PARALLEL MICROARRAYING OF MICROFLUIDIC DROPLETS FOR HIGH-THROUGHPUT INTEGRATION WITH MATRIX-ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY

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ABSTRACT

Droplet microfluidics affords researchers the ability to rapidly generate discretized conditions at kHz rates cheaply and controllably. We leveraged a simple microwell array to enable a parallel process of droplet microarraying, droplet sample deposition through surfactant removal, and thermally actuated device disassembly for facile integration with MALDI mass spectrometry analysis.

INTRODUCTION

Droplet microfluidics has dramatically expanded the throughput of microfluidic screening tools. One of the limitations of droplet microfluidics is that it introduced new challenges in interfacing with non-optical readout modalities. The minute volumes of droplets can contain insufficient sample masses for some molecules to be detected with label-free modalities – such as mass spectrometry detection. Further, the droplet form factor and their sheer numbers require a unique interface to discretely detect the contents each individual droplet. Recent developments of directly coupling droplet microfluidics with mass spectrometry has dramatically advanced the types of molecules that can be detected in droplet microfluidics. Notable examples include the ~1 Hz droplet injection rate in electrospray ionization [1] and the ~7 Hz robotic droplet spotting onto a matrix-assisted laser desorption ionization (MALDI) plate [2]. While these advances are impressive, they still fall an order of magnitude below the capability of laser based mass spectrometry instrumental throughput [3]. Thus, the critical bottleneck in droplet microfluidics mass spectrometry throughput remains the droplet mass spectrometry interface. To address this bottleneck (Figure 1), we leveraged a simple microwell array to enable a parallel process of droplet microarraying, droplet sample deposition through surfactant removal, and thermally actuated device disassembly for facile integration with MALDI mass spectrometry analysis.

METHODS

Materials and Chemicals

We purchased acetone, isopropyl alcohol (IPA), perfluorooctyltrichlorosilane (FOTS), dextromethorphan,

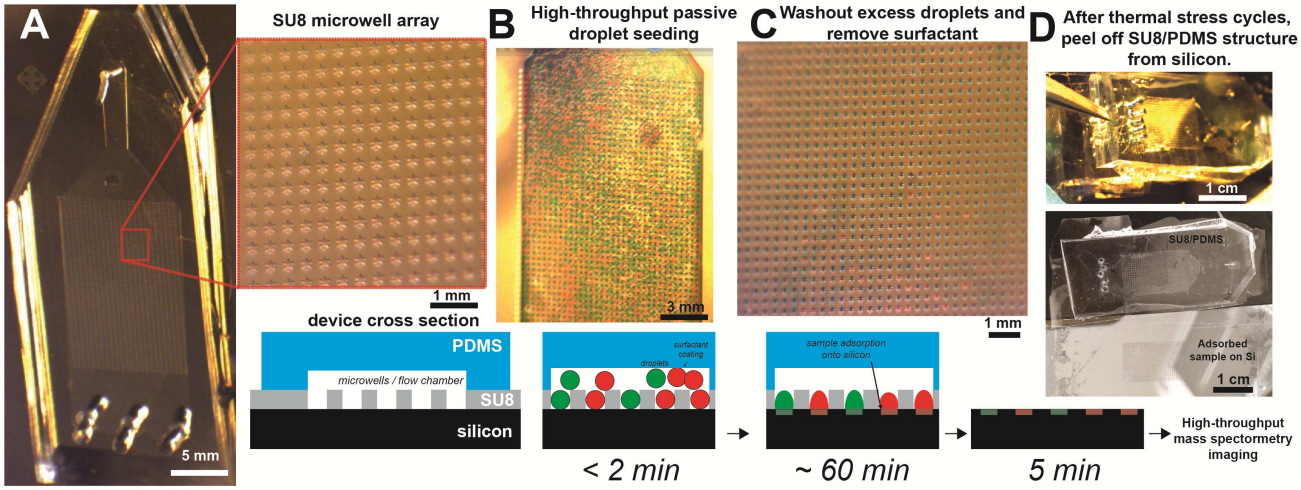

Figure 1: Parallel droplet microfluidic-MALDI integration is accomplished using a simple PDMS flow channel atop a SU8 microwell array (A). SU8 fabrication is performed after a reduced dehydration step (90° C, 3 min) with no O_2 plasma on the silicon wafer to prevent an excessively strong silicon/SU8 bond. The 45 µm SU8 layer is patterned with microwells (sizes range from 50 to 1000 µm in length, variety of shapes). The PDMS channel (45µm in height) is attached covalently to the SU8 using a N_2 plasma bonding technique [4]. (B) High-throughput seeding of aqueous droplets in a hexadecane oil (stabilized by 2% Span 80) is accomplished rapidly (~ 2 min) with density based droplet settling (1.00 to 0.77 g/cm^3). (C) Once excess droplets are washed away (~10 min), hexadecane without surfactant is added to dilute the existing surfactant and promote adsorption of the content of the droplet onto the silicon surface (minimum incubation of 60 min). (D) To gain access to the underlying deposited droplet sample, the wafer is exposed to cycles of 175° C and room temperature to weaken the SU8-silicon bond. Afterwards, the SU8/PDMS piece can be easily lifted off the silicon with minimal residue left behind.

Span 80, hexadecane, and 2,5-dihydroxybenzoic acid from Sigma Aldrich (St. Louis, MO, USA).

Device Fabrication

The SU8 microfluidic mold was fabricated on a four-inch diameter silicon wafer using the following procedure: (1) O_2 plasma treatment at 700 mTorr for 5 min. (2) Dehydration at 90º C for 10 min. (3) Spin coat a 45 µm thickness layer of SU8 2075 (Microchem, Westborough, MA, USA). (4) Soft bake at 95º C for 5 min. (5) UV exposure through a transparency mask (CAD/ART Services, Bandon, OR, USA) for 250 mJ/cm^2. (6) Post exposure bake at 90º C for 10 min. (7) Development in SU8 developer (Microchem), spray IPA rinsed, and N_2 gun dried. (8) O_2 plasma treatment at 700 mTorr for 5 min. (9) Chemical vapor deposition of FOTS in a desiccator under house vacuum for 1 hour. The resulting SU8 mold was used to cast the corresponding polydimethylsiloxane (PDMS) microfluidic device using traditional PDMS fabrication protocols.

The SU8 microwell array was fabricated on a silicon wafer using the following procedure: (1) Dehydration at 90º C for 3 min. (2) Spin coat a 45 µm thickness layer of SU8 2075. (3) Soft bake at 75º C for 5 min. (4) UV exposure through a transparency mask for 250 mJ/cm^2. (5) Post exposure bake at 75º C for 5 min. (7) Development in SU8 developer, spray IPA rinsed, and N_2 gun dried. The reduced bake temperatures are necessary to realize a strong PDMS-SU8 bond.

To bond the PDMS microfluidics to SU8 microwell array we followed a previously published protocol [4]. The PDMS was treated with a nitrogen plasma at 500 mTorr for 5 min. Then, the PDMS was aligned and placed into contact the SU8 microwell array and baked on a hot plate for 1 hour at 100º C.

Device Operation

Thousands of droplets were injected simultaneously into a PDMS flow cell and, due to the large density difference between the oil and water, they were quickly assembled in parallel into SU8 defined microwells (Figure 2). We settled ~ 2,000 droplets in less than 2 minutes. Due to the simplicity of passive density based settling, this approach can easily be scaled to tens or hundreds of thousands of droplets without appreciably increasing the seeding time. Once droplets were settled atop the silicon wafer and excess droplets were removed, a surfactant free oil was injected to dilute and remove existing surfactant from the droplets and incubated for 1 hour to promote adsorption of their contents onto the silicon wafer. Longer incubation times will likely be required for higher sensitivity applications.

Critical to our platforms operation, we designed a sealed microfluidic device - stable at high-flow rates and in the presence of concentrated surfactant – that can be opened up on-demand for laser desorption ionization mass spectrometry (LDI-MS) interrogation of droplet contents. To gain access to the microarray below the microfluidics, we utilized thermal expansion of SU8 by heating the device from room temperature to 175º C for 30 s, and then back to room temperature. By expanding and contracting the thin SU8 layer, we weaken the non-covalent association with the silicon wafer. This process was repeated at least three times. Tweezers could then be used to easily peel off the SU8/PDMS structure, leaving behind the microarrayed contents of the droplets on the silicon wafer.

RESULTS

As a proof of principle, we settled droplets containing 83 µM dextromethorphan and 23 g/L 2,5-

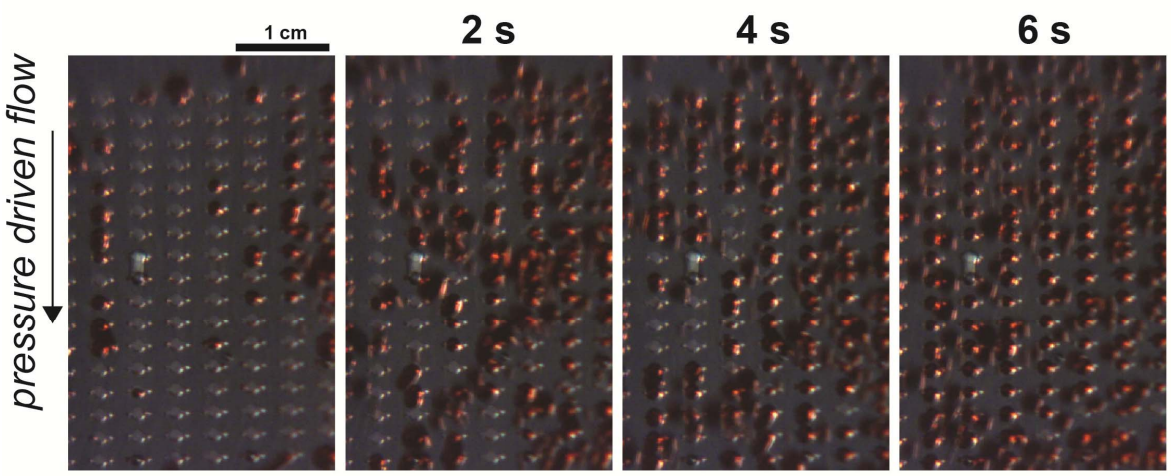

Figure 2: Microwell based droplet settling is accomplished rapidly across the large array. This sequence of photos demonstrates the settling of 70 empty microwells within 6 seconds of pressure driven flow. Due to the nature of this approach, it can be easily scaled to trap thousands of droplets with relative ease. In oils denser than water, e.g. HFE 7500 (1.5 g/cm^3), the settling process is easily completed by inverting the device (data not shown here).

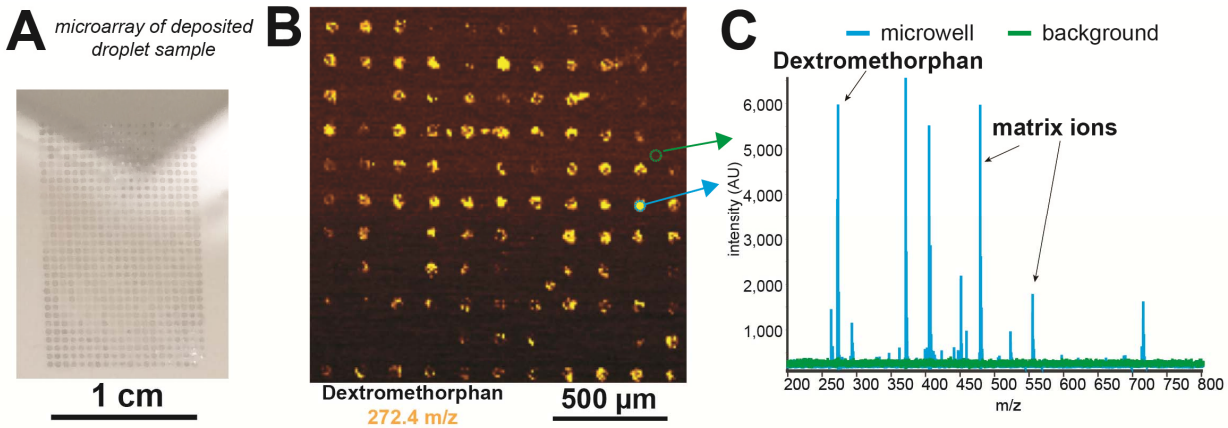

Figure 3: Deposited samples from droplets that contained 83 µM dextromethorphan and 23 g/L 2,5-Dihydroxybenzoic acid (MALDI matrix) on (A) the silicon substrate is (B) imaged at a resolution of 35 µm per laser shot with MALDI. Yellow color intensity indicates the relative abundance dextromethorphan (272.4 m/z). (C) Clear ions are detected in the microwell regions, and minimal signal is detected outside of the pattern.

dihydroxybenzoic acid (a MALDI matrix) and performed mass spectrometry analysis on the deposited sample using an ABSCIEX 4800 MALDI mass spectrometer. Mass spectrometry imaging (Figure 3A) with a 35 µm pixel spacing showed that the detected sample was concentrated in the microarray pattern. In future implementations, we will apply the matrix onto the microarray directly using a matrix sprayer [5].

DISCUSSION

We have developed a simple low infrastructure platform that integrates droplet microfluidics with MALDI analysis. The parallel nature of droplet microarraying in this approach makes it amenable to massive scaling towards analyzing hundreds of thousands of droplets with mass spectrometry.

ACKNOWLEDGEMENTS

The DOE Joint BioEnergy Institute are supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-FC02-07ER64494 and through contract DE-AC02-05CH11231, respectively.

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