MULTI-VOLUME DIGITAL PCR QUANTIFICATION WITH MICROFLUIDIC DROPLET PRINTING ROBOT

Wen-Wen Liu[1†], Ying Zhu[1†], Yi-Ming Feng[2], Jin Fang[2] and Qun Fang[1]*,

[1] *Institute of Microanalytical Systems, Department of Chemistry, Zhejiang University, Hangzhou, 310058, China*

[2] *Department of Cell Biology, China Medical University, Shenyang, 110001, China*

ABSTRACT

We presented a droplet-based multi-volume digital polymerase chain reaction (digital PCR) system for absolute gene quantification with wide and tunable dynamic range. Over one thousand droplets with volumes from 1.2 nL to 150 nL could be automatically generated in 8 min using a microfluidic droplet printing robot, achieving a wide dynamic range for digital PCR quantification of 357 to 3.7×10^6 molecules/mL. This system was applied in absolute quantification of PIK3CA gene.

KEYWORDS: Multi-volume, Digital PCR, Droplet, Gene Quantification

INTRODUCTION

Digital PCR has been shown to be a promising tool for profiling gene expression, studying gene variations, and sample preparation of next-generation sequencing. In digital PCR, sample would be randomly partitioned into hundreds to thousands of reaction chambers. And the number of target molecules approximately corresponds to the number of "positive" chambers utilizing Poisson Distribution. Most of current digital PCR systems reply on microfabricated microvalves, oil-separated microchambers, and continuous-generated droplets for large scale PCR reactions. However, these methods required complicated and expensive microfabrication. Large number of reaction chambers were needed to achieve high dynamic detection [1]. Here, we developed a low cost, droplet-based multivolume digital PCR method capable of quantifying gene with a high dynamic range of 357 to 3.7×10^6 molecule/mL, with a 10-fold decreased reaction number compared with single-volume method.

EXPERIMENTAL

As schematically drawing in Figure 1, specially-designed hydrophilic micropillar array were fabricated on a glass chip for rapid generating multi-volume droplet arrays, as well as robust droplet immobilization for thermal amplification. The patterned part on the chip was protected by chromium layer after photolithography in order to maintain inherent hydrophilic property of glass, while other exposed part became hydrophobic via surface modification.

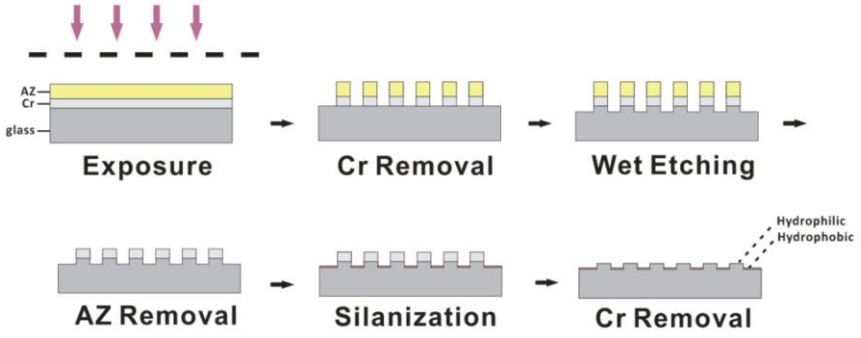

Figure 1. Schematic diagram of chip fabrication process.

The droplets were generated using our previously-developed droplet robot method [2]. The microfluidic droplet printing robot mainly composed by a capillary probe, syringe pump and x-y-z

translation stage. By combining the micropillar array chip with the robot system, droplets with different volumes could be automatically formed in high throughput (average 125 droplets/min) and high precision. Figure 2 shows bright field and fluorescent images of single volume droplet array and multivolume droplet array respectively, using sodium fluorescein as a model sample.

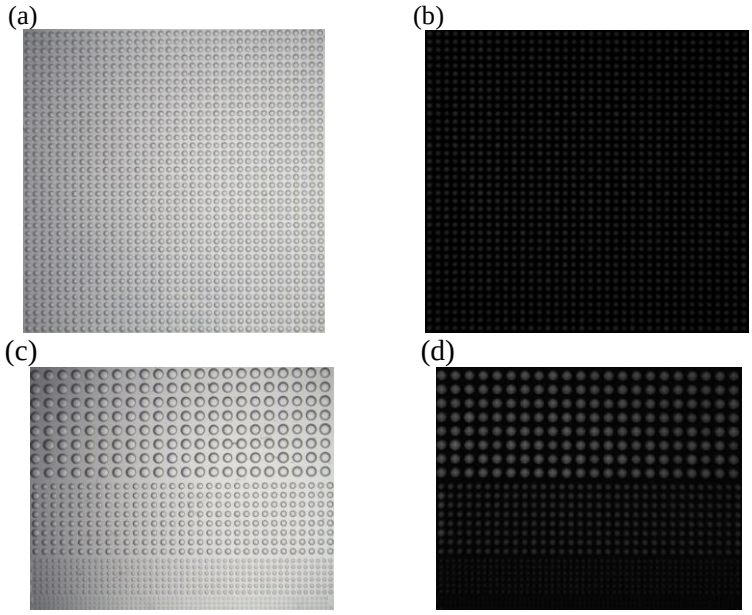

Figure 2. (a) Bright field image of a 10 nL droplet array (38×38). (b)Fluorescent image of the 10 nL droplet array (38×38). (c) Bright field image of a multi-volume droplet array. (d)Fluorescent image of the multi-volume droplet array.

RESULTS AND DISCUSSION

We preliminarily applied the system for quantification of PIK3CA gene (Figure 3). The sample solution was distributed into a multiple-volume droplet array with volumes of 1.2 nL, 6 nL, 30 nL, and 150 nL and corresponding number of 260, 294, 264, and 176. The end-point fluorescent image of the droplet array was obtained as shown in figure 3. After statistical analyzing the results of four-volume droplets, the quantification result were in agreement with theoretical value measured with spectrophotometry. We also demonstrated a high dynamic range of 4 orders of magnitude can be achieved with the system.

Figure 3. End-point fluorescent image of multi-volume digital PCR assay. Concentration calculated from Poisson equation is 1.9×10^4 molecules/mL.

CONCLUSION

In this work, we have developed a multi-volume digital PCR system for absolute gene quantification. The system is characterized as high precision, robustness, low cost, large dynamic range, and good compatibility with currently-used liquid-handling robots. In addition, the droplet array has a semi-open structure, which brings the potential of integrating sample pretreatment procedures on chip in the future.

ACKNOWLEDGEMENTS

Financial supports from Natural Science Foundation of China (Grants 21227007, 21475117, and 21435004) are gratefully acknowledged.

REFERENCES

[1] " Multiplexed Quantification of Nucleic Acids with Large Dynamic Range Using Multivolume Digital RT-PCR on a Rotational SlipChip Tested with HIV and Hepatitis C Viral Load," F. Shen, B. Sun, J.-E. Kreutz, E.-K. Davydova, W.-B. Du, P.-L. Reddy, L.-J. Joseph and R.-F. Ismagilov, JACS, 133, 44 (2011).

[2] " Sequential Operation Droplet Array: An Automated Microfluidic Platform for Picoliter-Scale Liquid Handling, Analysis, and Screening," Y. Zhu, Y.-X. Zhang and Q. Fang, Analytical Chemistry, 85, 14 (2013).

† Wen-Wen Liu and Ying Zhu contributed equally to this work.

CONTACT

* Qun Fang ; Tel: +86-571-88206771; Email: fangqun@zju.edu.cn