DROPLET-ON-DEMAND PLATFORM FOR BIOCHEMICAL SCREENING and DRUG DISCOVERY

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ABSTRACT

We present new biochemical applications for an automated droplet screening system (the Mitos Dropix). This droplet-on-demand robotic platform was previously shown to perform automated dilution gradients that can be detected by absorbance or fluorescence in a time-resolved fashion without the need for a chip. We now present reproducibility data for the Dropix and show that it can be integrated with microfluidic devices to merge and split specific sequences of droplets. It can also be used to encapsulate cells and multi-cellular organisms.

KEYWORDS: Droplets, Dropix, Cells, Organism, Microdroplets, Merging, Droplets-on-demand

INTRODUCTION

Our droplet-on-demand robotic platform (Dropix) was shown to generate high quality kinetic data from nanolitre volumes [1]. The instrument has now been interfaced with microfluidic designs that allow merging and splitting of long droplet sequences, bringing to bear high control and versatility on compound screening applications. Previously, in-line merging was limited to specific fluidic parameters. The new set-up can generate large sequences (up to 500 droplets) of droplets (1-50 nL) from multiple samples linked by a merging chip, thus allowing access to large numbers of different reagent combinations. The platform precisely controls droplet size, frequency, content and spacing. The droplets are directly fed into a microfluidic chip thereby keeping spatial encoding of the sequence. Interfacing with microfluidic chips allows for a much wider range of applications: merging, dilution [2], splitting, on-chip incubation or immobilization and analysis. Aside from drug discovery and enzymatic reactions, this novel platform can be used for controlled encapsulation of cells under a range of conditions. Here, we show the applicability of the platform to encapsulate cells (*S. cerevisiae*) and multi-cellular organisms (*C. elegans*).

EXPERIMENTAL

The method for generating specific sequences of droplets using our Dropix system was previously described [1]. We now show that the Dropix can be used in several modes to enable a wider range of applications (Figure 1). Mode 1: Droplet sequence production or droplet library generation. Sequences of droplets can be generated from 24 different fluids. Mode 2: Mixing or concentration gradients from 2 or more wells using a merging chip. Utilizing a droplet merging chip, ratiometric mixing from up to 24 wells, results in up to 300 droplet combinatorial reactions. Mode 3: Either in-line with mode 2, or independently, mode 3 extends Dropix from nanolitre technology to picolitre technology. This is most effectively achieved with the use of a droplet production microfluidic device.

978-0-9798064-7-6/μTAS 2014/$20©14CBMS-0001 1214

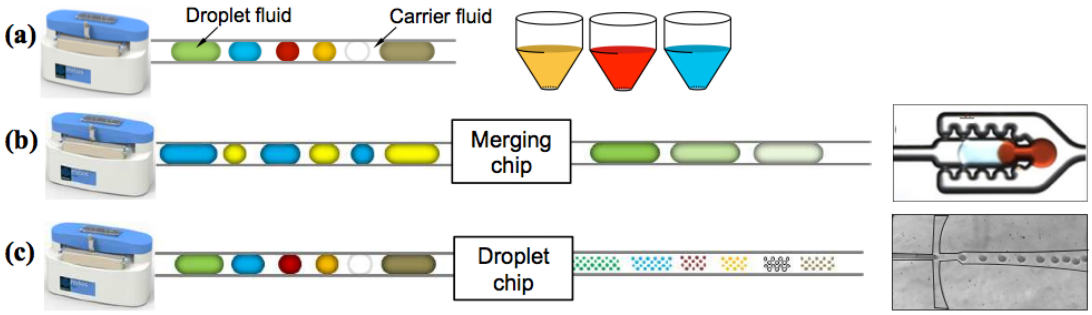

Figure 1: The Dropix can be used in 3 modes. (a) for the generation of droplet sequences, (b) for merging pairs of droplets and (c) to split droplet sequences into smaller droplets through a standard flow focusing chip.

RESULTS AND DISCUSSION

We assessed the reproducibility of droplet volume and repeated sequences (Figure 2). Previously, we relied upon measuring the size of each droplet after their formation, to specifically determine the volume and final concentration of merged droplets. Here, we calibrate our instrument so that 10 droplet repeats of 11 different target volumes correlated with the measured droplet volumes: precise volumes of droplets (± 4%) are now generated (Fig. 2a). This translates also in reproducibility of specific sequences of different contents and different volumes (Fig. 2b).

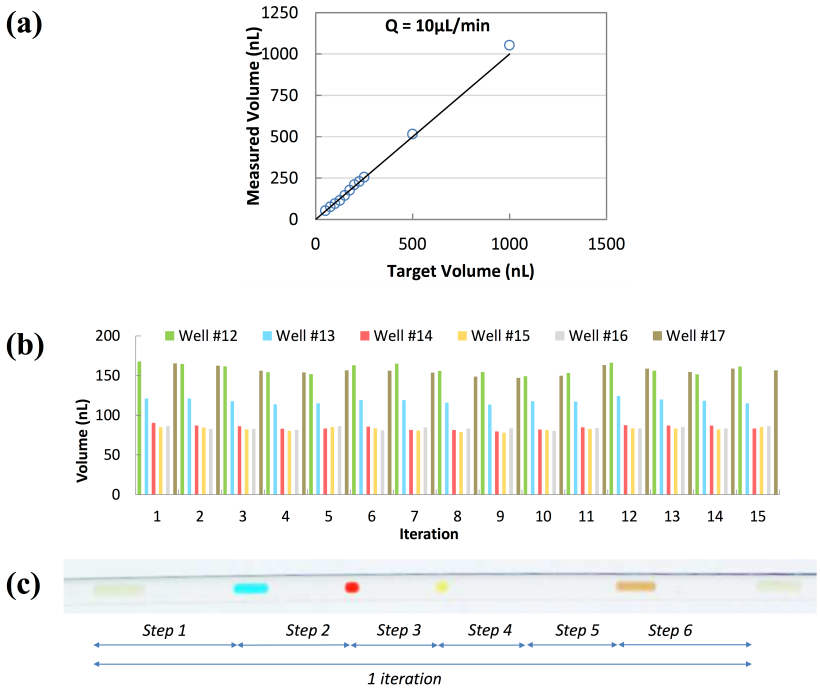

Figure 2: Calibration and reproducibility of multiple iteration. 10 droplets of 11 target volumes were generated and show good correlation with the measured volume (a) 15 repeat steps of sequencing 6 reagents over approximately a 5 minute period (b). Target volumes are: green (200 nL), blue (150 nL), red (100 nL), yellow (100 nL), clear (100 nL) and brown (200 nL). Image of the droplets sequence generated in the tubing (c).

The Dropix was use to encapsulate pools of engineered *S. cerevisiae* that were used to screen for anti-parasitic compounds [3]. The cells expressed DHFR proteins from *Homo sapiens* (^{Hs}DHFR), *P. falciparum* (^{Pf}DHFR), pyrimethamine-resistant *P. falciparum* (^{PfR}DHFR) and *Schistosoma mansoni* (^{Sm}DHFR) and each expressing a specific fluorescent protein (mCherry, Sapphire, Venus and CFP, respectively and GFP for control *S.cerevisiae*). As seen in Figure 3(a), the cells showed good growth for all cell strains overnight. *C. elegans* were also encapsulated and were stable in droplets overnight (Figure 3b).

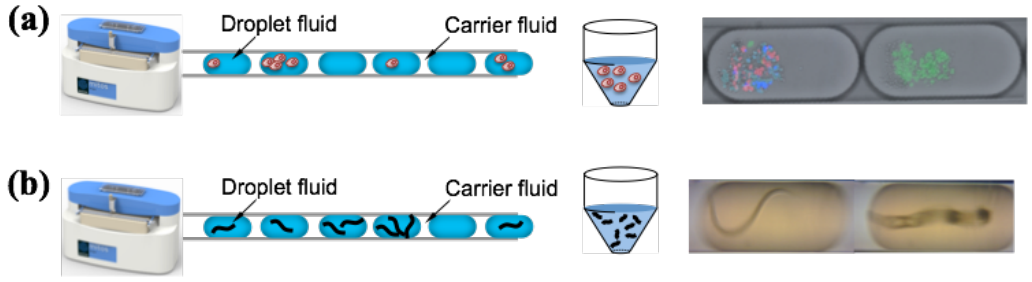

Figure 3: Cells and multi-cellular organism can be encapsulated. Pools of fluorescently-labeled yeast cells can be merged to follow growth curves (a). C. Elegans can be encapsulated and immobilized on chip for analysis (b).

CONCLUSION

Merging yeast cells with different drugs has the potential to show different growth patterns of pooled yeast strains. This will be a valuable tool for drug selectivity studies [3]. *C. Elegans* can be encapsulated using our system, and merging encapsulated organisms with drugs could be use to study effects on neural mechanism. Our system enables combinatorial screening in nanolitre volumes and we have demonstrated its application to drug screening whether *in vitro* or *in vivo.*

ACKNOWLEDGEMENTS

This work was supported in part by the EPSRC in part by The Dolomite Centre. The authors thank the University of Cambridge for use of its instruments and facilities.

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