SMART OPEN MICROFLUIDICS: AN AUTOMATED PLATFORM FOR THE DYNAMIC GENERATION OF FLUIDIC STRUCTURES DOWN TO THE SUB-nL-RANGE

T. Gleichmann[1], L. Gutzweiler[1], P. Koltay[1,2], R. Zengerle[1] and L. Riegger[1,2]
[1] University of Freiburg, Lab for MEMS Applications, Freiburg, Germany
[2] BioFluidix GmbH, Freiburg, Germany

ABSTRACT

We developed an open microfluidic (OM) [1] platform for the computer-assisted generation of fluidic structures virtually on demand. On using a set of piezo-driven dispenser modules, namely PipeJet™ [2] and Nano-Jet [3] (BioFluidix GmbH, Freiburg, Germany), and a peristaltic pump as well the system enables handling of liquids from the mL- down to the pL-range covering up to nine orders of magnitude in volume. The integration of multiple structuring methods like semi-contact writing (SCW) or non-contact dispensing allows for processing droplet arrays or continuous geometries of desired dimension, using low to medium viscous fluids and a number of different substrate materials.

KEYWORDS

Open microfluidics, non-contact dispensing, semi-contact writing, picoliter injection

MOTIVATION & INTRODUCTION

Since most microfluidic chips comprise a repertoire of common structures and operational units like reservoirs or channels one drawback is the necessity to structure such elements into silicon/glass or polymers. Thus, if a design flaw becomes obvious, redesign of the underlying structure is required and often accompanied with a time consuming or even expensive process chain.

Open microfluidics counteracts this disadvantage by creating fluidic elements on an open surface providing the ability of dynamic structure design and modifications. For this purpose we developed an integrated device to design, redesign or simply to repeat the application of almost arbitrary fluidic structures giving users the ability to test new fluidic approaches or to establish them using this platform.

While certainly not any microfluidic device or mechanism can be processed by such a system, a few promising applications have been identified covering PDMS prototyping, capillary gel electrophoresis or droplet-based PCR.

DEVICE CONFIGURATION

Serving as platform for process-automation, the 3-axis manipulator Biospot-BT600™ (BioFluidix GmbH, Freiburg, Germany), was modified towards OM-requirements (Fig. 1 A). The axes-bound printhead was equipped with a 5-dispenser-slot to install either PipeJet™- or Nano-Jet-modules in accordance to properties and volumes of desired liquids (Fig. 2 B). Based on a piezo-driven piston droplets are released *via* direct liquid displacement either through a polyimide tube nozzle (PipeJet™) of varying diameter addressing the nL-range from 200 nL to about 2 nL or a silicon micro-machined nozzle of a chip (Nano-Jet) with nozzle-diameters of 100x70 µm² down to 10x20 µm² covering volume-ranges of several 100 pL down to 30 pL. While PipeJet™- and Nano-Jet-modules address the range from nL to pL, larger volumes can be released by an integrated peristaltic pump exhibiting a flow rate up to 50 ml/min (Fig. 1 C).

Moreover, single dispenser can be moved pneumatically along a second z-axis to handle several PipeJet™-modules simultaneously required for semi-contact writing as explained later on [4][5].

Two high-resolution cameras (5 Mpx) were introduced for process observation but also giving the opportunity to acquire visual data. The substrate – a planar polymer or glass slide for instance – is mounted on the substrate carrier which encloses a FPH1-1270AC Peltier element (Z-Max Co. Ltd., Tokyo, Japan) to adjust the surface temperature between -5°C and 60°C. As surface-cooling helps to reduce evaporation, possible issues due to condensation are avoided by a dew-point-based feedback-regulation. Heat is drained both passively and actively by a heat-sink and a multi-fan rack as well. In order to ensure a planar processing surface the substrate is fixed by vacuum aspiration. A three-point screw adjustment with fine threads enables substrate leveling to set a constant dispenser-to-substrate distance supported by lateral camera-observation along the x-y plane.

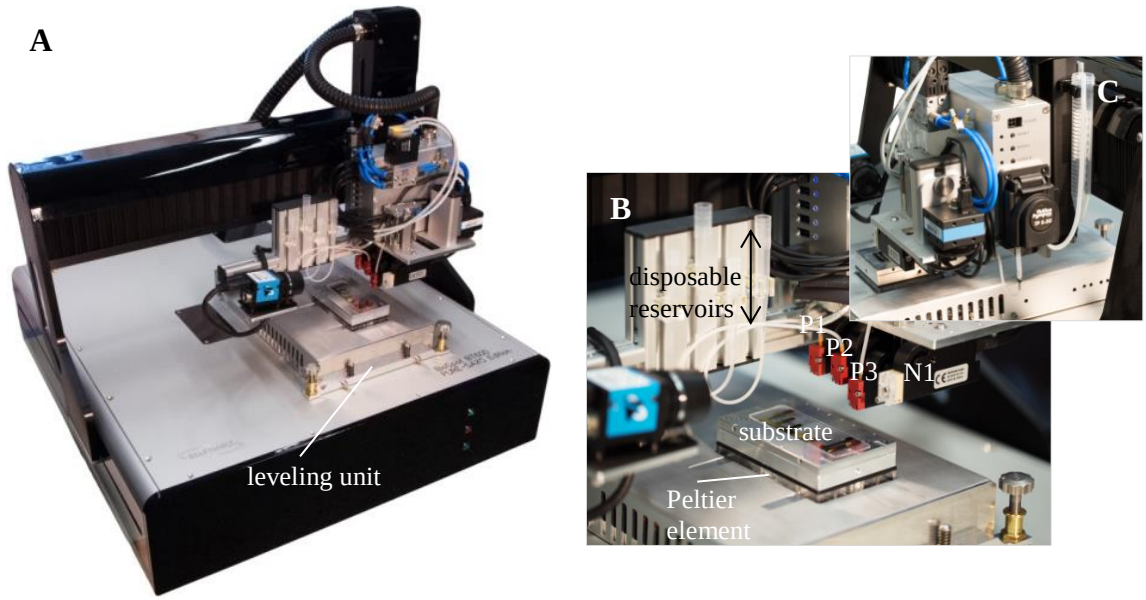

Figure 1: Custom made liquid handling device configured for electrophoretic applications. (A) Total view of the processing platform: a BioSpot-BT600™ (Biofluidix GmbH) serves as basic 3-axis manipulator. Substrate carrier is fixed by a spring-loaded passive 3-point leveling unit. (B) Close-up of the OM-adapted printhead and substrate carrier including a pL-nL-dispenser rack (1x fixed PipeJet™: P1, 2x lowerable PipeJet™: P2/P3, 1x Nano-Jet: N1, 1x open slot), an associated reservoir holder and a high-resolution camera for vertical alignment. Planar substrates are fixed by vacuum aspiration on a temperature-adjustable aluminum surface (88x54 mm). (C) Peristaltic pump with disposable reservoir for high-volume supply and second camera for process observation and horizontal alignment.

PROCESSING

System Initialization

Desired substrates with a maximum area of 88x54 mm² (just restricted by current design) are mounted onto the substrate carrier workspace. Vacuum aspiration is turned on to fix flexible substrates onto the metal surface. Dispensers are supplied with liquids *via* silicone tubing and standard pipet tips as reservoirs for PipeJet™-modules, the integrated reservoir of the Nano-Jet cartridge (80 µL) or 15-mL Falcon tubes connected to the pump. Especially when working with multiple PipeJet™-modules and tubes of different length an adequate z-alignment is important. Working with multiple fluids one would like to stack structures or inject droplets into an existing reservoir or channel, inherently possible with the presented device. For this purpose, a precise x-y alignment of each dispenser module to a reference point and thus to each other is conducted. Both vertical (z) and horizontal alignment (x-y) can be realized by camera-based optical inspection or image pattern recognition.

Fluidic elements are designed using an integrated visual batch-processor were the user is able to define droplet-, plane- or channel-based structures or even to combine them. Once the desired surface temperature is reached the batch can be executed to process the fluidic elements previously defined.

Generating Fluidic Structures

Fluidic structures are applied onto substrates either as single droplets of dynamic volume dispensed in a non-contact manner or deposited forming a liquid bridge between the pipe-nozzle of a PipeJet™-dispenser and the substrate surface (Fig. 2). The later method, namely SCW enables the generation of arbitrary planar structures as channels or filaments by continuous capillary flow out of the dispenser nozzle while displacing the axis-mounted dispenser. Usually supported by a software integrated batch processor, even more complex structures as grids or reservoir coupled injection channels as well as arbitrary sized droplet-arrays can be generated (Fig. 3). Moreover, planar filled areas of particular extension are applied by writing overlapping channel-structures side-by-side. Hydrostatic pressure can be adjusted by raising or lowering dispenser-associated reservoirs. By additionally adapting displacement velocity the flow rates affecting channel diameter can be set dynamically. Especially when handling aqueous solutions in the sub-µL range evaporation might cause issues as open fluidic compartments become unstable. For this purpose, if necessary, OM structures are

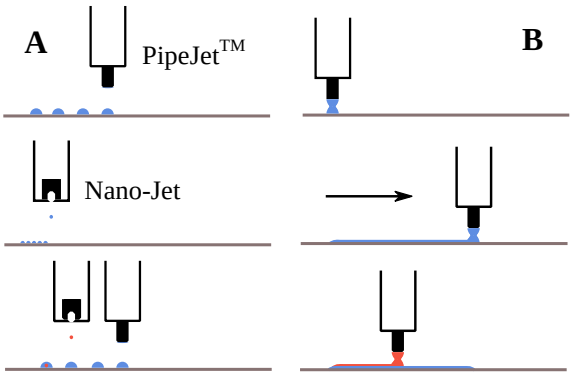

Figure 2: Strategies for open fluidic structure design. (A) Droplet ejection in non-contact mode either by PipeJet™ or Nano-Jet dispenser. The bottom sketch exemplifies a combined use of both dispenser types: pL-injection into nL-droplets. (B) Once a liquid bridge is formed (top) between the substrate and the pipe-nozzle dispenser displacement allows for the generation of continuous structures (middle). Stacking of structures (bottom) is used to protect functional fluidic elements from evaporation or to enlarge dimensions.

covered with immiscible liquids like protective oil layers deposited by the peristaltic pump supplementary to the Peltier-cooled surface.

APPLICATION EXAMPLES

To demonstrate system capabilities we designed and processed a variety of structures of different size and geometries using SCW, non-contact dispensing or droplet injection. We combined aqueous solutions, oil and gel-like fluids upon polymer or glass substrates.

Figure 3 exemplifies two common structures. An alginate-grid was designed to form squared traps for cell-culturing (Fig. 3A) by SCW (tube diameter: 200 µm). Reducing the pitch down to 200 µm between two parallel channels results in stable squares with wall heights of about 7 µm (width: 100 µm / 10 stacks) after processing and up to 50 times higher after rehydration in a CaCl-solution (alginate-structure previously dried). DMSO was used to print a 19x22 droplet array by non-contact dispensing with a 20x40 µm² nozzle-chip of a Nano-Jet-module at a nozzle-to-surface distance of ~1 mm (Fig. 3B). Volumes of single droplets were determined by gravimetric measurement techniques as shown by *Liang* et al. [5].

Finally, combined PipeJet™ and Nano-Jet droplet dispensing was used to demonstrate the ability for OM droplet-based PCR by droplet encapsulation. An arbitrary sized array (here 1000) of oil droplets (~5 nL each) was applied on

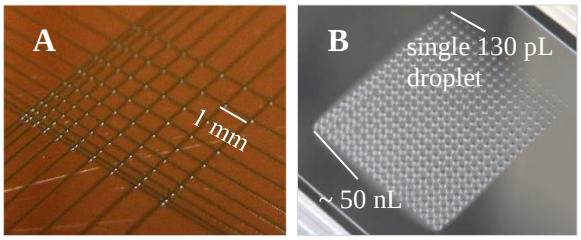

Figure 3: Examples of open microfluidic structures generated with the presented device. (A) Semi-contact written grid structure of alginate on polyimide used as delineation for cell culturing. (B) DMSO volume-gradient array on cooled cyclic-olefin copolymer foil (~10 °C). Array was generated in non-contact mode using a Nano-Jet-dispenser. Droplets are multitudes of 130 pL starting with n=1, where n is the number of single-spot dispensed droplets, up to n=400 corresponding to an overall volume of about 50 nL. For purpose of illustration a black polyimide foil was inserted between substrate foil and mounting plate.

standard microscopic glass slides first using a 200 µm tube of a PipeJet™-dispenser (Fig. 4A). Simulating the PCR-reaction mixture a Nano-Jet

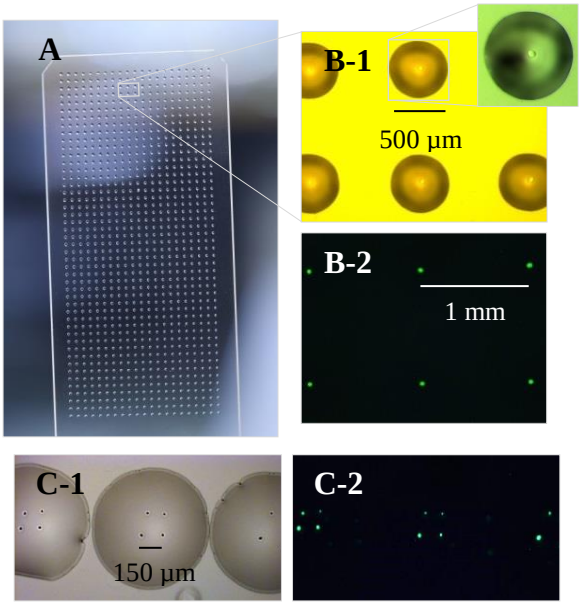

Figure 4: Precise nL-injection of an aqueous Fluorescein solution into oil arrays printed on standard microscopic glass slides. (A) 1000x-spot array applied with a PipeJet™; 0.5 mm nozzle-to-surface distance. (B-1) Close-up of (A) at incident light acquired by inverted fluorescence microscopy. (B-2) Close-up of (A) without incident light and fluorescence excitation of the enclosed ~60 pL spots previously injected by a Nano-Jet-dispenser. (C-1)(C-2) Nested arrays demonstrated by inverted fluorescence microscopy. The continuous shift between the upper and lower row might be owed to an axis backlash which can be compensated by optimizing the batch processing.

equipped with a 20x20 µm² nozzle was used to inject an aqueous solution of about ~60 pL into the oil droplets forming distinct phase boundaries (Fig. 4B-1, close-up). Here, we used a 3 µM Fluorescein solution from Fluorescein sodium salt (Sigma-Aldrich Inc., Saint-Louis, USA) for better visualization of the injection precision, exciting and observing the oil-enclosed droplets with a OLYMPUS CKX41 (Olympus Corporation, Tokyo, Japan) inverted fluorescence microscope (Fig. 4B-2). Increasing the level of complexity nested arrays of oil and the mentioned Fluorescein solution were processed (Fig. 4 C-1 & C-2), thus one can imagine to place several compartments of a single reaction next to each other. The reaction is started by fusing those compartments by gentle vibrations. Due to the integrated Peltier-cooling down to sub-zero degrees nested arrays of such a composition could be frozen (using DMSO for cryo-conservation in case of proteins or cells) and stored subsequently at e.g. -80 °C for later use.

CONCLUSION & OUTLOOK

Taken together the OM-platform presented here provides a plurality of integrated and combinable features to handle liquids. Among others:

- three species of liquid dispenser covering 9 orders of magnitude in volume
- on demand design of fluidic structures in non-contact or semi-contact mode
- processing liquids of various physico-chemical properties like low to medium viscosity
- using substrates of diverse materials in accordance with the demands of the user e.g. to process microarrays

Based on the work of *Tanguy* et. al. [6] open capillary gel-electrophoresis of proteins should be realized in the near future using the open microfluidic platform. Further applications might include medium to high-throughput droplet-based PCR – even of single cells [7] – using droplet-injection as demonstrated here.

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CONTACT

* T. Gleichmann tobias.gleichmann@imtek.de