

# A PDMS Microfluidic Spotter for Fabrication of Lipid Microarrays

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**Abstract-** A PDMS microfluidic spotter system has been developed for the patterning of lipid microarrays that require individually addressing each spot area and high spot density. Microarray spot concentration and uniformity are improved by forming array spots through continuous flow whereby a greater amount of lipid in solution will come in contact with and have the opportunity to bind to the substrate surface. Additionally, flow deposition offers more control over spot formation, since the flow rates and deposition times could be uniquely varied for each spot, producing spots with unique concentrations on the surface. The system is significant in that it facilitates the fabrication of high-density arrays with more dilute lipid solutions, which are difficult and sometimes prohibitively expensive to isolate in sufficient concentration for solid pin and ink-jet spotting. Preliminary test deposition using a fluorescent dye has demonstrated a 5-fold increase in deposition density as compared to conventional pin spotting.

**Keywords** – PDMS, spotter, microfluidic, lipid, microarray.

## I. INTRODUCTION

LIPIDS are central to the regulation and control of several cellular functions. They form many of the important structural features of cells, and are critical members of cellular signal transduction pathways. Cellular dysfunction is often caused by errors in lipid signaling; therefore, the proteins that interact with, synthesize or metabolize the lipids are potential therapeutic targets. Lipid microarrays will allow researchers to obtain a comparable fingerprint of the proteins from a cell or tissue that bind to lipids, and also enable the identification of functionally important lipid-binding proteins [1]. On a lipid microarray, lipid molecules are deposited onto a solid surface in micro-sized spots and act as baits for the entities that interact with lipids. The proteins captured by a lipid chip could generate a pattern of the protein-lipid interaction profile. Depending on the design of the specific array format, lipid microarrays could provide the general information of lipid-associated proteins in a cell, tissue or biological fluids. However, the inherent difficulty of producing high-concentration lipid solutions for use in conventional pin or ink-jet spotters essentially prevents the fabrication of these arrays, and thus provides the driving force to develop the PDMS flow spotter.

## II. METHODOLOGY

### A. PDMS Spotter Fabrication

The PDMS flow spotting system developed in this study uses continuous flow of low-concentration lipid solutions over isolated substrate areas to gradually build up the deposited lipids to a higher surface density. This process is

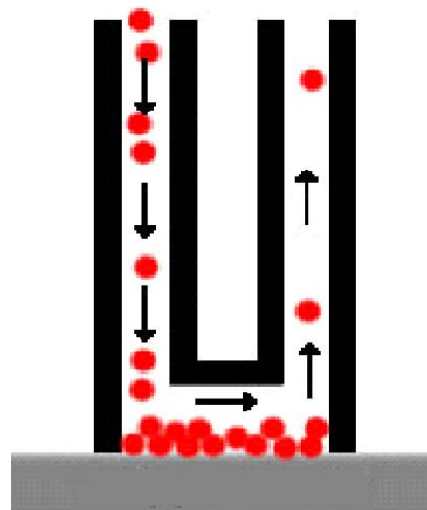


Fig. 1. Diagram of continuous-flow lipid spotter operation. Lipid molecules in low-concentration solution are flowed past the substrate and deposited in a controlled area. The remaining carrier fluid is either discarded or it can be recirculated through the spotter to minimize waste of precious solutions.

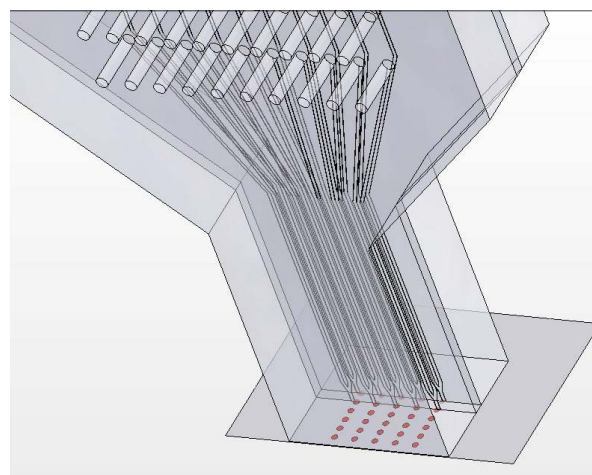


Fig. 2. 3-dimensional schematic of a multi-channel PDMS spotter. Each spot on the substrate is formed by continuous flow through coupled inlet and outlet channels.

illustrated in Fig. 1. The advantage of the continuous flow system over conventional pin and ink-jet spotting systems is that a large volume of fluid can interact with the substrate surface, so that an almost limitless number of lipid molecules can potentially bind to the substrate surface [2-6]. The spotter is fabricated using a lithographically-created SU-8 mold, which is composed of a pair of channels leading to V-shaped intersection. Once the channel is molded in PDMS, it is covered and sealed with a slab of PDMS using

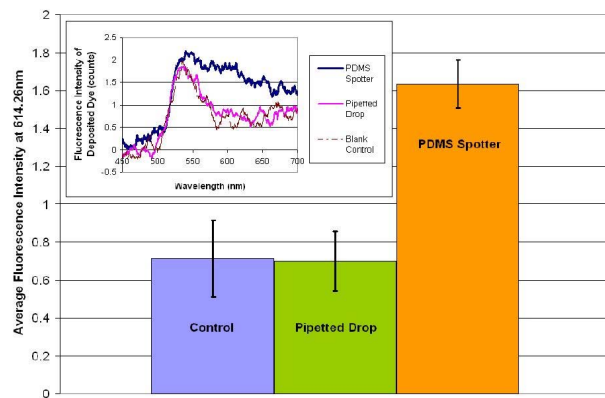
an oxygen plasma. The spot area is defined by the cross-sectional area of the PDMS channel intersections at the tip of the spotter. To access the spotting tip, a single section through the PDMS is made at the channel intersection, exposing it on the cut face. The cut face is then pressed against the substrate and lipid solutions are flowed past the intersection via fluidic packaging at the distal ends of the channels. Parallel channels can be manufactured on the same mold, with all interfaces sectioned at the same time. Multiple PDMS channel molds can also be stacked on top of each other, bonded and sectioned simultaneously, allowing massively parallel 2-dimensional arrays to be fabricated, with each spot individually-addressed (Fig. 2). The spotted area dimensions and spacing is determined solely by the SU-8 mold pattern, and the microarrays can be arranged in a repeatable fashion to suit the needs of the user.

### B. Deposition Density Testing

To test the spotter, a positively-charged fluorescent dye tris(2,2'-bipyridyl dichlororuthenium) hexahydrate was used as a probe molecule. The dye was dissolved in water at 2 $\mu$ g/L and flowed for 60 minutes through a macroscale version of the spotter, which had a 2mm x 2mm spotting area to facilitate easy optical detection. The substrates used were glass slides prepared with the negatively-charged polyion poly(sodium styrenesulfonate) (PSS) to ensure dye molecule adhesion. A pipetted drop of the same dye solution used in the spotter of the same area as the spotter was used to simulate a conventional pin or ink-jet spotter, and both spots were compared to control slides with no dye spots.

## III. RESULTS

Comparison of the flow-deposited drops, simulated pin-spotted drops and control substrates show a minimum 5-fold increase in dye density based on fluorescence intensity (Fig. 3). More significantly, the simulated pin-spotted drops show insignificant deposition, which is representative of conventional lipid array spotters.



A. Fig 3. Preliminary spotter data, demonstrating increased deposited dye density based on fluorescence as compared to a pipetted drop. Error bars show one standard deviation for the data set. Inset shows the spectrograph for each test sample.

## IV. CONCLUSIONS

A PDMS continuous-flow spotting system has been developed to produce high surface density lipid microarrays. Test spots using a model dye solution have demonstrated the fundamental mechanism of the spotter to fabricate high density microarrays. Further testing for density and spot uniformity will be carried out using low-concentrate dye-labeled lipid solutions, using a linear array of 300 $\mu$ m-square spots.

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