

Affect of Air Bubbles on Filling and Metering in a Microfluidic Device

J. Podczerviensky, J. McDowell, and L. Levine

ALine, Inc., 19500 Rancho Way, Ste 107, Rancho Dominguez, CA 90220; info@alineinc.com

ABSTRACT

Microfluidics are critical to the development of high sensitivity, multiplexed, and quantitative point-of-care diagnostic products that promise to improve the quality of healthcare while lowering costs.

Fundamental to a shift from qualitative screening tests to quantitative measurements near the patient is the need for a robust fluidic system that is tolerant of the method of manufacture, has robust interconnects to the instrument, with optimized actuation routines.

In this paper, we will discuss the variability of metering by measuring the size and number of air bubbles trapped in a channel. We will discuss the impact of air bubbles and channel geometry on metering performance and provide suggestions for improved performance.

Keywords: Microfluidics, Lab-on-a-Chip, polymer laminates, bubbles

1 INTRODUCTION

Multiple fabrication approaches can be applied to the development of Lab-on-a-Chip devices. Among the most popular approaches are PDMS-based methods that use standard lithography, injection molding, embossing, etched glass, and polymer laminate technology. PDMS-based approaches are popular for laboratory research and are suitable for cell culture applications, while glass is very well suited to laboratory-based platforms, but are not well-suited to disposable applications. Embossing and injection molding have been shown to be very good at producing fine features and complex geometries along with the promise of low production costs as sufficient volumes; however tooling costs and turn around times often frustrate development for anyone on a limited budget and timeline.

Polymer Laminate Technology (PLT) offers an attractive alternative for both development and volume production. The method uses stacks of thin film materials that are patterned using a CO₂ laser to create channels, valve and vent features. Volume production is achieved through roll-to-roll processing with special techniques to achieve the required alignment tolerances.

In an earlier technical article [1], we discussed the affect of stacking alignment tolerances and channel variability on repeatable pumping and metering in test fluidic devices. We also evaluated the inter- and intra-device variability.

In this paper we further that effort to evaluate the materials and geometries that affect bubble formation and evaluate their impact on device performance, with an eye toward developing design strategies that minimize the impact of air bubble on device performance.

1.1 Polymer Laminate Technology

The devices are constructed from plastic sheet and film substrates, such as PMMA, PET, COP and PC. Material thicknesses range from 3 mm to 12.5 microns. The design is divided into different layers to route the fluid and to incorporate valves, vents and pumps. Devices are designed using a CAD package. The features in various layers are laser cut into the substrates using a two dimensional CO₂ laser cutter. The laser cut sheets are cleaned using isopropyl alcohol or detergent and water. The layers are then aligned, stacked, and bonded together with pressure sensitive or thermal bond adhesives to produce complex three dimensional fluidic circuits.

Polymer laminate technology (PLT) offers rapid prototyping of complex microfluidic devices. Because the fabrication process does not require tooling, designs can be iterated in days rather than weeks. The laminate readily interfaces to injection molded caps containing reagent and waste storage, a pneumatic manifold for pump and valve actuation, and features for bursting blister packs as well as to an optical or electroactive sensor for detection. Functional components such as vents and filter membranes can easily be embedded within the device via pick and place methods for increased functionality. Integration with the various components allows the laminate to serve as the "fluidic motherboard" for a complex, self contained lab-on-a-chip device.

1.2 Effects of Bubbles in an Assay

Air bubbles can negatively impact microfluidic experiments in a variety of ways depending on where the bubble becomes trapped or whether the bubble is pushed through the entire microfluidic system. Bubbles can be a troublesome issue which are difficult to remove if they are not dealt with in the preliminary designs of the device.

When air bubbles become trapped in a metering channel, the volume of the dispensed reagent will be less than the intended volume. Metering a lower than expected volume of reagent can fail to completely fill a downstream sensor chamber or, if the reagents are to be mixed, will produce an

incorrect concentration of reagents. This can lead to a false response in the desired analyte measurement by impacting assay conditions (ie wrong pH, incorrect ratio of reagents, sensing errors) or create non-ideal environments for cell culture. Air bubbles that become trapped in a detection region, particularly in an optical detection system, impact quantitative measurements. Electrochemical measurements are less sensitive to the impact of air bubbles.

1.3 Sources of Bubbles

There are many sources of air bubbles in a microfluidic card. Gas bubbles can form when the temperature of the solution increases, altering the solubility of dissolved CO₂, causes bubbles to form at nucleation sites, such as the edges of a laser cut channel. Air can be trapped while actuating on-board pumps and valves. As the flexible membrane layer is actuated, there is a small amounts air that remains around the perimeter of the valve. Another source of air bubbles is un-even wicking of liquid along the side walls of the channels. The surface tension of the fluid and varying surface roughness of the laser cut channel walls can cause asymmetrical progress of the fluid through the channel. The liquid will fill along the sides of the channels ahead of the center of the channel. This characteristic can be seen in Figure 5. If enough fluid flows along the side of the channel, the fluid can build up on one or both side walls and eventually bridge the gap to the other side of the channel thereby trapping a pocket of air. The result is air that is trapped in the liquid as the fluid streams along the sides of the channels meet. It is expected that this effect to go away as height and width of a channel are reduced. Thin channels presumably would not allow the stream to split into two along the sidewall

The biggest potential source of air bubbles occurs when introducing multiple fluid streams at different times, as is the case with sequential and non-serial additio of reagents in a standard bioassay. A typical assay consists of mixing the sample with antibody, washing this with buffer, and then adding the conjugate. During mixing of the sample and antibody, followed by dispensing to a detection location, bubbles can be trapped along the sidewalls at bends and at vias. As two streams meet, a few bubbles may be trapped at the intersection, while further down the combined streams, no further bubbles are formed. If the wash is introduced without priming, then the air in the pre-primed channel is pushed through the system, and bubbles can often become trapped in critical areas.

One method to control air bubbles is to follow a fluid fill with air, however, droplets of liquid clinging to the side walls of the channels promotes capillary flow along the channel walls with a second filling , with air being trapped in the center of the channel.

Each source of bubble formation can be mitigated through a choice of actuation and fill routines, along with the correct choice of device geometry.

The goal is to design a device optimized for either flushing with air or priming with liquid, along with fluid actuation routines that use vacuum and/or pressure to move the fluids through the device.

1.4 Test Platform

ALine's ADEPT Platform was used to establish actuation routines, and visually observe device performance. The platform consists of a pneumatic controller, a moving x-y stage with an air manifold connection, and an optical comparator for viewing and measuring. The ADEPT can be programmed to perform user-defined actuation routines on the microfluidic device, including on-board diaphragm valves and pumps. The actuation routines were written in a text document and saved to a micro SD card, and then loaded into the instrument.

In combination with the controller and an x-y stage, a USB video camera was used to observe and record fluid movement in the device. In this experiment we also used the optical comparator feature of the ADEPT platform to measure bubble volumes in a metered channel.

The pneumatic controller incorporates miniature solenoid valves to provide pressure, vacuum, or venting independently for each air line in the manifold. Pressure sensors and regulators are used to monitor and adjust the actuation pressure and vacuum. Manual toggle switches permit 100% user control for trouble shooting on the fluid card.

We used house air to regulate the pressure and a Venturi tube to create vacuum.

Figure 1: ALine's ADEPT Development Platform used for operation and testing of microfluidic devices.



Figure 2: Picture of test device. A series of channels with the same valve and vent design were arrayed on a sheet and tested for both inter and intra device varitability.

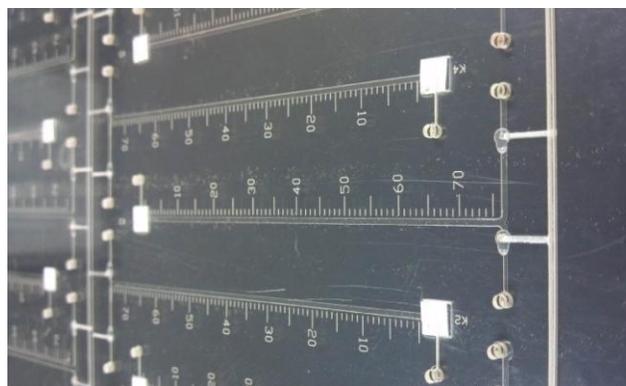
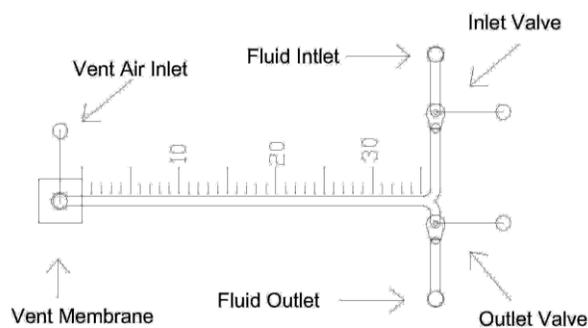


Figure 3: Schematic of the Test Device. The metering channel is laser etched with graduations to simplify quantitation of volumes metered.



1.5 Test Methods

We evaluated the effect of bubbles on one of the most important steps in an assay, metering. As seen in Figure 3, test devices were built with a “T” shaped channel containing an inlet, outlet and channel of fixed volume for metering. A vent membrane was located at the end of the metering channel to allow a vacuum to be pulled on the metering channel to draw in liquid through the opened inlet valve. The length of the metering channels was fixed at 75 mm and the aspect ratio (width:height) of the channels were varied from 1:1 to 8:1, which covers typical aspect ratios in our devices. To test the devices, the inlet valve was opened while maintaining the outlet valve closed and a 5 PSI vacuum was pulled on the vent membrane which drew liquid from the inlet into the metering channel. The inlet valve was closed and the bubbles were observed and measured under a 22.5x optical comparator (Sciencescope). Bubbles extend across the full channel height. The area of the bubbles were measured and multiplied by the height of the channel to get the approx. bubble volume. The volume of the individual bubbles in a given channel were then summed together to get the total bubble volume. After the bubbles were measured, the outlet valve was opened and the liquid was then pushed out of the metering channel using positive air pressure. The process was repeated five

time for each channel and the bubble volume averaged together for each channel. Eight identical channels for each geometry were investigated and averaged together.

2 RESULTS

The data is summarized of the following table and graph. The average bubble volume was below 0.5% of the total channel volume for all of the geometries measured. The channel volume varied from 2 to 18.5 uL. The bubble volume was under 0.75% of the total liquid volume for all trials in all of the channels tested. The variability of bubble volume is high; some bubbles 10 x larger than others. However, the average bubble volume is very low compared to the channel volume.

Figure 4: Graph of Bubble Volume as Percentage of Metering Volume vs. Metering Channel Width

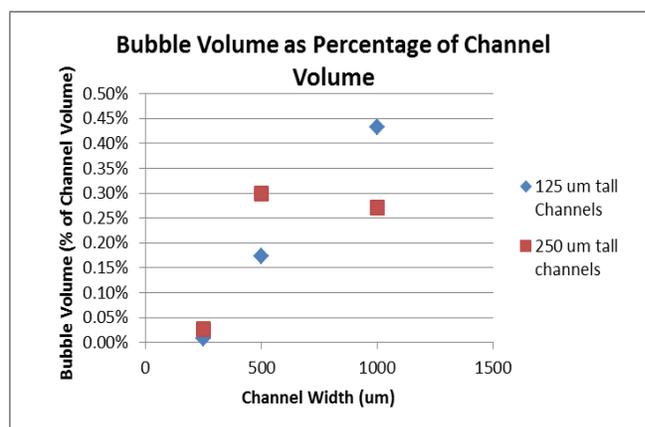


Table 1: Summary of Metering Channel Geometries and Bubble Volume

Channel Height (um)	Channel Width (um)	Channel Volume (uL)	Average Bubble Volume (uL)	Bubble Volume (%)
125	250	2.344	0.000	0.01
	500	4.687	0.008	0.17
	1000	9.375	0.041	0.43
250	250	4.688	0.001	0.03
	500	9.375	0.028	0.30
	1000	18.750	0.051	0.27

3 DISCUSSION

The air bubble volume in the metering step is below 1% of the total liquid volume for all channels. As was expected, as the width of the channel decreased, so did the percentage of volume taken up by air bubbles. When the channels are smaller, on the order of 250 um wide and less than ~250 um

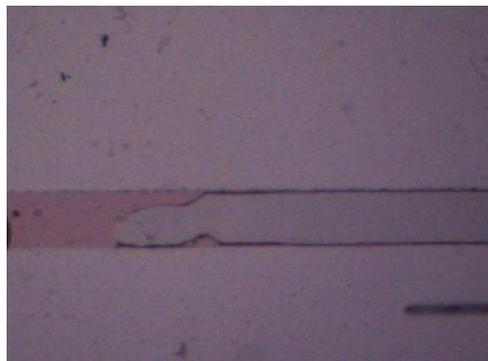
deep, the liquid is not able to split into two streams along the sidewalls and then merge back together, trapping a bubble of air in the process. Figure 5 shows this effect in a 1 mm wide channel. The air bubbles formed during metering via the method used are due to this effect of liquid traveling down the sidewall and merging together to trap air pockets.

The air bubbles were almost always circular in the XY plane. The majority of the bubbles in the channel were under 75 micron in radius. Typically there would be under ten of these bubbles in the 75 mm length of the channel. If larger bubbles of air were present, they were mostly between 100 and 300 micron in radius. Typically there were five or fewer of this size in a given channel. The bubble size variation is high, but is less than 1% of the total liquid volume. The size of the bubbles and the overall volume and variability does not present a large issue for measurements taken later in the assay.

Larger air bubbles (> 100 um diameter) tend to stick to walls and corners of the device rather than being pushed through the system. Tiny air bubbles can travel throughout the device and be pushed out to waste or remain within the liquid while posing little obstruction of measurements.. With the flowrates and volumes pumped through the devices, the air bubbles that become trapped along sidewalls cannot be effectively pushed out of the system. For the most part, this can be beneficial as many bubbles are trapped before entering the detection region, but if they are trapped in the detection region, they can be very difficult to remove. Design factors needs to be optimized to ensure these bubbles do not enter or form in the detection zone.

Design strategies to mitigate bubble formation or reduce their impact have been explored by looking at the affect of channel width on bubble formation using water with a water soluble dye as the liquid. Our results suggest that narrower channels minimize bubble trapping by liquid preferentially wicking up the sidewall. In this experiment we only evaluated water, however, by modifying the surface energy of the materials, or modifying the surface tension of the liquid by adding surfactants or a low concentration of an alcohol, we would expect to see changes to the bubble forming propensity. Another strategy for mitigating iar bubbles is move the fluid to a holding chamber with a vent membrane over the surface, which acts a de-bubbler when vacuum is applied.

Figure 5: Image of dyed water traveling unevenly along the side walls of a 1 mm wide channel.



4 CONCLUSION

For the device configuration used in these experiments, the bubbles play little role in the ability to meter reproducible volumes of fluid. This metering method is a reliable way of metering up to 20 uL and potentially more. Having thin channels (~250 um wide, < 250 um deep) is one way of reducing air bubbles in a microfluidic devices fabricated using our PLT platform. Further experiments are in progress to incorporate pumped metering as well as evaluate the affect of different fluids that better mimic the solutions used for bioassays.

REFERENCES

- [1] J. Podczerviensky, L. Levine, "Modular Designs for Reproducible Performance of On-Board Pumps," Proceedings for the NSTI Conference, Boston, MA 2011