A HIGH THROUGHPUT DROPLET-BASED MICROFLUIDIC BARCODE GENERATOR

Y. Ding[1], S. Stavrakis[1], X. Casadevall i Solvas[1], and A. J. deMello[1,*]

[1]Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland

ABSTRACT

The labelling (or barcoding) of microdroplets so that each unit can individually be identified and monitored is an important missing link for the application of droplet-based microfluidic platforms in high throughput chemical and biological assays. We present an efficient droplet barcode generator based on a simple T-junction design modified with four aqueous phase inlets. Codes are generated by combining two fluorophores at different concentrations, which is achieved by dynamically adjusting the ratio of the four aqueous flow-rates (two dyes, two buffers) using programmable syringe pumps. In a proof-of-concept experiment over 500 distinguishable codes were generated in less than 20 seconds.

KEYWORDS: Barcoding, Fluorescence, Microdroplet, Microfluidics

INTRODUCTION

Droplet-based microfluidics has the potential to revolutionize high throughput chemical and biological assays. Of high current concern is the ability to identify and monitor each individual droplet within a large droplet population during a complex experimental process, *i.e.* barcoding. Currently, the most reliable barcoding strategies involve dosing fluorescent species, such as quantum dots, fluorescent dyes, photonic crystals, lanthanide and nanophosphors into droplets. Several microfluidic barcoding methods have recently been demonstrated [1–5], with a maximum of 100 barcodes being produced during one processing step (without switching samples) [3]. Despite these achievements, significant effort is still required to increase the number of individually recognizable barcodes for high-throughput applications. Progress in this task requires improvement in labeling efficiency and simplification of the barcode generation and detection processes. Herein we present a simple and efficient microfluidic method capable of generating over 500 uniquely barcoded droplets in less than 20 seconds by combining distinguishable concentrations of two fluorescent dyes.

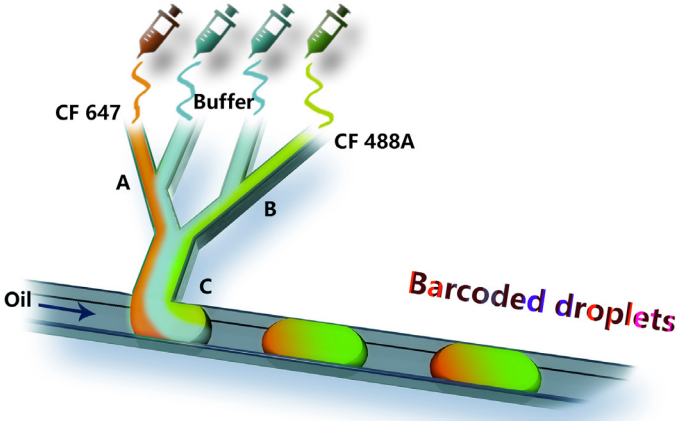

Figure 1: Schematic of the droplet barcode generator

EXPERIMENTAL

Droplets were generated using a PDMS microfluidic device containing a T-junction. The device was fabricated by using standard soft lithographic techniques. The design of the device and its operational principle are illustrated in Figure 1. The structure comprises four aqueous inlets and one oil inlet. The inlet flows of two fluorescent dyes (CF 647 and CF 488A - Biotium, Hayward, USA) were first paired with an individual buffer flow (PBS 1X, pH 7.4, Invitrogen, UK) each (Figure 1, A and B), before being joined together upstream of the droplet generator (Figure 1, C). These aqueous flows were generated using four neMESYS syringe pumps programmed to dynamically change the ratio of flow rates, whilst ensuring that the total aqueous flow-rate at C was constant. FC-40 (Sigma Aldrich, Dorset, UK) was used as the continuous oil phase to shear off the aqueous stream and form isolated droplets. Since the flow upstream of point C was laminar, the aqueous phase was only mixed after droplet formation. Once generated, each code (defined by the fluorophore payload) was read-out using a high-speed fluorescence camera (Orca Flash 4.0, Hamamatsu) combined with a dual light splitter to allow simultaneous imaging of two colors in the same frame (Figure 2). The green signal was recorded on the left half of the image and the red signal on the right half. The fluorescence intensities of the two signals for each droplet were later measured using a custom ImageJ macro.

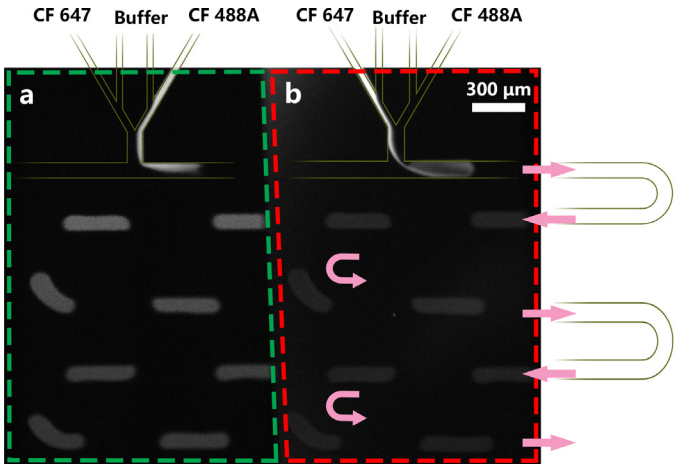

Figure 2 Fluorescence image of barcoded droplets. The left half of the frame (a) records the green signal and right half (b) records the red signal. Channels and the direction of flow are marked by lines and arrows respectively.

RESULTS AND DISCUSSION

In a proof-of-concept experiment 615 barcoded droplets were generated with a cycle time of 20 s. The flow-rate profiles programmed on the pumps of the four aqueous phases are shown in Figure 3a. To individually dose each droplet with a unique combination of concentration of fluorophores, the red dye flow-rate was linearly increased while the green dye flow-rate oscillated periodically. By adequately regulating the additional buffer inputs the total flow-rate was kept constant. Theoretically this strategy can deliver all possible combinations of concentrations of two dyes within a single cycle, *i.e.* for two processed colors and N recognizable intensity (or concentration) levels, N^2 unique codes can be generated. The success of this approach relies on two key factors. First, the actual flow-rates at the meeting point (C) need to match their defined values in the pumps. This is mainly determined by the performance of the pumps and it is also affected by the properties of the syringes, tubing and chip (*e.g.* soft elements could induce unpredictable lag effects on a dynamic flow). Herein glass syringes and hard tubing were used to reduce these effects to the largest possible extent. Second, in order to reduce the amount of droplets with similar fluorophore concentrations (*i.e.* with identical barcodes) and facilitate post-processing of the sample, the droplets produced need to be monodisperse. To assess this potential hurdle, the size (pixel area) of generated droplets was measured. The results show that the droplets obtained with this method were highly monodisperse (CV < 2%, Figure 3b).

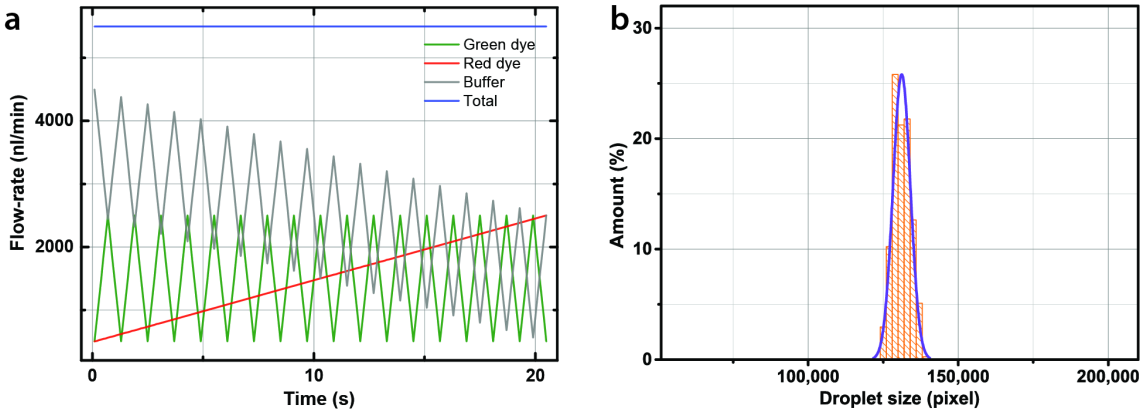

Figure 3: a) Programmed flow-rate profile during droplet generation.b) Size distribution for the population of generated droplet.

Figure 4 shows the fluorescence intensities (that report fluorophore concentration) for each droplet during a barcoding cycle. As expected, the generated codes fit the programmed flow-rates, with small deviations due to the variation-inducing factors described above. In particular, the concentration of green dye seems to fit well with the pump program. This may be due to the faster rate of change of the flow-rate applied by the green pump (when compared to the red one), which may contribute to reduce environmental instabilities.

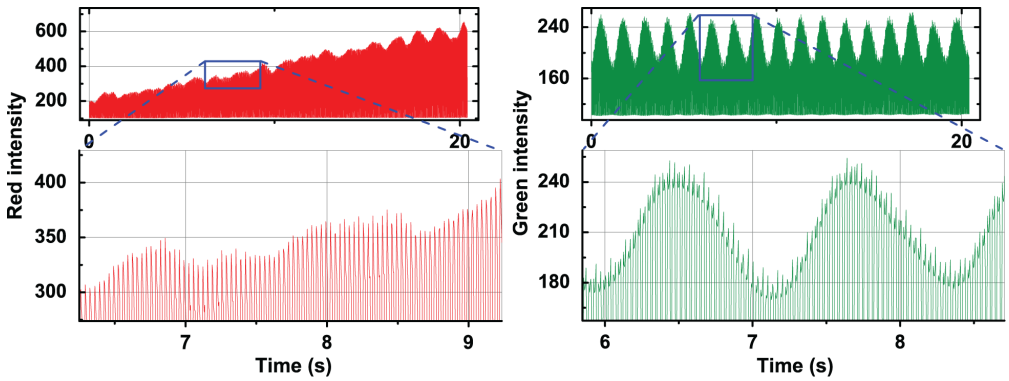

Figure 4: Variation of red- and green-channel intensities during a single droplet generating cycle.

A distribution map of the 615 barcodes obtained is shown in Figure 5. Under ideal conditions, the dots (codes) should distribute on the map evenly. The actual code map, though, has presents a higher density of dots on both the top and bottom areas. That is because the concentration profile of green dye (green curve in Figure 4) is more similar to a sine function than to the triangular function originally inputted on the pump (Figure 3a). Hence, smaller absolute values of the derivative exist near local maximums and minimums. Besides, both of the top right and bottom right corners are empty of dots, which is likely due to a slight desynchronization between the red and green dye inputs (*i.e.* the programmed red concentration suffers from a slight delay). Here, the sensitivity of the system is defined as the standard deviation of the fluorescence intensity of droplets generated by a single dye concentration ($\sigma = 1.17$). Based on a 99.7% confidence interval (3σ), 92.2% of the barcodes generated (567) were unique and distinguishable.

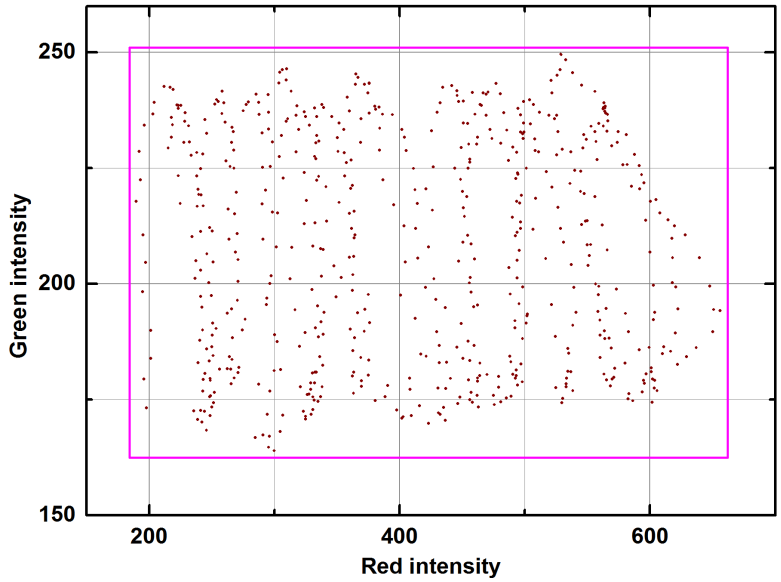

Figure 5: Distribution map of barcoded droplets. Each dot represents an individual barcode. The red value of a barcode can be read from the abscissa, and green from the ordinate.

CONCLUSION

We have demonstrated a simple and highly efficient droplet-based microfluidic barcode generator. Such barcoded microdroplets can potentially be stored as a library and subsequently merged with other sample-containing droplets, thus providing a means to label and identify individual experiments. These microdroplets might also be solidified by chemical crosslinking or photocuring to generate barcoded microparticles. By applying the same methodology to a mixture of four distinguishable fluorophores, we expect to produce in excess of one million unique barcodes in a simple and direct fashion in the future.

ACKNOWLEDGEMENTS

We would like to thank the support from China Scholarship Council (fellowship to Y.D.).

REFERENCES

[1] R.E. Gerver, R. Gómez-Sjöberg, B.C. Baxter, K.S. Thorn, P.M. Fordyce, C.A. Diaz-Botia, et al., *Lab. Chip*. 12 (2012) 4716–4723.
[2] Y. Zhao, X. Zhao, C. Sun, J. Li, R. Zhu, Z. Gu, *Anal. Chem.* 80 (2008) 1598–1605.
[3] X.-H. Ji, W. Cheng, F. Guo, W. Liu, S.-S. Guo, Z.-K. He, et al., *Lab. Chip*. 11 (2011) 2561–2568.
[4] S. Fournier-Bidoz, T.L. Jennings, J.M. Klostranec, W. Fung, A. Rhee, D. Li, et al., *Angew. Chem. Int. Ed.* 47 (2008) 5577–5581.
[5] X.-H. Ji, N.-G. Zhang, W. Cheng, F. Guo, W. Liu, S.-S. Guo, et al., *J. Mater. Chem.* 21 (2011) 13380–13387.

CONTACT

* A. J. deMello, Tel: +41 44 633 66 10; andrew.demello@chem.ethz.ch