

SINGLE-MOLECULE PCR IN A PICOWELL ARRAY SIMULTANEOUSLY IMMOBILIZING PCR PRODUCTS TO A PDMS COVERSLIDE

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ABSTRACT

For the first time, we amplified single DNA-molecules (“Digital PCR”) randomly distributed in a picowell array and simultaneously immobilized the generated PCR-products to the surface of a PDMS coverslide (“solid-phase PCR”) which was used as sealing of the picowells during PCR. First, by this unprecedented technique PCR-products can be recovered for both, further reactions/analysis and counting the number of initial DNA-molecules from digital signals with a good signal to noise ratio of 10. Second, our experiments demonstrate the currently smallest low-volume on-chip PCR in an array of 18.5 pL wells. This may enable single-cell PCR experiments by filling PCR microreactors with truly single-cells due to geometrical constriction.

KEYWORDS: Digital PCR, solid-phase PCR, parallelization

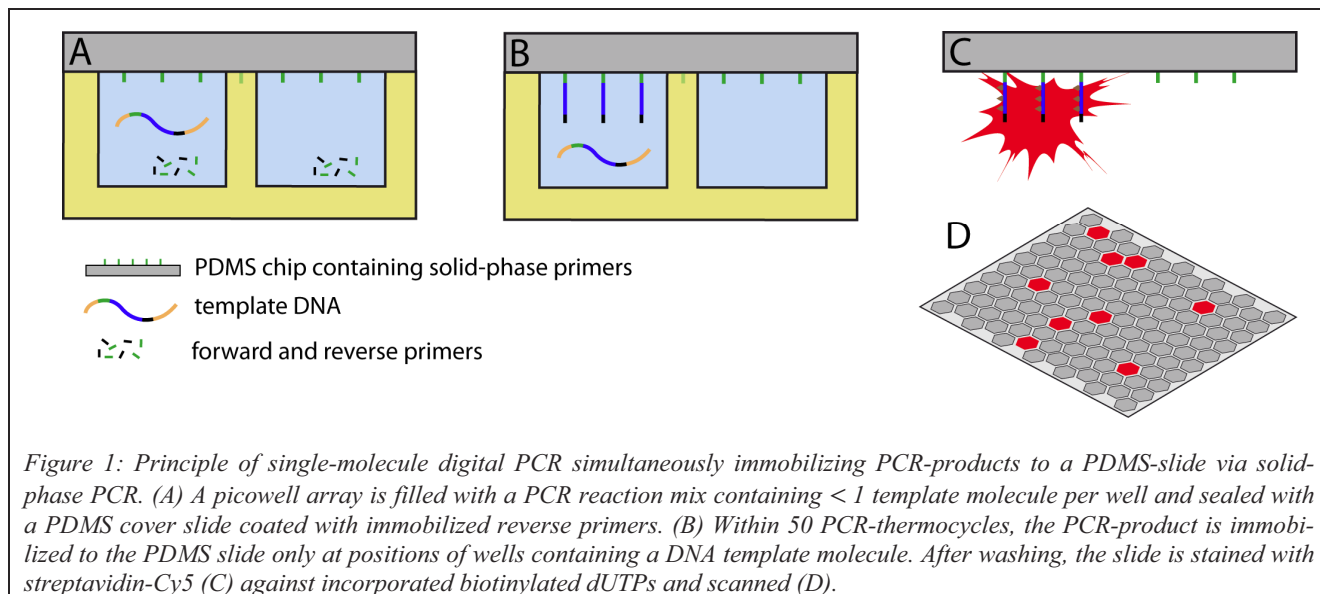
INTRODUCTION

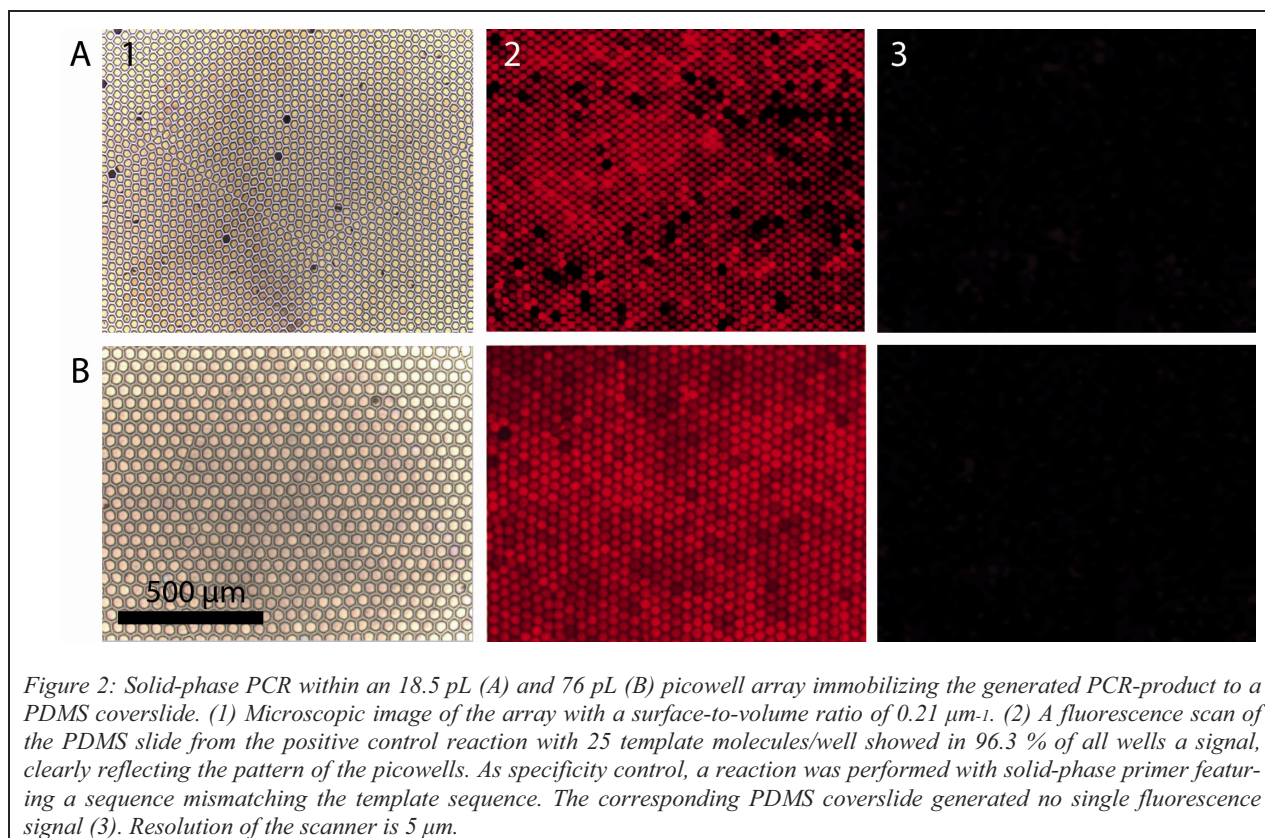
The first primer-directed PCR published in 1988 by Saiki *et al.* was performed in a reaction volume of 100 μ L [1]. Meanwhile, PCR reaction volumes have been scaled down (86, 23, 1.3 pL) while increasing the number of reaction wells (10^4), but PCR failed in volumes of 23 and 1.3 pL [2]. Among the major applications of low-volume PCRs in on-chip microreactors are Digital PCR [3,4] and single-cell analysis [5,6]. Significant shortcomings are inherent to the latter systems. First, PCR is often detected by TaqMan probes [5] or intercalating dyes [3], generating low and in some cases unspecific signals; second, low-volume PCR-products are discarded after PCR and cannot be further analyzed [3,5]. Our system overcomes these disadvantages by immobilizing PCR products spatially-resolved to a standard slide format for intensified visualization and a vast range of other downstream protocols.

REACTION SETUP

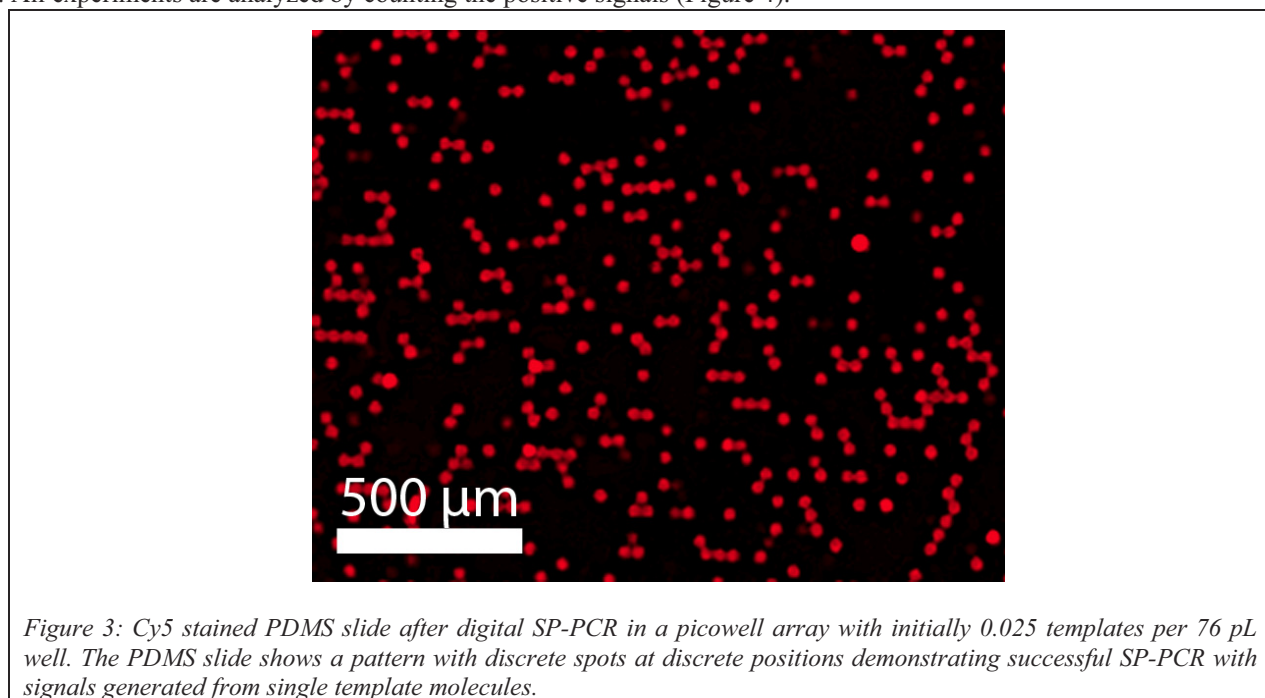
Solid-phase PCR (SP-PCR) is performed in glassy picowell arrays with well diameters of 29 μ m (18.5 pL) or 44 μ m (76 pL) (Figure 2, 1). A PCR mixture with 0.025 (digital PCR) or 25 (positive control) DNA molecules/well is centrifuged into the picowells, and sealed with a PDMS coverslide coated with solid-phase primers (Figure 1 A, B). After SP-PCR, the PDMS slide is detached, washed, stained, and scanned with a microarray reader (Figure 1 C, D).

EXPERIMENTAL RESULTS





First, we optimized the composition of the low-volume PCR reaction mixture for performing SP-PCR within on chip reactors. Careful balancing of BSA and Tween 80 enabled successful PCR within picoliters. SP-PCR with calculated 25 molecules/well generated a signal in 96.3 % of all wells on the PDMS chip showing the hexagonal pattern of the picowells (Figure 2, 2). No signal was generated on a PDMS coverslide coated with mismatching solid-phase primers (Figure 2, 3). Second, signals at discrete positions are obtained from statistically single molecules (Figure 3) with a signal-to-noise ratio of 10. All experiments are analyzed by counting the positive signals (Figure 4).



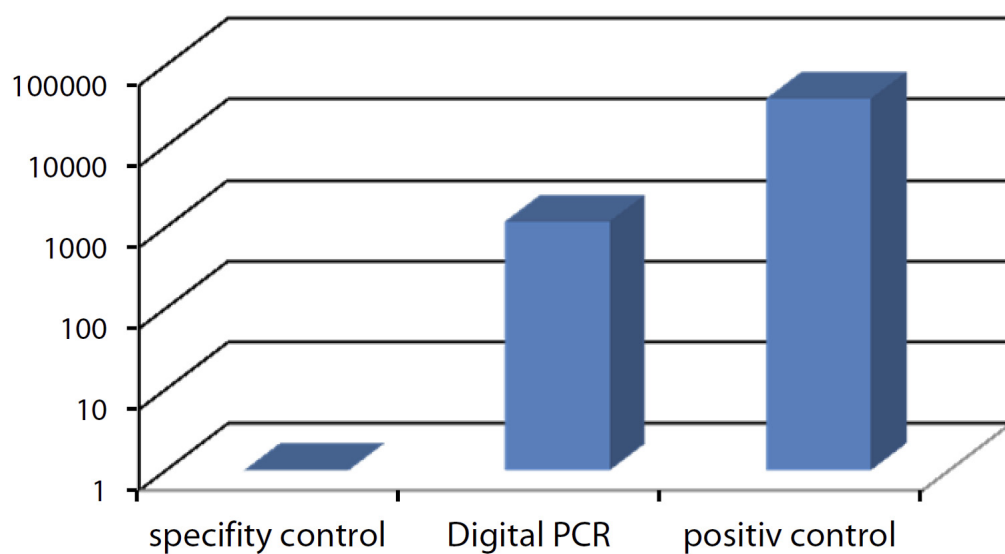


Figure 4: Analysis of Figure 2 and 3. Each array featured ~ 40.000 wells. No signals were obtained from the non-specificity control with a mismatching primer. In case of the positive control, the initially 1.000.000 DNA molecules yielded 96.3 % positive wells. In the case of digital SP-PCR, in total 1.000 DNA molecules were distributed in 40.000 wells in the array and we counted 1183 signals after SP-PCR.

CONCLUSION AND OUTLOOK

This work demonstrates two novelties. First, we performed low-volume SP-PCR in on-chip microreactors featuring the currently smallest reported volume of 18.5 pL. This can lead to highly parallel SP-PCR analytics for e.g. single-cell analysis [5] or digital PCR [3]. Second, we were able to immobilize PCR products from single molecules to a PDMS coverslide making them accessible for further downstream analysis. Novel applications can include the recovery of PCR-products from digital PCR chips from e.g. by Fluidigm [7] for downstream processing.

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