the fabrication characteristics, i.e. precision, were still unknown, and this modification was not performed. As in design C, a washing lane can be added before the outlet lane or a filter.



Figure 11 Improvements over design C

#### 2.4 Equivalent Resistance Circuit

After the initial selection of the circuit type, the design outline was replaced by a more detailed version that incorporated a washing lane to avoid clogging the outlet valve.



**Figure 12 Detailed Circuit Diagram** 

#### **Calculation of the Circuit**

Once the basic circuit was selected, the value of the hydraulic resistances can be established. The design parameters that were used for selecting the resistance ratio are the following ones:

Valves OFF

Iin < Iout	High Dilution Factor
Iin =< I2	No Selection when the valve is OFF
	Flexible Factor that depends on the Flow Focusing

Valves ON

I7 > I2	Effective Switching
Iin ON +- Iin OFF	Flow stability, will guarantee stable flow focusing

After numerical iterations, the following resistance ratios satisfy the basic requirements.

R1	R2	R3	R4	R7	R8	DF
5	99	1	99	1000	1	0,01
2,5	60	1,25	25	1,25	1	0,05
2,5	50	1,25	20	1	1	0,06
2,5	36	1,25	15	1,25	1	0,08
2,2	20	1	8,5	1	1	0,1

**Table 2 Resistance Ratios** 

The values are listed from top to bottom accordingly to their dilution factor, DF in the table. For the values satisfying the criteria, the DF is enhanced almost proportionally to R4/R3 within a single significant digit. For the same values, the actuation performance, I7/I2, was relatively stable, roughly 1.5 +-0.1.

Despite of having the best DF, the first resistances ratio may be abandoned in favor of one of the other choices because, for some fabrication methods, such a large difference may be not easily achievable.

#### 2.5 Final Design

Once the resistances desired are known, the exact cross sections can be calculated; for that purpose, an analysis of the relationship between rectangular cross sections is performed in the next section, followed by the final selected cross sections.

#### Calculation of the Channel Resistance Based on the Equivalent Radius

For calculating equivalent resistances of channels of varying cross section, the equivalent radius along with the friction correction factors for different geometries must be used. For such purpose, the following table, obtained from the book: "Fundamentals of Heat and Mass Transfer" written by Incropera and DeWitt, was used.

Cross Section	$\frac{b}{a}$	f Re <sub>B</sub>
$\bigcirc$		64
a 📄	1.0	57
a	1.43	59
a b	2.0	62
a b	3.0	69
d b	4.0	73
b	8.0	82
Heated	x	96
Insulated	x	96
$\bigtriangleup$		53

**Figure 13 Friction Factor for Different Tube Geometries** 

Along with this table, the following derived equations were also used. From the definition of friction factor:

$$\frac{-\left(\frac{dP}{dx}\right)^* D_{eq}}{\frac{\rho \overline{u}^2}{2}} = f \qquad (3)$$

Where:

$-\left(\frac{dP}{dx}\right)$	is the Pressure Gradient	
$D_{eq}$	is the equivalent tube diameter	
f	is the friction factor	
ρ	is the liquid density	
ū	is the average velocity	

Also, for a laminar flow the friction correction factor has the form

$$f = \frac{\#}{\operatorname{Re}(\overline{u}, D_{eq})} \tag{4}$$

Where # is a real number

Now, combining both equations:

$$\frac{-\left(\frac{dP}{dx}\right)^* D_{eq}}{\frac{\rho \overline{u}^2}{2}} = \frac{\#}{\operatorname{Re}(\overline{u}, D_{eq})} = \frac{\#}{\frac{\overline{u}\rho D_{eq}}{\mu}}$$
(5)

Where  $\mu$  is the viscosity

Now, simplifying for the pressure gradient to obtain an expression in terms of the pressure drop and the flow:

$$-\left(\frac{dP}{dx}\right) = \frac{\mu^* \overline{u}^* \#}{2D_{eq}^2} \quad (6)$$

Now, taking into account the definition of the average velocity,  $q = A^* \overline{u}$ , and that of equivalent diameter,  $D_{eq} = 4 * \frac{A}{P}$ , and substituting into the previous equation:

$$-\left(\frac{dP}{dx}\right) = \frac{\mu^{*\#}}{2^{*}D_{eq}^{2}*A}*(\overline{u}*A) = \frac{\mu^{*\#}}{2^{*}(D_{eq}*P/4)*D_{eq}^{2}}*q \quad (7)$$

Where

- P is the Perimeter of the tube cross section
- A is the area of the tube cross section
- Q is the volumetric flow

Finally, to obtain the resistance equivalent, both sides of the equation are multiplied by the length L.

$$\Delta P = -\left(\frac{dP}{dx}\right) * L = \frac{2 * L * \mu * \#}{P * D_{eq}^{3}} * q = R_{eq} * q \tag{8}$$

Where  $R_{eq}$  is the equivalent tube resistance.

Therefore, the equivalent resistance can be calculated as:

$$\frac{2^* L^* \mu^* \#}{P^* D_{eq}^3} = R_{eq} \qquad (9)$$

When dealing with only rectangular channels, only the functional dependence is important, therefore

$$R_{eq} \alpha \frac{L^{*\#}}{P^{*} D_{eq}^{3}} = \frac{L^{*\#}}{(a+b)^{*} 8^{*} \left(\frac{a^{*}b}{a+b}\right)^{3}} \alpha \frac{L^{*} (a+b)^{2}^{*\#}}{(a^{*}b)^{3}}$$
(10)

$$R_{eq} \alpha \frac{L^* (a+b)^2 * \#}{(a*b)^3}$$
(11)

Based on the fabrication process selected, as discussed in the next chapter, the z dimensions can be varied every 25 microns after the initial 50 microns. As for the channel width, the minimum is 150 microns, and can be increased every 25 microns. Based on these restrictions, the maximum resistance cross section is 150 by 50 microns. A reasonable cross section for the minimum resistance channels is 500 by 150 microns, which being so large, might be difficult to connect to the other channels. Finally, in order to maintain the resistances within the same length magnitude, and thus minimize the overall space, the remaining cross sections have to compensate for the higher resistance; based on this criterion, an intermediate cross section was selected too, figure 15.



**Figure 14 Channel Cross Sections** 

Now, using the expression for the resistance ratio and the channel cross sections, the following resistances ratios can be obtained, figure 16.

CHANNEL	$4\frac{(a+b)^2}{(a*b)^3}$	RATIO	RATIO F	
COMPENSATED				
500x150	4.006x10^-9	1	1	
250x75	6.409x10^-8	16	16	
150x50	3.793x10^-7	94	92.21	

**Table 3 Correction Factors and Resistance Ratios** 

Using these dimensions, it is clear that the design that is easiest to fabricate is the one at the bottom of the list; and particularly due to the process's unknown reliability in closing channels, this was the one selected for fabrication.

A more detailed view of the switching junction is shown next, first image of the figure. The junctions is not designed as a Y junction in order to use the inertia from the sample to create switching, although it turns harder to switch the selection mode off. Since the fabrication outcome is still unknown, the junction may have numerous variations that can greatly modify the unsteady state.



Figure 16 Probable Outcome from the fabrication process.

Additionally to this basic design, a flow focusing lane has to be designed. Due to the flow ratios, flow focusing of at least one third of the channel is needed. In order to calculate the flow focusing, the steady velocity profile was approximated as a double parabolic profile in the X, Y directions. Thus, the velocity profile can be described as:

$$V = K(1 - y^{2})^{*}(1 - x^{2})$$
 (12)



Figure 17 Double Parabolic Approximation

Using this model, further analysis of the flow rates required for focusing can be done. Given the cross sections of the channels, in order to focus the central flow, a channel with the smaller cross section must be crossed by a channel with the larger cross section. This crossing will produce vertical focusing as well as focusing along the width of the channel. If the focusing zone is assumed to produce a symmetrical flow along the X axis, the focused flow remains in contact with the upper surface, and the focused zone is assumed as a rectangular zone, an approximate model of the focused zone can be obtained.

During focusing, the flow coming from the sample channel has to be the same as the one in the focused region.

Using the double parabolic model, the following relationships can be obtained.

From the momentum conservation

$$16^{2} / (1+\#)^{2} = 2*(15a-10a^{3}+3a^{5})*(8+10b^{3}-15b-3b^{5})-M$$
(13)

From the mass conservation

$$16/(1+\#)=2*(3a-a^3)*(2+b^3-3b)$$
 (14)

#### Where

- # is the ratio of the total flow divided by the sample channel flow
- **a** is half the width of the focused flow
- **b** is the height of the bottom of the focused flow measured from the center of the channel
- **M** is the momentum gained



Figure 18 Flow Focusing Schematic

For the given dimensions, the approximate flow ratios are shown in the next graphs.



Figure 19 Possible a and b for a given 1/#


Figure 20 Possible a and b for a given log(1/#)



Figure 21 Flow ratios for the given a and b dimensions



Accordingly to the channel dimensions, the focusing in the X and Y dimensions should be similar, 1:1 - 1:1.5. In the plot the range of allowed a and b dimensions for this ratio are shown. Therefore, accordingly figures 21-24, the ratio of one to nine should suffice for the requirements.



Figure 23 Focusing Accordingly to the Approximation

After this initial selection, a basic AutoCAD<sup>TM</sup> Design was generated, Figure 5. Additionally to the resistances, during the geometrical design, other parameters were selected based on functionality.

*Input Holes* Ports were selected to be 10 mm in diameter in order to produce interconnection with the ports.

*Ports* Ports are 10-32 threaded ports, smallest dimension for standard chromatographic ports.



Figure 24 Inlet-Outlet Ports

*Vacuum Area* In order to enhance vacuum, the area fraction composing the vacuum cavity was set to 60%, 33 square centimeters.



Figure 25 Vacuum Chamber

*Channel Density* In order to provide enough area for the channels to be properly sealed, a distance of four times the channel width was left between the channel center lines.

Also a possible method for increasing the concentration of the cell samples was designed. The device consists of a large cavity that divides the stream in two rotating zones. The design was added to the collection lane. A basic diagram of the device functioning is shown in figure 28.



Figure 26. Basic Concentrator Actuation. Based on the cavity geometry, the flow can be set into a rotation that minimizes the flow velocity between the two cycles. The flow enters and leaves the cavity perpendicular to the surface. Besides these flow directions, 90 degrees rotations were also evaluated, but did not produce the desired rotation.

The basic circuit connection is shown in figure 29.



**Figure 27 Basic Circuit Connection** 

#### 2.6 **Optics and Electronics**

In order to acquire the images from the device, an inverted fluorescent microscope, Nikon TE2000, was used. After substituting filters, the microscope provided the basic optics needed for the switch activation. A photo multiplier tube, PMT, was used for signal acquisition, followed by a filtering and amplification signal.



**Figure 28 Optics Setup** 



Driving Circuit for Automatic Valving (Pressure Sources)



Figure 29 Pressure Regulating Line, Not to scale

In order to apply automatically the pressure, the following circuit was used to activate the pressure valves. The pressure valves used were Pneumadyne solenoid valves; in the following diagrams they are represented as R2 and the diodes. The

voltage needed for logical activation was provided by a USB port connected to a USB-Parallel Port Codifier. For the Power Supply, a 8 amperes and 24 volts variable voltage source was used.



#### **CIRCUIT OFF**



#### CIRCUIT ON

# Figure 30 Driving Circuit for Pressure Valve, ON and OFF positions. The actuation time is set to 0.3 ms.

### **Driving Circuit for the Lee Valve**

In order to activate the switching valves, the circuit shown in figure 33 was built. The input signal for testing was a signal generator working between 1-50 Hz. When a sample is flown, the signal is switched the signal coming from a PMT after amplification, which senses the flow of samples. In order to provide the circuit power, a 24 V, adjustable power supply was used. The Lee valves require for activation two voltages: spike activation voltage and hold voltage. The first voltage, activation, is higher, and is provided directly from the voltage supply. For the second one, hold, a voltage transformer was used. The signal generator sequences the voltage supplied accordingly to the signal provided from the signal line; first applies the activation voltage.







Figure 32 Voltage Source, Signal Generator and Transformer

# CHAPTER 3

# PROCESS DESCRIPTION AND CHARACTERIZATION



Figure 33 The Lab Was in the Mood

# 3. - Process Description and Characterization

Our objective was to be developed a debondable, true 3D microfabrication procedure. Most lithography based microfabrication does not allow true freedom in the depth dimension; hence it is termed "2.5 D". Our goal was to develop a procedure that allowed variable depth channels. We also wanted a reversible bonding process in order to allow cleaning plus recycling of high value actuated structures. The design factors during the device design of the fabrication were the reversible bonding plus a transparent cover for high quality imaging.

The fabrication process can be divided in two parts: channel fabrication, by milling, and channel sealing with an optical window. After experimenting with different methods, the fastest and easiest method is shown in figure 36.



backing piece of glass

**Figure 34 Final Process** 

#### **3.1 Channel Fabrication**

#### 3.1.1 Material Selection

Due to the thermal properties of metals, large heat conductivity and resistance to high temperatures (which could enable further laser actuation), a metallic material was chosen for the channel fabrication. Among the metallic materials, aluminium was chosen due to its machinability, and the further possibility of surface hardening by anodization. Anodization, the growth of an oxide layer by an electrolytic process, has the additional advantage of creating a dark surface that enhances the absorption of incoming luminous energy, and improves the contrast in images of specimens flowing though the channels. Anodization can also provide an additional layer of precise thickness and therefore can be used to further tailor the dimensions of the channels. Soft materials such as PDMS are often chosen for prototyping microfluidics, but these are very poor materials in terms of stability, permeability and thermal properties.

#### 3.1.2 Surface Characteristics

In order to achieve bonding in the next fabrication step and improve the quality of the surface milling, two extra parameters must be defined: surface roughness and surface flatness. The first parameter, surface roughness, is the local surface height variation, within a distance comparable to the dimensions of the channels. The second one, surface flatness, describes the variation of the surface average level between two different surface points separated by a prescribed distance, in this case, the device length.

Surface roughness modifies the local surface adhesion and the local channel geometry, but it could also influence the large scale behaviour if the surface forms distinctive patterns across large areas. Therefore, must be prevented by reducing surface roughness. Surface flatness can modify the overall quality of the device since a large surface variation could represent large variations in the channels height. Also, a large variation in surface flatness creates extra difficulties during bonding of hard substrates,

since the weaker layer, the borosilicate glass layer in this case, has to deform to resemble the non flat surface, creating extra stresses that will tend to debond the piece.

In order to create a flat surface, the MIT Machine Shop flying wheel was used. To improve the surface roughness, sanding, followed by diamond paste polishing were used to finish the bonding surface.

#### 3.1.3 Machining Process

Milling was selected as method of fabrication because it provides an economical, readily available and reproducible method of true 3D fabrication. Since commercial milling tips of up to 50 microns<sup>4</sup>, flat and spherical, are available, the existence of milling tools does not represent a limitation for the fabrication of most microfluidic channels for cell handling. Commercial milling machines can handle drill bits of up to 75 microns in diameter, 10 micron tolerances for the X-Y dimensions and can make up to 50 microns, 10 micron tolerances in the X-Y dimensions and make 75 micron depth channels. However, the machine limitations are not the real limiting factor; instead, the real problem is that it is difficult to convince most of the commercial machining facilities to make a device even with the larger specifications, since relatively few shops specialize in fine-scale milling. Therefore, the real constrain, at least initially, is the machining center.

After quoting, QC Drilling was selected for the milling process.

#### 3.1.4 Process Characterization

Aluminium stock was provided by the machining center. After the flying wheel cut performed at the MIT Machine Shop, the aluminium surface was flat within 15 microns for the cut, 10 cm by 10 cm, aluminium piece. The achieved flatness is compatible with the floated borosilicate glass flatness of 7-15 microns for the same dimensions.

<sup>&</sup>lt;sup>4</sup> The available milling tips depend on the specific provider and milling tips of up to 25 microns are available commercially, but the common range is up to 50 or 75 microns.



Figure 35 Aluminium Piece Surface before any Process

The roughness of the aluminium piece changed from 6.5 um to 7 um RMS. This was reduced with a complete cycle with sanding paper using a turning table to roughly 0.7 microns RMS. During sanding, more surface material is removed as the distance from the surface border decreases. In order to compensate for the distance effect, a larger aluminium piece, 10 cm by 10 cm, was originally cut for a final part of 7.5 cm by 7.5 cm. After sanding, the piece was cut to its final dimension and the borders rounded. Following this initial step, the channels were milled by the outside vendor.





Figure 36 Aluminium Piece after Sanding

Two chips were milled for the project. The first chip did not achieve the required dimensions since, as admitted by the company, there was no quality control implemented for the given dimensions (not even measuring was performed). After consideration of this initial drawback, it was decided to compensate the fabrication dimensions with an additional pressure source at the actuation port, and the process was continued. Next, the first chip was anodized, and a 20 microns oxide layer was grown,

increasing the surface level by roughly 11 microns, and decreasing the channel X-Y dimensions by 20 microns. Even with this layer, the channels were wider and deeper than requested as can be seen in the first graph of desired dimensions versus achieved dimensions, figure 39.



Figure 37 Anodized aluminium piece, First Iteration.



Figure 38 Aimed VS Fabricated Dimensions.



Figure 39 Desired Dimensions VS Absolute Error



Figure 40 Aluminium Piece Structure Next to the Channel

Besides the error in dimensions, the first piece milled presented other additional problems. The surface of the first chip was milled to create a working surface as a premilling step. This pre-step created one micron height stripes across the piece, which made the bonding step harder to achieve. Also, as an unforeseen but predictable side effect of the anodization, the surface roughness grew considerably due to the new grain structure, producing a roughness of nearly 15 microns. Both of these problems were compensated with an extra polishing step after milling. During polishing, roughly 15 microns of material were removed from the aluminium surface using diamond paste. The polishing created an almost mirror finishing, but it was not uniform; some grains were lost from the surface during the polishing process, producing a higher local roughness around these points of roughly 18 microns. Polishing also curved the surface, making the surface 5-10 microns lower near the edges than in the center. Further polishing may compromise the flatness of the piece and therefore, it was concluded that extra polishing was incompatible with anodization.



**Figure 41 Grinding and Polishing Process** 

On a second iteration with the machine shop a large improvement is noticeable. As it can be seen in figure 43, "Desired Dimensions VS Achieved Dimensions", the X-Y dimensions were just slightly smaller than requested. For the range shown in the graph, the Aimed Dimension VS Fabricated Dimensions have a linear relationship, a good process property for design. Also, as it can be seen from figure 43, "Desired Dimensions VS Absolute Error", the error for the requested channel dimensions interval, 150-500 microns, is always below 10%, and decreases as the aimed dimension increases.

The Z dimensions are smaller than requested, 80% of the requested/aimed value, but also follow a linear relationship. In contrast with the X-Y dimensions, z error remains non-negligible, around 20% for our channel dimensions, 50-200 microns.

Despite the discrepancy in the aimed VS fabricated Z dimensions, the process is susceptible of being used reliably in other designs provided this calibration curve is known.



Figure 42 Aluminium Piece, Second Iteration



**Figure 43 Desired VS Fabricated Dimensions** 



Figure 44 Dimensions VS Error %

For the second milling iteration, no pre-milling steps and anodization were performed; therefore the surface characteristics are roughly the same as the ones initially obtained, 0.5-0.6 microns roughness and 10-15 microns for the flatness. As the sanding process progress the surface roughness diminishes, as it can be seen in the next graphs, figure 45.



Figure 45 Aluminium Surface as Sanding Progress

Fly wheel cutting followed by sanding in circles has the advantage over the premilling step of not creating large zones of lower height. After sanding, surface roughness of up to 0.5 microns maximum can be obtained as shown in figure 46, "Final Aluminium Surface Roughness", which shows the final roughness obtained after sanding.



**Figure 46 Final Aluminium Surface Roughness** 

The milled final surface must also be characterized. Inside the channels, roughly one micron height marks left by the milling tip are visible. The exact height of the marks left depends on a combination of two factors: the milling tip and the milling machine used for the channel fabrication. For small tip diameters, smaller than 500 microns, a weak relationship was observed: the larger the milling tip used, the larger the roughness left by the tip. This behaviour can be seen in the next two graphs, figure 47, that show the surface roughness of a 250-micron-wide channel and the roughness of the concentrator channel surface. Special attention is required to interpret the second graph, the one that displays the concentrator roughness, since it combines the roughness due to the milling tip itself, and the process roughness, product of passing the milling tip over the surface several times in a discontinuous way to produce the cavity. The two sudden height changes, steps, are the result of the machine tip passing over the surface in a non continuous way, and therefore, the roughness created by the tip is just 0.9 microns RMS.



Figure 47 Aluminium Channel Surface; Left, outlet; Right, concentrator

As can be seen from the figure 47, "Aluminium Concentrator Roughness", the channel geometry can also modify the surface roughness achieved. This behaviour is more evident in the vacuum cavity, where changes in the milling direction and discontinuous milling produce large surface effects, figure 48. The first graph in figure 48, "Step Due to Change in Milling Direction", shows the 25 micron step due to the joint of two perpendicular milling directions in the vacuum cavity surface. The second graph shows the 2 micron steps due to discontinuous milling, i.e. milling of parallel lanes to produce a larger cavity.



Figure 48 Steps and Stripes on Aluminium Surface due to Milling

#### **3.2 Channel Sealing**

The method that we chose originally for closing the channels, vacuum, didn't produce the desired results. It leaked. Instead, two different methods were explored for sealing: gluing to a hard surface using PDMS and film sealing, with and without

vacuum. Before discussing both methods, a discussion of the vacuum approach is presented.

#### 3.2.1 Vacuum

In order to create a sealing surface, a 0.8 mm thick floated borosilicate piece of glass was coated with a spun 40 micron thick PDMS layer. After spinning and curing, the PDMS surface roughness was 0.25 microns, equal to that of the borosilicate glass. The rim resulting from the PDMS spinning was pushed outside the sealing area by increasing the size of the glass piece by 2.5 cm. After fabrication, the PDMS-glass layer is placed into contact with the channel surface, and then the vacuum is activated.

The vacuum cavity occupies 33 square centimeters of the total surface area, roughly 60%. Therefore, upon vacuum application, assuming perfect sealing, a pressure of 23 psi is applied to the surfaces in contact, sealing the channels. After the initial closing, liquid was flown through the channels using as a first attempt negative, and then positive pressure on the water column. In both cases, and after and before the diamond paste polishing step, leaking out of the water channel was observed, within the first five minutes of operation. The leaking rate was high enough to prevent the normal operation of the device.

Further analysis led to the conclusion that the leaking was due to fluid "channelling" through the local roughness and surface grain boundaries. As mentioned above, even the surface polishing didn't prevent the liquid in the channels from leaking into the vacuum cavity. Therefore the vacuum clamp method was discarded.

#### **3.2.2 Channel gluing to a transparent surface**

After the initial failure of the vacuum approach, a second method using a multi component polymer adhesive, PDMS, was used. PDMS is a well known and extensively used polymer; it is biocompatible and non fluorescent, excellent properties for the micro channels, particularly when used as a gasket. The process consisted of partially curing a PDMS film spun on a piece of borosilicate glass, and joining the partially-cured layer and the aluminium piece followed by a full curing process. Partial curing is performed in order to reduce the polymer flow into the channels and prevent channel obstruction before the pieces are glued by fully curing the PDMS.



**Figure 49 Fabrication Process with Gluing** 

## Spinning

In order to produce a thin film of PDMS over the borosilicate glass the following procedure was followed:

1. - In a clean environment, and using a clean container three times larger than the PDMS volume used, mix 20 grams of the polymer and curing agent that constitute the Sylgard 184 compound, fast curing PDMS, in a weight proportion of 10 to 1. Over mixing would introduce unnecessary gas to the mix, and therefore, should be avoided if possible. On the other hand, over mixing is preferable to insufficient mixing, which yields complete uncured film zones that won't provide adhesion. Normally around 150 strokes should suffice.

2. - Degas the mix for 1 hour using a vacuum desiccator connected to a vacuum pressure of 0.6 atm; almost no bubbles should be visible after this step. Normally using 1 atm of vacuum, just half an hour should suffice, but the used electrical pump did not provide enough vacuum and an increased time was necessary.

3. - Spin PDMS on the borosilicate surface.

The PDMS mix viscosity changes as the compound cures, and, as a result, the thickness obtained after degassing for different times is also different; thus the available curves in the literature are of little help unless the same time is used. Therefore, in order to produce a reliable fabrication process, the spinning was characterized.

Despite frequently omitted in the literature, spinning cycles must be described in detail in order to make the process reproducible because different spinning cycles greatly modify the surface properties. For this process, two spinning cycles were used. The first cycle, named 'half time cycle' in figure 50, lasts for 275 s, and drives the spinning surface at maximum speed for 25 seconds. The second one, named 'full time cycle' in figure 51, drives the spinning surface at maximum speed for 50 seconds, and last 600 seconds. Both processes achieve maximum speed with minimum acceleration, that otherwise could produce radial stripes product of local PDMS non uniformities. Intermediate speeds are used before and after the maximum speed to create a uniform layer for the high speed spinning, and to dry the PDMS after being spun, respectively.



Figure 50 Half Time Curing Cycle

Figure 51 Full Time Curing Cycle

Using the spinning processes above, the following calibration curves were obtained, figure 52. In this graph it can be observed that the processing time does make a notable difference. As the spinning time is increased, the final thickness of the film decreases, as expected. In order to bond the glass piece and the aluminium chip, the thickness of the PDMS layer has to be at least of the same thickness magnitude as the surface roughness and flatness; additionally, it also must the thin enough to be rapidly cured and decrease film distortion upon curing. A compromise between both is 24 microns, which occurs at 1200 rpm.



Figure 52 Thickness VS RPM, Sylgard 184

#### Additional considerations

Most of the spinners use a vacuum chuck for holding the pieces to spin, in this case, the spinner we used, SP100-BIDTEC, deformed the glass surface when used to hold the substrate as illustrated below. Due to the local deformation a circle of lower PDMS thickness was formed after vacuum release.



Figure 53 Film Distortions due to Vacuum Chuck

The circular depression prevented bonding of this zone. In general, local changes in the PDMS thickness have no significant effect on the overall bonding, but when the depressions form a continuous line, the material is hard to bond. In order to prevent the deformation due to vacuum, a backing dummy piece of glass was used to be hold by vacuum, and the window glass piece was hold to the dummy piece using duct tape. The new holding method completely prevented the appearance of the vacuum marks, but decreased the range of available spinning velocities. Even with careful alignment, the larger amount of mass hold by the spinner lowered the onset of vibration, which leads to the piece separation from the vacuum chuck at roughly 1300 rpm.



Figure 54 Spinner as the Dummy Layer is Placed

#### **Partial Curing**

The partial curing process must create a PDMS film hard enough to prevent leaking into the channels and leave enough curing agent to promote adhesion during the following full curing step. This requirement proved to be difficult to achieve initially, and upon the initial failure, the drying step was modelled to create a deeper understanding that could yield a successful process.

The curing of PDMS is an irreversible reaction between two compounds; therefore, the reaction can be modelled either as a first order or a second order irreversible reaction. The reaction is mainly a cross linking with a gas by-product. Accordingly to the datasheets the compound "fully cures"5 in 24 hours at room temperature (293 K) and in 1-2 hours at 338 K. Based on this information and experimental information a model of the curing reaction and its dependence on the temperature can be formulated.

If a first order reaction is assumed, the equation relating the remaining amount of cross linked compound, X, and time, t, takes the following form:

$$X = 1 - e^{-\lambda t} \tag{15}$$

<sup>&</sup>lt;sup>5</sup> PDMS achieves dimensional stability after this time; any further dimensional change is smaller than 2%, although mechanical properties as the Young Modulus continue to changes asymptotically at a different lower rate.

Where X is the linked fraction of the compound, and  $\lambda$  is the inverse of the reaction time constant. For chemical reactions, the reaction time constant has the following temperature dependence:

$$\lambda = \kappa e^{\beta T} \tag{16}$$

Where  $\kappa$  and  $\beta$  are appropriate constants for the reaction. Given these relationships, the time and temperature dependence of the reaction can be established. Combining these two equations, the following relationships can be obtained:

$$X = 1 - e^{-\kappa e^{\beta T}t}$$
(17)  

$$\kappa = \frac{-\ln(1 - X)}{t} e^{-\beta T}$$
(18)  

$$t = \frac{-\ln(1 - X)}{\kappa} e^{-\beta T}$$
(19)

From these equations and assuming a fixed cure fraction from the definition of a "fully cured" substance, a basic description of the process can be obtained based on the initial information. Using the data cited above, the following graphs were obtained for room temperature, 318 K, and 338 K.



**Figure 55 Curing Process at Room Temperature** 



Figure 56 Curing Process at 318 K



Figure 57 Curing Process at 338 K

If a second order reaction is assumed, the equation relating the remaining uncrosslinked compound with the time takes the form:

$$1 - X = \frac{1}{1 + \alpha t} \tag{20}$$

Where  $\alpha$  is a constant of the reaction that has the following time dependence:

$$\alpha = \gamma e^{\delta T} \qquad (21)$$

Where  $\gamma$  and  $\delta$  are appropriate constants for the process. Combining the last two equations, 20 and 21, the following expression can be obtained:

$$1 - X = \frac{1}{1 + \gamma e^{\delta^T t}}$$
(22)

From the above data, the following graph can be obtained:



Figure 58 Cured Fractions at Room Temperature as Time Progresses, Second Degree Kinetics

Comparing the two models, first and second order reactions respectively, it is noticeable that the total curing time doesn't change much, but the initial reaction rate does, being faster for a second-degree reaction, as expected. From observations of the curing process, the second model can be discarded in favor of the first-order-reaction model. For the given data, the second-order-reaction model predicts that the reaction is almost completed after 5 hours (>90% curing), which is much faster than what is observed, since PDMS still behaves like a fluid rather than a solid after 5 hours of drying.

Once the model was obtained, subsequent experiments yield better results. In order to produce a reproducible process, independent of thermal unsteady states that depend partially on the specific oven used, the half curing step was performed at room temperature, between 20 and 23 °C for the laboratory. In order to reduce the process time, using the already derived relationships, partial curing can be performed at higher temperatures as long as the unsteady state is taken into account too. Between 17 and 19 hours of curing at room temperature produced good results in terms of liquid not

flowing into the channels and adhesion, which is further analyzed in a subsequent section. In the subsequent analysis 18 hours is the standard time used, which correspond to an approximate 90% curing.



Figure 59 Example of the adjusted curing time for different temperatures. Top, using a first kinetics model, and bottom using a second degree kinetics. The Top one, first degree kinetic, is preferred since fits observations better.

Using the first order reaction graphs obtained from calibrations, it can be noticed that even at a relatively low temperature as 45°C, if the oven and glass thermal unsteady states are roughly half an hour, the remaining uncured fraction might change by a factor of 2, producing a PDMS layer that will flow into the channels or not adhere to the substrate. Thus, the reliability of a process depends highly on the unsteady thermal state as the temperature is increased, and is not likely to work if different ovens are used (as was done during the initial experimentation step).

After fully curing and debonding the PDMS layer, uniform film cross sections were verified on a Mitutoyo Micrometer Surftest 301, figure 60. The difference in the PDMS surface due to flow of the uncured polymer and full curing was smaller than 4 microns, smaller than the error from machining. An unexpected result is that the surface is not slightly higher, but slightly depressed. The depression could be the result of the difference in gas release efficiency by an open surface above the channel relative to a closed one. Therefore, the material adhered to the aluminium surface is less efficiently degassed, hence more "fluffy".



Figure 60 PDMS Surface After Debonding, Concentrator Zone

The same pattern as the one observed for the large cavity is seen in the smaller channels, but here, the step height is smaller. The height difference suggests a mechanism sensitive to the changes in channel dimensions as the responsible for the surface irregularities, and reinforces the hypothesis of a local PDMS depression in the channel zone due to gas release during drying.



Figure 61 PDMS Surface after Debondig, Zig Zag Channels Zone

### **Full Curing**

The main goal of the full curing step is to produce enhanced adhesion to hold the PDMS and the aluminium piece together, thus, it interacts with the previous step, which leaves an uncured fraction for adhesion. Once the maximum amount of possible uncured fraction is known from the previous process, the remaining unknown process parameters are the temperature and the pressure at which the process must be performed.

#### Temperature

Curing of the PDMS can be performed at room temperature, yielding higher adhesion strength, but the more time it takes to become fully solid, more material will flow into the channels. Also, the time for bonding should be minimized in order to speed the process.

During the curing process, PDMS has to remain below 180 °C to prevent the formation of formaldehydes, which sets an upper limit for the temperature. Also, as the

temperature is increased, degassing due to the blocked free surface becomes a problem and bubbles start to appear at the surface. Therefore, temperatures in this range are rarely used. Normally temperatures lower than 100 °C should produce fast enough curing.



Figure 62 Example of Bubble formed in the film

The temperature rise has as secondary effect: the creation of shear stresses due to thermal expansion. The materials used in the fabrication of the micro channels: aluminium, PMDS and borosilicate, have quite different thermal expansion coefficients. Thus, this difference has to be considered when dealing with the temperature increments during curing. For these materials, the reported values of the expansion coefficients are the following:

1/KAl23x10^-6Borosilicate3x10^-6PDMS<sup>16</sup>3.1x10^-4Table 4 Expansion Coefficients

After examination of these values, it is clear that PDMS is the material that experiences the maximum amount of expansion during a given curing process, and therefore stresses over its surfaces have to be considered. The adhesion force between the borosilicate and the PDMS is stronger than the adhesion force between the PDMS and the aluminum piece, since, for glass, covalent bonding provides stronger adhesion. Thus, the limiting factor is the adhesion of the PDMS to the aluminium piece. In addition to these factors, PDMS imposes an additional constraint: the uncured polymer experiences shrinking due to the evaporation of the curing agent. PDMS shrinkage, as the thermal stress, creates a shear stress on both faces of the cured PDMS. Given the order in which glass and aluminium are placed into contact with the PDMS, by the time that PDMS and the aluminium surface contact, almost all of the curing has occurred, and the shrinkage left is minimized. In a simple curing process, the shrinkage ratios of PDMS are "1.06, 1.52 and 1.94% for curing temperature of 65, 80 and 100°C, respectively"<sup>17</sup>.

Using the reported values for the shrinkage and the remaining curing fraction predicted by the model, a comparison of the influence of the shrinkage and the thermal expansion can be made. For a temperature difference of 100 °C and 10% uncured fraction, the linear shrinkage is roughly 0.2%, and the thermal expansion is 3.1%, therefore, for this temperature the predominant factor is thermal expansion. In general, and assuming shrinkage of 1% for temperatures lower than 65 °C, shrinkage does not become important till the temperature difference is smaller than 10 °C. As an alternative calculation, from the cited data it can be extrapolated that at room temperature the shrinkage reduces considerably, figure 63, and the thermal expansion effect remains the predominant factor even at temperature differences lower than 4°C.



Figure 63 Extrapolation of the Shrinkage Dependence VS Temperature

Initial bonding experiments at 100 °C produced unfavorable results. Just after curing, the pieces seemed to have bonded, but after cooling down, the pieces debonded, leaving just the central piece adhered. After cooling down, roughly 1/3 of the initial length remained bonded. Thus, knowing that the thermal expansion is the main factor in the process and, using the fact that thermal expansion is linear, an adequate temperature can be estimated. After performing such calculation, new trials were performed for temperatures below 50 °C; finally 35°C, curing for 2.5 hours, was selected for the last step of the curing process and new experiments produced positive results (up to 45 °C could be used, but would produce a less reliable process). As an optional process, in order to reduce the curing time, the initial curing temperature can be set higher, i.e. a ramping process, as long as most of the curing is realized at a lower temperature, or, the glass layer can be set at a higher temperature as the aluminium piece is maintained at a lower temperature.

Using the PDMS thickness; the Poisson ratio<sup>18</sup>, 0.5; and the Young Modulus<sup>19</sup>, an estimate of the order of magnitude of the failure stress can be obtained. Given that the film thickness is roughly 25 microns, and neglecting non linearities due to the large deformation, the thin film approximation can be used, giving roughly 30 kPa.

#### **Additional Factors**

In order to enhance the aluminium adhesion to the PDMS layer, experiments applying vacuum pressure during the final curing step were also performed. During them, 0.7 atm of vacuum were applied at different times during the curing process. As a side effect from the application of vacuum, bubbles were produced in the covered zones, as in the case of the pre-curing step when the temperature was increased. These bubbles had a dome like structure, indicating that most of them were nucleated in the proximities of the aluminium surface or the glass surface, and are the result of the accumulation of the gas by-product of the PDMS curing, figure 64.


**Figure 64 PDMS Bubble Structure** 

Even though the vacuum process seemed to improve the process adhesion, bubbles prevented the process from becoming reliable. Since the aluminium piece roughness traps small quantities of air upon contact with the PDMS layer, these surface imperfections become nucleation points for bubbles. As more and more gas is accumulated due to the curing process, it slowly diffuses creating air bubbles and eventually air channels, which appear when several air bubbles collide. The collision phenomenon is enhanced by scratches, machine tool marks and the non polished grain structure on the aluminium surface that provide close nucleation points. Eventually these nucleation points form bubbles that collide forming air channels that will produce imperfect bonding and leaking, figure 65.

Due to the formation of such bubble structures, the application of vacuum was abandoned. If further attempts of using vacuum to enhance adhesion are performed, a tighter control of the surface properties is required.



Figure 65 Air Channels Formation Process in PDMS

#### **Bonding Characterization**

In order to evaluate the process performance, visual inspection, leaking at 5 psi, and then bursting pressure tests were performed for the anodized aluminium piece.

Upon water filling at 3 psi, visual inspection of the channels was performed. Using an optical microscope, the following photographs were taken. The next images are from a 17 hours partially-cured and 45 °C fully cured piece. The images are shown because they represent the defects found during bonding, although are not present in most of the finally bonded pieces. As can be seen in the series of images of figure 66, bubbles were formed in the vicinity of the channel, colliding with the channel space once they had grown enough. The grain structure of the piece is visible upon polishing because the grain boundaries reflect the incoming light differently and the thickness of the aluminium oxide coating is different in these zones.



Figure 66 Bonding Defects Next to the Channels

The next image, figure 67, shows a bubble in one of the channels. In this picture, the coating layer of aluminium dioxide is more evident, as it surrounds the bubble. Given the channel roughness observed is evident that small bubbles will tend to accumulate in certain zones of the channels during actuation.



Figure 67 Bonding Defects Next to the Channels II

In order to perform the leaking test and visualize the liquid flowing through the channels, a solution of 1.25  $\mu$ M fluorescein was flowed through the closed channels. To take the photographs, a fluorescent microscope was used. As can be seen from the photograph, and its negative in figure 68, the channels do not present leakage. The only zones that present some illumination are the zones near the channel, where some grains are missing. Also, some grain boundaries that reflect the illumination light are seen since the optic filter is less effective with light not coming exactly parallel to the filter optical axis. This scattering effect is seen even when no fluorescent liquid is flowed through the channels and over the entire aluminium surface, with and without fluorescent die.



Figure 68 Fluorescein Leakage Test, Positive and Negative Image

In order to show the contrast with an unsealed channel, the following image of an improperly closed channel is presented, figure 69. In this case 2.5 micron green fluorescent beads were flown across this channel junction. The bright/dark spots are beads stuck in the aluminium surface, and the white/dark lines are beads flowing across the channel and the space between the aluminium and PDMS. Given the size of the beads, the space between the channels is at least 5 microns. Again, the grain structure is visible due to the reflected light.



Figure 69 Leakage after bonding

Finally, a pressure burst test was performed. Commonly leakage stops once the pressure has been balanced by the closed cavity, unless leakage starts between different lanes conducting fluid. If leakage between the channels and the vacuum cavity is severe, it will push apart the aluminium piece from the PDMS-glass substrate. As expected, the zones with smaller distance between the lanes were the ones prone to fail, zig-zags and the focusing zone. The bursting pressure for well adhered pieces was roughly 30 PSI, maximum pressure used in most micro fluidic devices, figure 70.

FAILURE PRESSURE FOR PDMS + GLASS (PSI). Aluminium not coated with enhanced adhesion chemicals	ZONE
34	OUTLET ZIG-ZAG
30	FOCUSING ZONE
30	WASHING ZIG-ZAG

Table 5 Failure Pressure For PDMS + Glass



Figure 70 Failure Pressure for PDMS + Glass Layer

Finally, the additional use of enhanced adhesion chemicals for PDMS, i.e. DOW CORNING P5200 and 1593 CLEANER/PRIMER, can make the process more reliable, but also produces an undesired layer inside the channels, and therefore it is not recommended unless additional strength is required.

# 3.2.3 Channel closing using polymer films.

Different kinds of polymer films are available commercially, but in order to be usable for sealing the channels, they must have the following properties:

- 1. Low fluorescence.
- 2. Good adhesion and confomality to the surface upon application.
- 3. High stiffness and non delamination during the application of pressure.

PDMS was used as a benchmark for fluorescence properties, but is unsuitable for the application in thin films due to the stiffness requirement.

### Fluorescence

Different commercially available adhesive-backed films were considered. We measured their optical properties and compared them to PDMS. The following films were considered.

FILM THICKNESS	mils"	microns
FLOATED BOROSILICATE GLASS, TELEC		
1mm	40	1016
PDMS (Sylgard 184)	0,94	24
PARAFILM M (AMERICAN NATIONAL CAN)	4	101,6
Optical Adhesive Cover (Applied Biosystems		
4311971)	4,5	114,3
GLASS+MicroAmp Clear Adhesive Films		
(Applied Biosystems 4306311)	9	228,6

GLASS+FEP FILM WITH ADHESIVE BACK	3,5	88,9
GLASS+SILICONE RUBBER SHEET WITH		
ADHESIVE BACK	20	508
GLASS+STATIC CLING PVC (TYPE 1) FILM	2	50,8
GLASS+ADHESIVE BACKED POLYESTER		
(PET) FILM	2	50,8
SCOTCH TAPE	2,5	63,5
MAGICTAPE	4,5	114,3

#### **Table 6 Film Thickness**

In order to measure the fluorescent properties of the adhesive backed films, a Nikon Eclipse TE2000 U Inverted Fluorescent Microscope with blue excitation and green excitation light and a Hamamatsu Camera Model C4742-95-12NR were used. The sample intensity sensed by the camera is an arbitrary measurement; but along with the measurement of the intensity of a common fluorescent liquid, fluorescein, provides an adequate standard to compare the luminicence of the films relative to that of a selected arbitrary staining method. For that purpose, measurements of fluorescein for 25-ms exposure were taken for different fluorescein concentrations.



Figure 71 Calibration Curve for Nikon Microscope

As can be seen from figure 72, the camera reaches a saturation point around 2  $\mu$ M, and therefore a lower concentration must be used to perform any measurements.

Also, for concentrations below 1  $\mu$ M, the relationship between the concentration and intensity is almost linear.

Most of the films showed a significant fluorescence, as can be seen in the complete set of florescence graphs in the Appendix. Here, only the ones containing the Optical Adhesive Cover (OAC) used for RNA fluorescence tests are shown, since it showed the best performance. Since the intensity may depend on the sensibility of the system to the exposure time, the measurements were performed for different exposition times.



Figure 72 Film Fluorescent Intensity Relative to Vacuum, Blue Excitation



Figure 73 Film Fluorescent Intensity Relative to Vacuum, Green Excitation

From the figures 73 and 74, it can be seen that the intensity for the highly fluorescent samples follows an exponential curve in the log scale shown, and therefore shows direct proportionality to the incoming light and exposure time. Thus, the ratio of intensities should be relatively independent of the exposure time for the fluorescent samples. Although, for the low fluorescence samples, the intensity remains almost at constant level and then rises slightly, making it hard to fit properly to a model.

As expected, the PDMS shows relatively low levels of fluorescence. The fluorescence intensity values for the PDMS-glass layer are practically the same as those for the bare glass piece in the case of green excitation, and low for the blue excitation. The only other film that had a similar performance, slightly better for blue illumination, was the Optical Adhesive Cover (OAC).

In the figures, for illustrative purposes, the intensity of a random dust particle is shown. The particle is relatively non fluorescent; even though, a good contrast is achieved for all exposure intervals is obtained if the glass-PDMS substrate or the OAC is used as a substrate. In order to show the dependence of the contrast vs. the exposure time of the system, the parameter of interest in most systems, the following graphs were obtained.



Figure 74 Contrast VS Exposure Time, Blue Excitation



Figure 75 Contrast VS Exposure Time, Green Excitation

As can be seen from the figures 75 and 76, the contrast is nearly constant for low exposure times, and improves for long exposure times. The slight contrast decrease that is observed for low exposure times is due to the slight increase in fluorescence level of the PDMS and OAC, and is at least partially due to the expected measurement error for low intensities.

After the initial testing, full absorption emission and emission spectrums were obtained for the films. Spectra for all the analyzed materials are shown in the Appendix. The OAC was selected for closing the channels based on its low fluorescence, and then was tested for mechanical properties.

# Adhesion



Figure 76 Leak Test using food colorant

To measure adhesion, a bursting pressure test was performed. The film was initially adhered applying pressure with the fingers, which produced a local pressure between 1 to 7.5 atm. Although high pressure was locally applied, checking for nonadhered parts had to be performed constantly to avoid non adhered zones. Next, pressure was applied. As can be seen from the figure 78, "Pressure Failure for Optic Film", initially the failure pressure is larger, but then decreases slowly due to the accumulation of adhesive residues, which eventually degrade the adhesion to the surface. The failure pressure stabilizes at roughly three quarters of the initial value, 15 PSI. The decreasing adhesion strength phenomenon can be observed more clearly in the graph for Static Cling Film (SCF), second to the OAC in fluorescence performance, in the Appendix Section.



Figure 77 Failure Pressure for OAC, bare hands bonding

From these trials, it can be observed that the failure zones are stress concentration zones, as expected. The failure occurs in regions of large channel area is or where channels join together. The OAC film exhibited slightly less adhesion force than other films normally used for covering plates.

FAILURE PRESSURE FOR OPTIC FILM (PSI). FORMATION OF A BUBBLE FOLLOWED BY SUDDEN DEBONDING	ZONE
20	CONCENTRATOR
16	FOCUSING
25	CONCENTRATOR
18	CONCENTRATOR
17	CONCENTRATOR

**Table 7 Zones of Failure Pressure for OAC** 

The failure pressure of the film can be compared with the higher obtained for BS film and shown in the Appendix.

In order to increase the values of pressure failure of the plastic film, two methods were used: a) vacuum enhanced adhesion and b) uniform pressure preloading.

### A) Vacuum Enhanced Adhesion

In order to improve adhesion, vacuum was used after initial bonding, deforming the film near the vacuum cavity that had been designed into our device for "Vacuum Chuck Bonding to PDMS Gaskets" in chapter 2. Vacuum applied extra pressure just where the film was not completely bonded; making the sealing more uniform and reliable, but did not increase the bursting pressure noticeably.



Figure 78 Vacuum Enhanced Adhesion Process

#### B) Uniform Pressure Loading

As an alternative to vacuum pressure application, upon initial contact with the aluminium piece, uniform pressure was applied mechanically over the film to increase bond strength. As the loading pressure was increased, the failure pressure increased too, as can be seen for the shown figures for 1 and 5 atm of loading pressure.



Figure 79 Uniform Pressure Enhanced Adhesion Process

For 1 atm, the film resisted more than with the application of a higher local pressure with the bare hands. This effect is due to the improved uniformity of the bonding, although no uniformly adhered zones were still present. The failure zones were located again where geometrical stress concentration zones are present, i.e. the focusing zone, or where the film had a smaller contact area, e.g. the zig-zags of the washing lane.



Figure 80 Failure Pressure for 1 atm Pressure Enhanced Adhesion



For 4.5 atm of external bonding pressure, the film didn't debond at the maximum internal channel measurable pressure, 35 PSI. This is enough for most microfluidic applications; but, by the time the maximum pressure was applied, the film had already suffered deformation that enlarged the channel dimensions, more than 30%. However, all the plastic film was completely uniformly adhered to the aluminium surface. After the film removal, few small fragments of the adhesive part of the film layer remained adhered to the aluminium surface, and removal of them was necessary before each trial.

FAILURE PRESSURE FOR OPTIC FILM (PSI), 90 KG				
LOAD DURING BONDING, 4.5 atm				
>35				
>35				
>35				
* uniform bonding				

Table 8 Pressure for 1 atm Pressure Enhanced Adhesion

### Deformation

After the application of external pressure the OAC deforms considerably. By the time pressure reaches six psi, the height of the concentrator channel is increased by 16 microns, and becomes more important than the machining errors. This appreciably changes the device flow patterns and is not acceptable. Even more significantly, some of the deformation is non elastic, and using high pressure will permanently affect further performance of the channels when cycled back at lower pressures. In the next graph, the maximum height increment of the concentrator zone versus the pressure applied is shown, along with a cross section of the concentrator obtained with the Mitutoyo micrometer Surftest 301.



Figure 81 Maximum Deformation for Concentrator Channel

In order to compensate for the deformation, the film was backed with a piece of glass borosilicate that prevented long range deformations. An additional benefit from backing the film with a glass layer is the enhance range of pressures before film separation. After glass backing, for the first process, vacuum enhanced adhesion, separation occurred between 30 and 35 psi, and for the second one, pressure loading at 4.5 atm, separation occurred at pressures higher than 35 psi.

### **Bonding Characterization**

In order to evaluate the process performance, leak inspection was performed using the OAC film adhered to the non anodized aluminium piece using a bonding pressure of 4.5 atm. Testing was by visual inspection using 1.25 uM fluorescein pumped at 3 PSI. Inspection was done using a Nikon TE2000 inverted fluorescent microscope. As it can be seen in the series of images no major leakage is observed. Some zones with small scratches around the channels seem to have higher intensities, but the effect disappears after a short a distance from the channel.



Figure 82 Fluorescein Leak Test, Outlet Zig-Zag and Outlet Port

# Additional Considerations

Since the vendor was uncooperative in supplying even rudimentary information about the film, short series of forensics were performed to identify the polymers` composition. An estimation of the Young Modulus, and measurement of the absorption and emission spectrums were performed.

Although the film was too thick for the thin film approximation to be used, the geometry of the concentration zone could be used to perform a Bulge Test20 to get an approximation of the order of magnitude of the film's Young Modulus. In any case, since the OAC film is likely a compound layer where the adhesive layer is a soft polymer, even if the exact solution is used, the calculated Young modulus would be an approximation too. Usually, if the adhesive layer is present, the layer is about 25 microns, one quarter of the total thickness; therefore, a calculation neglecting this soft layer would give the right order of magnitude.

The Youngs modulus estimation of the film can be obtained using nondimensional analysis and matching the local first derivative of the model to that of the linear approximation of the data around the same point. The equation obtained is:

$$\Delta P \sim 2Eth/a^3 * \Delta h \qquad (23)$$

Where:

ΔP	is t	he	Pressure	Change
----	------	----	----------	--------

- $\Delta h$  is the Film Height Change
- t is the Film Thickness
- a is the Concentrator Channel Width
- E is the Youngs modulus

After substitution, the Young modulus was estimated to be 3 GPa. Therefore, polystyrene or COC (cyclic olefin copolymer) are both consistent since their Youngs moduli is similar as the unknown OAC film.



Figure 83 Linearization of the Pressure VS Maximum Displacement Relationship

After comparison of the measured transmission spectrum of polystyrene and the OAC film to that reported of COC and Polystyrene, COC, figure 87, is considered to be most likely material composing the Optic Film.



Polystyrene Transmission Spectrum

OAC Transmission Spectrum



Figure 84 Transmission Spectrum<sup>21</sup>

# 3.3 Summary

In summary, after experimenting with partially-cured PDMS gaskets, we successfully developed a bonding process and characterized its adhesion. We found that the curing conditions were difficult to control, and time consuming. An alternative method of channel sealing using pressure sensitive adhesive on, putatively, COC films was successful.

# **CHAPTER FOUR**

# **SWITCH OPERATION**



Figure 85 Microfluidics is a hippie field too...

# 4. - Switch Operation

#### 4.1 **Pre-operation Problems**

To operate the device, it first has to be fully filled with a pre-filtered and degassed liquid. First, the zones prone to accumulate bubbles are filled, the focusing zone and concentrator. Then, pressure is increased up to 30 PSI to make the bubbles leave the fluid circuit. Next, the previously filled fluid lines leading to the valves are connected and operated at different frequencies. Normally, bubbles are hard to evacuate from the channels; if bubbles remain, without disconnecting the valves, the cycle is repeated. Bubbles also shed from the input ports, where they accumulate in the flat bottom ports, so flowing extra time after no bubbles are observed is advisable. The bubble problem is in part created by the grain structure of the aluminium surface, figure 89, which created specific zones prone to accumulate bubbles, especially when one grain was lost next to the channel surface.



Figure 86 Grain Structure Defects that made the channels prone to bubble clogging.



Figure 87 Bubble in Concentrator, place prone to bubble accumulation.

In the next images, figure 91, bubbles in the inlet and outlet ports are shown, bubbles at the outlet ports are of no concern as long as the flow is not reversed. Bubbles in the ports are common in many microfluidic devices, and is associated more with the geometry of the port rather than the fabrication process.



Inlet Port



Figure 88 Bubbles at Inlet and Outlet Ports

Normally the bubbles are hard to observe using fluorescent light, and visual inspection is preferable. For the oxidized aluminium piece, due to the enhanced contrast, bubbles can be seen with naked eyes, whereas, for the bare aluminium piece a microscope was used with reflected light. This characteristic made it hard to distinguish between circuit clogging due to the bubbles and other kinds of malfunctions.



 Reflected Light
 Fluorescent Light

 Figure 89 Bubble Images Using Reflected and Fluorescent Light

# 4.2 Concentrator

Within the concentrator, bubbles experienced a lower drag force due to the lower velocity, thirty times smaller. Therefore this cavity was prone to bubble accumulation. Bubble accumulation prevented the proper function of the concentrator design most of the time.



**Figure 90 Flow in Concentrator** 

For the maximum velocity of 5 mm/s, the Reynolds number in the cavity is 0.01. At this Reynolds number, the flow still exhibited a non-rotational behaviour, as can be seen from the figure 93. At higher velocities, images of the particles were hard to obtain, and the flow was no longer compatible with the function of the device, switching. Some differences from the simulation are due to a miscalculation of the Reynolds number when handling the transformation to a 2D simulation. The use of this

rotational flow pattern for cell concentration is still a possibility, but it would require the use of higher velocities than we used in this design.

# 4.3 Flow Focusing

Due to the fabrication errors during fabrication of the first fabrication iteration, a lower degree of focusing was achieved by 20-30% relative to the design.



Figure 91 Flow Focusing without compensation

For the first fabrication iteration, the error in the dimensions was in the order of 50%, as shown in figure 95, but, since what is important for focusing is the resistance ratio, not the absolute value, the resistance error was slightly smaller, around 40%, as shown in figure 96. This error produced the difference in hydrodynamic focusing. For the fabricated ratio, 1:6 instead of 1:9, the focusing should be 40 % instead of the expected 30%, which agrees with the observed results.

	DIMENSIONS:	Microns		
CHANNELS	AIMED	OBTAINED	ERROR %	ABSERROR
BUFFER	500	506	1,2	1,2
	150	181	20,6666667	20,6666667
INLET	150	233	55,3333333	55,3333333
	50	75	50	50
FOCUSED INLET	150	220	46,6666667	46,6666667
	50	68	36	36
CONTROL FLOW IN	250	266	6,4	6,4
	75	94	25,3333333	25,3333333
WASTE	250	233	-6,8	6,8
	75	68	-9,33333333	9,33333333
OUTLET	150	222	48	48
	50	77	54	54
WASHINGLINE	150	227	51,3333333	51,3333333
	50	71	42	42
OUTLET VALVE	500	511	2,2	2,2
CELL TRAPZ	100	94	-6	6

Table 9 Error in the Machined Pieces Dimension, First Iteration

	FA	BRICATED		
	DESIGNE	Ð		%ERROR
	R1	2,2	1,57	40,1
-	R2	20	14,3	40,1
	R3	1	1	
	R4	8,5	6,07	40
	R7	1	1	
	R8	1	1	
VALVEOFF	lin	0,31	0,4	21
	lout	0,04	0,06	41,2
	11	0,03	0,04	20,9
	12	0,31	0,38	17,7
	lin/Itotal	0,9	0,9	0,01
	lout/Itot	0,11	0,14	25,6
VALVEON	Itotal	0,88	0,95	7,84
	Vo	0,42	0,44	5,79
	lín	0,27	0,36	25,4
	lout	0,05	0,07	32,7
	11	0,03	0,04	25,3
	12	0,42	0,44	5,79
	17	0,58	0,56	4,56
	18	0,42	0,44	5,79

Table 10 Error in the Resistance Obtained, First Iteration

In order to compensate and achieve stronger focusing, a higher pressure can be supplied to the buffering lane, as shown in the next images, figure 47.



Figure 92 Pressure Adjusted Flow Focusing. The pressure applied to the sample lane was 2.5 PSI. Pressures applied to the buffer lines are increased approximately every 0.5 PSI, limit of the pressure gauge resolution, above the sample-lane pressure.



Figure 93 Flow Focusing Stream Lines

For the second fabrication iteration, the problem was reduced, and the resistance error was smaller, as it can be seen in figure 99.

	DIMENSIONS:	Microns		
CHANNELS	AIMED	OBTAINED	ERROR %	ABSERROR
BUFFER	500	495	-1	1
	150	164,705882	9,80392157	9,80392157
INLET	150	165	10	10
	50	41,1764706	-17,6470588	17,6470588
FOCUSED INLET	150	148	-1,33333333	1,33333333
	50	41,1764706	-17,6470588	17,6470588
CONTROL FLOW IN	250	240	-4	4
	75	60,0490196	-19,9346405	19,9346405
WASTE	250	231	-7,6	7,6
	75	68,627451	-8,49673203	8,49673203
OUTLET	150	165	10	10
	50	51,4705882	2,94117647	2,94117647
WASHINGLINE	150	165	10	10
	50	51,4705882	2,94117647	2,94117647
OUTLET VALVE	500	495	-1	1
CELL TRAPZ	100	90,9313725	-9,06862745	9,06862745

Table 11 Second Iteration Aluminium Piece, Not anodized

FABRICAT	ſED		
DESIGNED	)		%ERROR
R1	2,2	2.4	9
R2	20	25.8	26
R3	1	1	
R4	8,5	11.05	29
R7	1	1	
R8	1	1	

Table 12 Resistance Ratio for the Second Iteration Aluminium Piece

For this second iteration, the focusing was better because in general the dimensions were smaller than designed, and since the relationship between the resistances is nonlinear, the resistance ratios were larger. For this piece the flow ratio was approximately 1:9, which increased the focusing to roughly 25%, as it can be seen in figure 101, where fluorescein is flown through the sample port. The width of the focused liquid stream is in agreement with the value predicted by the model (28%).



Figure 94Hydrodynamic Focusing using fluorescein, second chip closed using the OAC layer. The first image was taken just after the focusing zone. The second image is from a point after the concentrator, where the geometry increased the diffusion length, and thus decreased the focusing.



Figure 95 Hydrodynamic Focusing using food colorant, second fabrication iteration closed using OAC. The images show the focusing zone. Here, the vertical focusing is more evident than with the fluorescent dye.



Figure 96 Hydrodynamic Focusing using food colorant, second fabrication iteration closed using OAC. Zones where the closing adhesive did not adhere properly can be seen as slightly green areas next to the channel.

### 4.4 Switching

The geometry for switching was quite different from the design. To ensure connection, a critical requirement, the washing lane was joined to the collection lane, which had a smaller cross section, creating a depressed zone. This flow crossing created a zone prone to bubble accumulation. As a secondary affect, the particles crossing this area had a reduced velocity. Both effects created an irregular flow within this area. Also, the milling process did not create a sharp edge between the waste and collection lane. Instead of a sharp edge, a 200-micron-radius-of.curvature rounded shape was formed.



Figure 97 Switching Zone Geometry

During the actuation, images of the 33% focused fluorescein liquid were acquired with an ORCA Hamamatsu Camera. The images were acquired stroboscopically for different actuation frequencies, from 1 Hz up to 48 Hz. In the next images, actuation at 10 and 48 Hz is presented. As can be seen from the images, the flow ratio between the waste and the collection lane is insufficient to cause total flow switch between the collection and waste lanes.



Switch about to be activated, due to the reduced velocity, some fluorescein is still present in the channel





Fluorescein is switched into the selection channel, the intensity of the flow between the sample and collection lane is noticeable





As the fluid enters the crossing between the washing and collection lane, it goes into a larger cross sectional area that decreases the flow velocity and lets the fluorescein accumulate

As the switch is deactivated, the accumulated fluorescein leaves the crossing and the fluid circulates into the waste lane again.







Figure 98 Switching at 10 Hz. As it can be seen in the images, the flow ratio is insufficient to cause the total switch of the flow into the collection lane.



Figure 99 Switching at 48 Hz, as can be seen, the actuation was greatly minimized compared to 10 Hz. Actuation at higher frequencies was limited by the unsteady state behaviour of the liquid. Also, in order to make the system work at higher frequencies, the flow velocity had to be increased.

Next, HeLa cells were flown through the channel, as it can be seen in the following images. In the case of the HeLa cells, stained with Sytox Orange, the contrast was good for a 25 ms exposure time. In the images, the cells appear as lines in the central focused region, roughly 1/3 of the channel.



Figure 100 HeLa cells flowing through the sample lane.

The images of figure 108 show the flow of cells when the switch is deactivated. The first image shows cells flow when 3 PSI where applied. The second image shows cells at a higher velocity and pressure, 6 PSI. As the velocity increases, the inertia of the cells makes them take tighter turns, and makes them easier to switch.



Figure 101 Cells flowing through the channel with the switch off.

The images of figure 109 show cells selected at 10 Hz. As the switching time was random with respect to the position of the cells down the flow channel, the cells ended up in different zones of the collection channel. Here, the undesired effects of the flow crossing can be appreciated. As can be seen form the image, the cells tend to accumulate in the crossing next to the wall and to the washing lane.



Figure 102 Superposition of images of cells selected when flowing through the channel

Switching at higher velocities and visualization was hard even after increasing the flow velocity due to the exponential behaviour of the average velocity. As can be seen from the figure 110, because the exponential decay of the velocity is slower than the flow acceleration on valve opening, the average velocity increases overtime. Thus, if the valve half on is activated at a higher frequency than 48 Hz, the switching is replaced by a steady state after a small number of cycles.



Figure 103 Exponential behaviour of the average velocity

In order to characterize the switching performance of the valve, the valve was activated at different frequencies as fluorescein was flown through the sample port, and the fluorescein concentration from the collection and waste lanes were measured.

First, the Hamamatsu camera was calibrated to measure the intensity of different concentration fluorescein samples, figure 111. In order to have reliable measurements, the sample concentration flown has to be smaller than 1.25  $\mu$ M. For smaller concentrations a linear relationship can be used for relating concentration to fluoresce intensity.

An additional factor to consider is the possible error due to photobleaching. As can be observed from the following graph, figure 111, photobleaching can have a significant effect, especially for higher concentrations. The intensity error can be as high as 15% for the higher concentrations.

When extrapolating the concentration from the intensity in the nonlinear region, of high concentrations, the intensity variation due to photo bleaching can lead to concentration errors in the order of 25%. In the linear region, the intensity error has the same approximate magnitude as the extrapolated concentration, roughly 15%.



Figure 104 Effect of photo bleaching in the light intensity

In figures 112 and 113, the concentration obtained for the waste and collection lanes is presented. During this process, 1.25-micromolar fluorescein is flown through the sample channel; the required dilution will come from the buffer, which will decrease the intensity to the linear zone, maintaining a large signal.



**Figure 105 Frequency VS collection Intensity** 



Figure 106 Frequency VS Waste Intensity

As can be seen from figure 112, as the frequency is increased, the efficiency of the switch increases. This effect is due to the increased pressure and thus velocity used for the valve actuation. This higher pressure was needed for adequate actuation, as observed before for the particles. In the graph, the sample pressure was increased from 1.5 psi to 3 psi. Due to the increased inertia, the flow is switched more easily to the collection lane. After the initial increment of the flow switched, the flow fraction switched dropped before the unset of the transient state of valve operation. This drop is consistent with the reduced open time, which is comparable with the on-set of flow at high pressure oscillation rates. On-set is preventing switching times of less than 18 ms.

From the data, the final concentration for a given sample can be obtained. For a given concentration, the final purified concentration will be around 1/40 of the original concentration within the collected volume.

# 4.5 Channels after Debonding

Once the channels were clogged, either with the sample or particles not removed during filtering, the channels were opened and cleaned. The cleaning process starts with flowing distilled water when possible, followed by opening of the channels. Next, the channels were sonicated using filtered water for 20-30 minutes. After this, the channels were cleaned using a bleach solution, 1:5, followed by 10 minutes of sonication in
distilled water. If particles remained, the process was repeated or the remaining particles were removed using a sharpened plastic straw. The use of metals should be avoided since it can scratch the surface, in any case, the Moss Hardness Scale must be consulted first.

After the cleaning process, almost no particles remained except when adhesion chemicals were used, as explained in the fabrication chapter; it left a thin film over the channel surface, which caused irregular flow in the channels and increased the particle shedding when cleaning the aluminium piece.



#### Figure 107 Aluminium Piece being Sonicated



**Figure 108 Clogging in the Channels** 

109

# **CHAPTER FIVE**

# DISCUSSION AND CONCLUSIONS



Figure 109 The Lab after cleaning, or it was before?

# 5 Discussion and Conclusions

#### 5.1 Fabrication

Our objective was to develop a de-bondable fabrication process which could allow a microfluidic part to be classically micro-milled (e.g. into aluminum), then bonded with a low fluorescence window. We tested parts with and without anodization and tried three different approaches to reversible bonding.

The anodization step created many irregularities over the surface, but also provided a harder surface with higher optical contrast. In order to decrease the irregularities, anodization needs to be introduced at the correct order in the fabrication sequence. If an anodization of up to 200 microns thickness step is performed after the flywheel cut followed by sanding and polishing, the irregularities during milling should de diminished. Any anodization causes some enhancement of grain boundaries, and the best procedure is probably to avoid anodization and use an alternative blackening method if required.

The use of square channels, created by micromachining as opposed to isotropic etching, made the analysis and design of the device easier, but at the same time, did not eliminate zones of low velocity and pressure loss at the channel junctions. Semicircular channel cross section, may reduce the creation of separated flow and loss of velocity, and may decrease the accumulation of undesired particles and bubbles at low-flow regions. Additionally, the crossing of channels of different width should be avoided, and channel extensions should be used between the channels of smaller width and those of larger width. This eliminates tool changing in the machining step, which was found to be the largest contributor to machining errors. If only semicircular channels are used, no compensation due to the friction factor is needed.

The bonding process using PDMS was proven successful, resisting up to 30 psi, but time consuming and difficult to control. As an alternative, sealing techniques using adhesive backed polymers were tested. In order for the film to adhere, the roughness has to be small as possible, but 0.6 micron RMS should suffice. Finally a 4.5 psi precompressed AOC film was shown to be successful, withstanding up to 34 psi. The AOC film reduced the bonding time to a couple of minutes, but needed a back support to reduce deformation.

Based on the previous observations, the fabrication process can be redesigned as shown next in figure 117.



backing piece of glass

**Figure 110 Fabrication Process** 

#### 5.2 Design

#### **Inlet/Outlet Ports**

The flat-bottom ports, created by the end mill, were a constant reservoir of bubbles during operation. Flat bottom ports could have practically no dead volume as long as they are precisely aligned to the hole inside the cavity, but the alignment was not precise in many channels, and large dead volume was observed frequently. It would be possible to replace the flat bottom ports with conical ports, which self align and have a constant dead volume. Also, the diameter of the inlet ports could be decreased to 500 microns, half of the current value, which would increase the flow speed and would facilitate the evacuation of bubbles.

#### **Flow Focusing**

Stronger flow focusing using two independent lanes for the buffer would give a more flexible design. This would reduce the accumulation of particles and bubbles, and decrease the filling time. A stronger focusing, with at least an order of magnitude larger flow ratio, would decrease the requirements on the pressure pulse rise time, and would reduce the actuation time. Additionally, most of the focusing should be done using length to obtain the resistance ratio. In this way, the flow should be much more reliable than balancing with different pressure sources. Using the model developed in chapter 2, a curve for predicting the needed flow ratio was obtained, figure 118. Accordingly to this graph, a flow ratio of 1 to 70 or 1 to 90 should be enough to center the 10-20 micron cells in a line at the center of the channel.



Figure 111 Focusing for different flow ratios

#### Switching

The switching circuit can be modified to obtain a lower time response, using the optional circuit described in figure 119. In this design, the Lee valves can be used to change the path of the particle. This approach requires a smaller displacement of the particle and thus, permits a faster reaction time. In this improved design, in order to protect the valves, filters can be added before the valves or the pressure can be reversed during the second part of actuation. This change should reduce the net amount of flow between the valves to near zero.



**Figure 112 Alternative Flow circuits** 

For both circuits, the resistances rv should be minimized as possible to decrease the flow resistance and achieve a faster final flow speed. The pressure source should include a pre-focusing step as discussed before. The main part of the circuit, i.e. the resistances shown in the diagram, should be machined with the same cross section as much as possible, to decrease the error during fabrication.

#### **Resistance Ratio**

Now that a reliable high performance reversible bonding process has been found, a higher channel density can be used in future designs. For the aluminum pieces, a distance of four times the channel width between the centers of the channels was used in our studies here. Accordingly to what was observed, this distance can be decreased to roughly three times the channel width, but no much further, because those areas will be prone to failure and leakage. The resistance ratio between collection and waste should be increased by at least a factor of 10 to increase the final concentration of the sample.

Based on the observations, the following designed rules can be recommended:

## Design Rules

1. - Minimize the number of different cross sections used; whenever possible, use length to obtain the resistances ratio required; this will decrease the error during fabrication.

2. - Don't cross channels of different cross sections unless necessary; crossings will create zones of reduced speed and pressure drop that increase bubble clogging. Consider using semicircular cross sections instead of rectangular ones to reduce junction dead zones.

3. - Do not directly connect channels of different cross sections, use extensions from the smaller cross section to the larger cross section.

4. - Separate the channels leaving at least three times the channel diameter between center lines.

5. - Use conical input ports; this will create self aligning ports that will help decrease the trapped volume at ports.

6. - Use 0.5 mm or smaller input holes, this will decrease the volume of air trapped.

7. - Before adding extra ports, think of the way bubbles will escape the channels and design accordingly.

8. - When joining a large cavity to the rest of the channels, do it gradually, otherwise bubbles will clog near to the cross section discontinuity.

#### 5.3 Future Applications for Cell Sorting

Initial cell switching was successful, as shown in the images acquired in section 4. The florescent light from the cells was collected using a photomultiplier as shown in chapter 2.

The signal from the PMT was weak and filtering and amplification was needed in order to obtain sufficient signal for switching. The filtering stage consisted of a differentiator that sampled the increase of signal over time, and the amplification was a variable 1000 gain amplification. For this signal acquisition scheme, the alignment of the optics to obtain a reliable signal proved to be achievable but difficult, and an alternative method such as scattered laser light should be investigated.

This selection method could provide an easy to fabricate method for large cells, as 200 microns wide cardiocytes, because, unlike clean room methods, is easy to scale up.

#### 5.4 Laser Actuation

Laser remains as an alternative for faster actuation, sub-millisecond range. For light interaction, the anodization step is required. This layer of oxide enhances absorption, and decreases immediate heat conduction to the substrate, till the heat wave reaches the aluminium substrate. The absorption coefficient of light for the aluminium oxide in the visible spectrum is in the order of 14%. Once the surface is heated, roughly 40% of the heat is transferred to the water above the channel, and 60% is conducted. From the literature, an estimate of the order of magnitude of the laser power can be performed. From figure 120, for a 5-10 ms bubble, a laser of 200 mW is needed. As the actuation time decreases, the power required for the laser increases, and for a couple of ms, a laser of roughly 1-2 W is needed.

Author	Paper	length (nm)	Pulse Time (ns)	Energy (uJ)	Diameter (bubble) (um)	Power Density (uW/um^2)	Energy Density (uJ/um^2)
Daniel Palanker, Igor Turovets, Aaron Lewis	Dynamics of ArF Excimer Laser- induced Cavitation Bubbles in Gel Surrounded by a Liquid Medium <sup>22</sup>	193 nm	200 ns	30-300	250	0.0025	0.0005- 0.004
Igor turovets, Daniel Palanker, Yu Kokotov, Itzhak Hemo, Aaron Lewis	Dynamics of cavitation bubble induced by 193 nm ArF excimer laser in concentrated sodium chloride solutions <sup>23</sup>	193	200ns- 30 us	50~350	50-250		0.00072 (min theo) 0.0018
HanQun Shangguan, Lee W. Casperson, Alan Shearin, Dennis Paisley, Scott A. Prahl	Effects of Material Properties on Laser- induced Bubble Formation in Absorbing Liquids and On Submerged Targets <sup>24</sup>						
C. C. Chen, J. S. Wang, O. Solgaard	Micromachined bubble-jet cell sorter with multiple operation modes <sup>25</sup>	577	1.3 us	<u>50 000</u> 2.5-91	2000	0.4	9000
Tudor N. Buican, Miriam J. Smyth, Harry Crissman, Gary Salzman, Carleton Steward, Jhon Martin	Automated Single Cell Manipulation and sorting by light trapping <sup>26</sup>						

 TABLE 13 Bubble actuated Sorters

### FLUID MECHANICS BASIC THEORY

#### Flow Resistance in a tube

When a liquid moves inside a tube and the flow reaches steady state, the pressure drop across the length of the tube can be linearly related to the flow through it. The resistance of the fluid to move in a circular pipe is proportional to the length of the channel, inversely proportional to the fluid's viscosity and inversely related to the radius of the tube. This relation is expressed precisely for circular tubes by the Poiseuille's Law:

$$\Phi = \frac{dV}{dt} = v\pi R^2 = \frac{\pi R^4}{8\eta} \left(\frac{-\Delta P}{\Delta x}\right) = \frac{\pi R^4}{8\eta} \frac{|\Delta P|}{L} \quad (A1)$$

Where:

- $\Phi$  is the volumetric flow
- $\eta$  is the viscosity
- $\Delta P$  is the pressure drop across the tube
- L is the total length of the tube
- R is the radius of the tube

The specific geometrical dependence on the cross section for non circular channels is most of the time hard to calculate exactly; for these cases, an equivalent circular cross section can be used instead. The equivalent circumference must preserve the inertia to viscous force ratios, which is proportional to the area and perimeter respectively, therefore:

$$Req=2*A/P$$
 (A2)

Where:

<b>n</b>	1 - 41		A	ma diana
Keq	is the	equival	lent	radius

A is the cross section area

P is the perimeter of the cross section

### Unsteady Flow through a Tube

For the case of a tube with a difference of pressure applied between the extremes, the unsteady equations describing the behavior of the system can be obtained solving the Navier-Stokes equations applying the appropriate boundary conditions (unidirectional flow).

From the Navier-Stokes Equation

$$\frac{\partial v}{\partial t} + \left(\bar{v} \cdot \nabla\right)\bar{v} = -\frac{1}{\rho}\nabla P + \frac{\mu}{\rho}\nabla^2 \bar{v} *$$
(A3)

And the continuity equation:

$$\nabla \cdot v = 0 \qquad (A4)$$

Now, in index notation the NS equation can be simplified as:

$$\frac{\partial v_i}{\partial t} + v_j \frac{\partial v_j}{\partial x_j} = -\frac{1}{\rho} \frac{\partial P_i}{\partial x_i} + \frac{\mu}{\rho} \frac{\partial^2 v_j}{\partial x_j^2} \qquad (A5)$$

Now, since the velocity in the in the x and y directions are null due to the assumption of unidirectional flow plugged into the continuity equation and that the profile in the x direction doesn't change:  $\frac{\partial v_j}{\partial x_i} = 0$ , the NS equations are:

$$\frac{\partial v_i}{\partial t} = -\frac{1}{\rho} \frac{\partial P_i}{\partial x_i} + \frac{\mu}{\rho} \frac{\partial^2 v_i}{\partial x_i^2} \qquad (A6)$$

Also, since the flow is unidirectional,  $\frac{\partial v_i}{\partial t} = 0$ , therefore, the equation for the  $i \neq 3$ 

first two coordinates is null.

$$\frac{\partial v_z}{\partial t} = -\frac{1}{\rho} \frac{\partial P_z}{\partial x_z} + \frac{\mu}{\rho} \left( \frac{\partial^2 v_z}{\partial x^2} + \frac{\partial^2 v_z}{\partial y^2} + \frac{\partial^2 v_z}{\partial z^2} \right) \quad (A7).$$

But, again, since the flow profile doesn't change in the x direction

$$\frac{\partial v_z}{\partial t} = -\frac{1}{\rho} \frac{\partial P_z}{\partial x_z} + \frac{\mu}{\rho} \left( \frac{\partial^2 v_z}{\partial x^2} + \frac{\partial^2 v_z}{\partial y^2} \right) \quad (A8)$$

When a pressure difference is applied across a small long channel, the lubrication approximation is valid and the pressure gradient can be considered as constant.

$$\nabla P = \frac{\Delta P}{L} \tag{A9}$$

Now, since the geometry is complex and could change due to fabrication process, let's analyze the problem using an equivalent radius for the cross section.

$$r' = 2A/P = \frac{2ab}{2(a+b)} = \frac{ab}{(a+b)}$$
 (A10)

Now, considering the reduced equations in circular coordinates:

$$\frac{\partial v_z(r,t)}{\partial t} = k + \upsilon \left( \frac{1}{r} \frac{\partial}{\partial r} r \frac{\partial v_z(r,t)}{\partial r} \right) \quad (A11)$$

Since the equation is theta invariant:  $v_z = v_z(r, t)$ ,

$$\Rightarrow \nabla^2 v_z = \frac{1}{r} \frac{\partial}{\partial r} r \frac{\partial v_z(r,t)}{\partial r} \qquad (A12)$$

Now, if the velocity is decomposed in two terms, an steady and unsteady term  $v_z = v_1 + v_2$ ,

$$v_1 = k_2 r^2 + c$$
 (A13)

$$\Rightarrow \frac{\partial v_{z}(r,t)}{\partial t} = 0$$
  
$$\Rightarrow 0 = k + \upsilon \left( \frac{1}{r} \frac{\partial}{\partial r} r \frac{\partial (k_{z}r^{2} + c)}{\partial r} \right) = 4k_{z}\upsilon + k$$
(A14)  
$$\Rightarrow k_{z} = -\frac{k}{4\upsilon}$$

$$v_1 = -\frac{k}{4\nu}r^2 + c \qquad (A15)$$

On the other hand, for the unsteady term:

$$\frac{\partial v_2}{\partial t} = \nu \left( \frac{1}{r} \frac{\partial}{\partial r} r \frac{\partial v_2(r,t)}{\partial r} \right)$$
(A16)

Now, using the method of separation of variables:

$$v_2(r,t) = R'(r)T(t)$$
 (A17)

$$R\frac{\partial T}{\partial t} = \frac{\upsilon}{r}T\frac{\partial}{\partial r}\left(r\frac{\partial R}{\partial r}\right)$$

$$\frac{1}{T}\frac{\partial T}{\partial t} = \frac{\upsilon}{Rr}\frac{\partial}{\partial r}\left(r\frac{\partial R}{\partial r}\right) = -\alpha$$
(A18)

From the firsr part:

$$\frac{1}{T}\frac{\partial T}{\partial t} = -\alpha$$

$$\Rightarrow T = T_0 e^{-\alpha t}$$
(A19)

Now using the boundary conditions for the steady part and T, since the unsteady part will disappear as t gets large

$$v_1 = \frac{k}{4\upsilon} (r'^2 - r^2),$$
 (A20)

Where  $k=(\Delta P/L)\rho$ 

Since the velocity at the wall is zero, as it was derived before in the notes From the second equation:

$$\frac{\upsilon}{R'r}\frac{\partial}{\partial r}\left(r\frac{\partial R}{\partial r}\right) = -\alpha$$
(A21)
$$\frac{\partial^2 R}{\partial r^2} + \frac{1}{r}\frac{\partial R}{\partial r} + \frac{\alpha}{\upsilon}R = 0$$

This is a Bessel equation with solution:

$$r = c_1 J_0(\lambda r) + c_2 Y_0(\lambda r)$$
, with  $\lambda = \sqrt{\frac{\alpha}{\nu}}$  (A22)

But in order to get convergence when r approaches 0+,  $c_2 = 0$ .

$$\Rightarrow r = c_1 J_0(\lambda r) \tag{A23}$$

Now, substituting back in the reduced equation

$$v_{2} = \sum_{i=0}^{\infty} T_{0} e^{-\alpha_{i} t} C_{i} J_{0}(\lambda_{i} r) = \sum_{i=0}^{\infty} Q_{i} e^{-\alpha_{i} t} J_{0}(\lambda_{i} r)$$
(A24)

Now applying the boundary condition in r=r',  $v_z=0$  y  $v_1(r')=0$ ,:

$$\Rightarrow v_2 = \sum_{i=0}^{\infty} Q_i e^{-\alpha_i t} J_0(\lambda_i r^t) = 0 \qquad (A25)$$
$$\Rightarrow J_0(\lambda_i r^t) = 0 \qquad (A26)$$

Then  $\lambda_i r'$  are the eigenvalues of  $(\chi_i s)$ 

$$\Rightarrow \alpha_i = \left(\frac{\chi_i}{r'}\right)^2 \upsilon \tag{A27}$$

Using the orthogonality property of the Bessel polynomials for t=0, vz=0

$$0 = v_z = v_1 + v_2, \ v_1 = \frac{k}{4\nu} (r^2 - r^2), \qquad (A28)$$

$$v_{2} = \sum_{i=0}^{\infty} Q_{i} e^{-\alpha_{i}t} J_{0}(\lambda_{i}r) \qquad (A29)$$
$$\Rightarrow -\frac{k}{4\nu} (r^{2} - r^{2}) = \sum_{i=0}^{\infty} Q_{i} J_{0}(\lambda_{i}r) \qquad (A30)$$

Multiplying by the weight function and integrating:

$$-\int_{0}^{r'} \frac{k}{4\nu} (r'^{2} - r^{2}) J_{0}(\lambda_{n}r) r dr = \int_{0}^{r'} \sum_{i=0}^{\infty} Q_{i} J_{0}(\lambda_{i}r) J_{0}(\lambda_{n}r) r dr$$
(A31)

$$-\int_{0}^{r'} \frac{k}{4\nu} (r'^{2} - r^{2}) J_{0}(\lambda_{i}r) r dr = \int_{0}^{r'} Q_{i} J_{0}^{2}(\lambda_{i}r) r dr$$
(A32)

$$Q_{i} = \frac{-\frac{k}{4\upsilon} \left[ r^{2} \int_{0}^{r} J_{0}(\lambda_{i}r)rdr - \int_{0}^{r} r^{3} J_{0}(\lambda_{i}r)dr \right]}{\int_{0}^{R} J_{0}^{2}(\lambda_{i}r)rdr}$$
(A33)

but

$$\int_{0}^{r'} J_{0}^{2}(\lambda_{i}r)rdr = \frac{r'^{2}}{2} J_{1}^{2}(\lambda_{i}r')$$
 (A34)

For the second integral

$$\int_{0}^{r'} J_0(\lambda_i r) r dr = \frac{1}{\lambda_i^2} \int_{0}^{r'} J_0(\lambda_i r) (\lambda_i r) (\lambda_i dr) = \frac{1}{\lambda_i^2} J_1(\lambda_i r) (\lambda_i r) \Big|_{0}^{r'} = \frac{r'}{\lambda_i} J_1(\lambda_i r')$$
(A35)

Using integration by parts

$$\int_{0}^{r'} r^{3} J_{0}(\lambda_{i}r) dr = \frac{(r^{3})}{\lambda_{i}} J_{1}(\lambda_{i}r) \Big|_{0}^{r'} - \frac{2}{\lambda_{i}} \int_{0}^{r'} r^{2} J_{1}(\lambda_{i}r) dr \qquad (A36)$$

$$\frac{(r^{3})}{\lambda_{i}} J_{1}(\lambda_{i}r) \Big|_{0}^{r'} - \frac{2}{\lambda_{i}} \int_{0}^{r'} (r\lambda_{i})^{2} J_{1}(\lambda_{i}r) (\lambda_{i}dr) = \frac{(r^{3})}{\lambda_{i}} J_{1}(\lambda_{i}r) \Big|_{0}^{r'} - \frac{2}{\lambda_{i}} (r\lambda_{i})^{2} J_{2}(\lambda_{i}r) \Big|_{0}^{r'} \qquad (A37)$$

$$= \frac{(r^{3})}{\lambda_{i}} J_{1}(\lambda_{i}r) \Big|_{0}^{r'} - \frac{2}{\lambda_{i}} (r\lambda_{i})^{2} J_{2}(\lambda_{i}r) \Big|_{0}^{r'} = \frac{(r^{3})}{\lambda_{i}} J_{1}(\lambda_{i}r') - \frac{2}{\lambda_{i}}^{2} r^{2} J_{2}(\lambda_{i}r')$$

Now substituting back to find the function

$$Q_{i} = \frac{-\frac{k}{4\upsilon} \left[ r^{\prime 2} \int_{0}^{r'} J_{0}(\lambda_{i}r)rdr - \int_{0}^{r'} r^{3} J_{0}(\lambda_{i}r)dr \right]}{\int_{0}^{r} J_{0}^{2}(\lambda_{i}r)rdr} = -\frac{k}{4\upsilon} \frac{\frac{2}{\lambda_{i}^{2}}r^{\prime 2} J_{2}(\lambda_{i}r^{\prime})}{\frac{r^{\prime 2}}{2} J_{1}^{2}(\lambda_{i}r^{\prime})} = -\frac{k}{\lambda_{i}^{2}\upsilon} \frac{J_{2}(\lambda_{i}r^{\prime})}{J_{1}^{2}(\lambda_{i}r^{\prime})}$$

(A38)

But noticing that the lambdas are the zeros of the function

$$\frac{2}{\lambda_i r'} J_1(\lambda_i r') = J_2(\lambda_i r') + J_0(\lambda_i r') = J_2(\lambda_i r')$$
(A39)

Substituting back into the previous equation:

$$Q_{i} = -\frac{2k}{\lambda_{i}^{3}\upsilon r'} \frac{J_{1}(\lambda_{i}r')}{J_{1}^{2}(\lambda_{i}r')} = -\frac{2k}{\lambda_{i}^{3}\upsilon r'} \frac{1}{J_{1}(\lambda_{i}r')}$$
(A40)

Then Vz is equal to

$$V_{Z} = \frac{k}{4\nu} (r'^{2} - r^{2}) - \sum_{i=0}^{\infty} \frac{2k}{\lambda_{i}^{3} \nu r'} \frac{1}{J_{1}(\lambda_{i} r')} e^{-\alpha_{i} t} J_{0}(\lambda_{i} r)$$
(A41)

Now, obtaining the average velocity

$$\overline{V_{Z}} = \frac{\int_{0}^{r^{2}2\pi} \left[\frac{k}{4\upsilon}(r^{\prime^{2}} - r^{2}) - \sum_{i=0}^{\infty} \frac{2k}{\lambda_{i}^{3}\upsilon r^{\prime}} \frac{1}{J_{1}(\lambda_{i}r^{\prime})} e^{-\alpha_{i}t} J_{0}(\lambda_{i}r)\right] r d\theta dr}{\pi r^{\prime^{2}}}$$
(A42)

$$\overline{V_{Z}} = \frac{2\pi \left[ \int_{0}^{r'} \left[ \frac{k}{4\upsilon} (r'^{2} r - r^{3}) dr \right] - \int_{0}^{r'} \left[ \sum_{i=0}^{\infty} \frac{2k}{\lambda_{i}^{3} \upsilon r'} \frac{1}{J_{1}(\lambda_{i}r')} e^{-\alpha_{i}t} J_{0}(\lambda_{i}r) r dr \right] \right]}{\pi^{*} r'^{2}}$$
(A43)

$$\overline{V_Z} = \frac{2\left[\left[\frac{k}{4\upsilon}(\frac{r^{\prime 4}}{4})\right] - \int_0^{r}\left[\sum_{i=0}^{\infty}\frac{2k}{\lambda_i^{5}\upsilon r^{\prime}}\frac{1}{J_1(\lambda_i r^{\prime})}e^{-\alpha_i t}J_0(\lambda_i r)(\lambda_i r)(dr\lambda_i)\right]\right]}{r^{\prime 2}}$$
(A44)

$$\overline{V_{Z}} = \frac{2\left[\left[\frac{k}{4\upsilon}(\frac{r^{\prime^{4}}}{4})\right] - \sum_{i=0}^{\infty}\frac{2k}{\lambda_{i}^{5}\upsilon r^{\prime}}\frac{1}{J_{1}(\lambda_{i}r^{\prime})}e^{-\alpha_{i}t}J_{1}(\lambda_{i}r^{\prime})(\lambda_{i}r^{\prime})\right]}{r^{\prime^{2}}}$$
(A45)

$$\overline{V_Z} = \frac{2\left[\left[\frac{k}{4\upsilon}(\frac{r^{\prime^4}}{4})\right] - \sum_{i=0}^{\infty}\frac{2k}{\lambda_i^5\upsilon r^{\prime}}\frac{1}{J_1(\lambda_i r^{\prime})}e^{-\alpha_i t}J_1(\lambda_i r^{\prime})(\lambda_i r^{\prime})\right]}{r^{\prime 2}}$$
(A46)

Now simplifying using the following definitions:

$$\lambda_{i} = \sqrt{\frac{\alpha_{i}}{\upsilon}}$$
(A47)
$$\alpha_{i} = \left(\frac{\chi_{i}}{r'}\right)^{2} \upsilon$$
(A48)



(A49)

Now, substituting the value of k

$$\overline{v_{Z}} = \frac{kr^{\prime 2}}{8\upsilon} - \frac{4kr^{\prime 2}}{\upsilon} \sum_{i=0}^{\infty} \frac{e^{-\left(\frac{\chi_{i}}{r}\right)^{2}\upsilon t}}{\chi_{i}^{4}} = \frac{kr^{\prime 2}}{\upsilon} \left(\frac{1}{8} - 4\sum_{i=0}^{\infty} \frac{e^{-\left(\frac{\chi_{i}}{r}\right)^{2}\upsilon t}}{\chi_{i}^{4}}\right)$$
$$\overline{v_{Z}} = \frac{-\nabla P^{*}r^{\prime 2}}{\mu} \left(\frac{1}{8} - 4\sum_{i=0}^{\infty} \frac{e^{-\left(\frac{\chi_{i}}{r}\right)^{2}\upsilon t}}{\chi_{i}^{4}}\right)$$
(A50)

# CALIBRATION CURVES FOR HAMAMATSU CAMERA AND NIKON ECLIPSE MICROSCOPE



# THICKNESS OF FILMS USED FOR CHANNEL CLOSING



### FILM FLUORESCENCE RELATIVE TO PDMS













### FAILURE PRESSURE FOR DIFFERENT FILMS

FAILURE PESSURE (PSI) FOR BS PLASTIC FILM WITH ADHESIVE. LEAKAGE STARTS ALONG WITH DEFORMATION THAT PROGRESSES TILL COMPLETE DEBONDING	ZONE	PRESSURE FOR LARGE DEFORMATION THAT CAN CLEARLY BE OBSERVED VISUALLY		
34	CONCENTRATOR	12		
33	CONCENTRATOR	13		
30	CONCENTRATOR	10		
*VALUES FOR TOTA				
*PRESSURE FAILURE WITH OUT GLASS BACK				



FAILURE PESSURE FOR STATIC CLING FILM (PSI), 90 KG LOAD PRESSURE DURING BONDING, 4.5 ATM	ZONE
25	FOCUSING
14	FOCUSING
10	FOCUSING
12	FOCUSING
10	FOCUSING
10,5	FOCUSING
GLASS BACK	30-35 PSI



# **FILM OPTIC PROPERTIES**



OPTIC ADHESIVE COVER, 4311971, APPLIED BIOSYSTEMS



PDMS, SYLGARD 184<sup>TM</sup>



STATIC CLING FILM, PVC (TYPE 1) FILM, TRANSLUCENT, LOW TACK, 0.002" THICK, 12" WIDTH, 12' L, 7524T13 MCMASTER-CARR.



TRANSPARENT SILICONE RUBBER SHEET, ADHESIVE BACK, 0.020" THICKNESS, 12" x12", 40A DUROMETER, 86915K22 MCMASTER-CARR



FEP FILM WITH ADHESIVE BACK, 0.0035" THICK, 12" X 12", 5805T11 MCMASTER-CARR



Adhesive Backed Polyester (PET) Film, 0.002" thick, 27" Width, 20" Length. 8689K31 MacMaster-Carr

### References

1 David C Duffy, Olivier J A Schueller, Scott T Brittain and George M Whitesides, "Rapid prototyping of microfluidic switches in poly(dimethyl siloxane) and their actuation by electro osmotic flow", J. Micromech. Microeng. 9, 1999, pp. 211–217.

2 Asuncion V. Lemoff and Abraham P. Lee, "An AC Magnetohydrodynamic Microfluidic Switch for Micro Total Analysis Systems", Biomedical Microdevices, Volume 5, Number 1 / march 2003, pp. 55-60.

3 Lisen Wang, Abraham Philip Lee, "Dielectrophoretic Microfluidic Switching for Lab on a Chip Applications", Materials Research Society, Proc. Vol. 1004, 2007, 1004-P08-04.

4 Petra S. Dittrich and Petra Schwille, "An Integrated Microfluidic System for Reaction, High-Sensitivity Detection, and Sorting of Fluorescent Cells and Particles", Anal. Chem., 75 (21), 2003, pp. 5767 -5774.

5 K. Grujic, O. Hellesø, J. Hole, and J. Wilkinson, "Sorting of polystyrene microspheres using a Y-branched optical waveguide", Optics Express, Vol. 13, Issue 1, 2005, pp. 1-7

6 Holmes, D. Sandison, M.E. Green, N.G. Morgan, H., "On-chip high-speed sorting of micron-sized particles for high-throughput analysis", Nanobiotechnology, IEE Proceedings, Volume 152, Issue 4, pp. 129-135.

7 Alan H. Tkaczyk, Dongeun Huh, Joong Hwan Bahng, Yu Chang, Hsien-Hung Weil, Katsuo Kurabayashi, James B. Grotberg, Chang-Jin "CJ" Kim", Shuichi Takayama, "Fluidic Switching of High Speed Air-Liquid Two Phase Flows Using Electrowetting-On-Dielectric", 7th International Conference on Miniaturized Chemical and Biochemical Analysts Systems, October 5-9, 2003, pp. 461-464.

8 Thilo Brenner, Thomas Glatzel, Roland Zengerle and Jens Ducrée, "Frequencydependent transversal flow control in centrifugal microfluidics", LabOn a Chip, 2005, 5, pp. 146 - 150.

9 Jagannathan, H. Yaralioglu, G.G. Ergun, A.S. Khuri-Yakub, B.T., "An implementation of a microfluidic mixer and switch using micromachined acoustic transducers", Micro Electro Mechanical Systems, 2003. MEMS-03 Kyoto, pp. 104-107.

10 Sung-Yi Yang, Suz-Kai Hsiung, Yung-Ching Hung, Chen-Min Chang, Teh-Lu Liao and Gwo-Bin Lee, "A cell counting/sorting system incorporated with a microfabricated flow cytometer chip", Meas. Sci. Technol. 17,2006, pp. 2001–2009.

11 Chen, C.C. Zappe, S. Sahin, O. Zhang, X.J. Furlong, E.E.M. Fish, M. Scott, M. Solgaard, O., "Microfluidic switch for embryo and cell sorting", TRANSDUCERS, Solid-State Sensors, Actuators and Microsystems, 12th International Conference on, 2003, PP. 659- 662.

12 Chih-Ming Cheng, Cheng-Hsien Liu, "A capillary system with thermal-bubbleactuated 1/spl times/N microfluidic switches via time-sequence power control for continuous liquid handling", Journal of Microelectromechanical Systems, April 2006, Vol. 2, pp. 296-307.

13 Jesper Glückstad, "Microfluidics: Sorting particles with light", Nature Materials 3, 2004, pp. 9 – 10.

14 Marc A. Unger, Hou-Pu Chou, Todd Thorsen, Axel Scherer, Stephen R. Quake, "Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography ", Science 7 April 2000, Vol. 288. no. 5463, pp. 113 – 116.

15 Chih-Ming Cheng and Cheng-Hsien Liu, "A Capillary System with Thermal-Bubble-Actuated 1×N Micro Fluidic Switches via Time-Sequence Power Control for Continuous Liquid Handling," Journal of MicroElectroMechanical Systems, Vol. 15, n. 2, pp. 296-307, April 2006.

16 T. Buma, M. Spisar, and M. O'Donnell, 'A High Frequency Ultrasound Array Element Using Thermoelastic Expansion in PDMS', 2001, IEEE Ultrasonic Symposium, pp. 1143-1146.

17 Seok Woo Lee and Seung S. Lee, 'Shrinkage ratio of PDMS and its alignment method for the wafer level process', Microsystem Technologies, volume 14, number 2, February 2008, pp. 205-208.

18 Deniz Armani, Chang Liu and Narayan Aluru, 'Reconfigurable Fluid Circuits by PDMS Micromachinnig'. 12th International Conference on MEMS, MEMS 99, pp.222-227, Orland, FL, 1998.

19 Kyung M. Choi and John A. Rogers, Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey 07974, "A Photocurable Poly(dimethylsiloxane) Chemistry Designed for Soft Lithographic Molding and Printing in the Nanometer Regime", JACS Communications, March 18, 2003.

20 M. G. Allen, M. Mehregany, R. T. Howe & S. D. Senturia, "Microfabricated Structures for the In-Situ Measurement of Residual Stress, Young's Modulus, and Ultimate Strain of Thin Films", Applied Physics Letters, Vol 51, pp. 241, 1987.

21 Brochure for RNA Analysis, Aurora® Bio-Technologies, 7668 El Camino Real, Suite 104-61, Carlsbad, CA 92009 USA.

22 Daniel Palanker, Igor Turovets, and Aaron Lewis, "Dynamics of ArF Excimer Laserinduced Cavitation Bubbles in Gel Surrounded by a Liquid Medium", Lasers Surg Med. 1997;21(3), pp. 294-300. 23 Igor Turovets, Daniel Palanker, Yu. Kokotov, Itzhak Hemo, Aaron Lewis, "Dynamics of cavitation bubble induced by 193 nm ArF excimer laser in concentrated sodium chloride solutions", J. Appl. Phys., Vol. 79, No. 5, 1 March 1996, pp. 1689-2693.

24 HanQun Shangguan, Lee W. Casperson, Alan Shearin, Dennis Paisley, and Scott A. Prahl, "Effects of Material Properties on Laser-induced Bubble Formation in Absorbing Liquids and On Submerged Targets", Proc. SPIE 2869, 1997, pp. 783-791.

25 C.C. Chen, J.S. Wang and O. Solgaard, "Micromachined bubble-jet cell sorter with multiple operation modes", Sensors and Actuators B: Chemical, Volume 117, Issue 2, 12 October 2006, pp. 523-529.

26 Tudor N. Buican, Miriam J. Smyth, Harry A. Crissman, Gary C. Salzman, Carleton C. Stewart, and John C. Martin, "Automated single-cell manipulation and sorting by light trapping", Journal of Applied Optics, Vol. 26, No. 24, 15 December 1987, pp. 5311-5316.