Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

A Microfluidic Alternating-Pull-Push Active Digitization Method for Sample-Loss-Free Digital PCR

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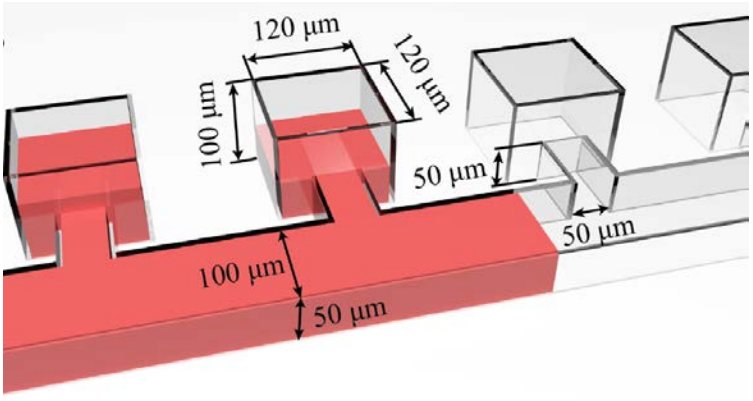

Figure S1. Dimensions of the microwells and the main channel in the µAPPAD device.

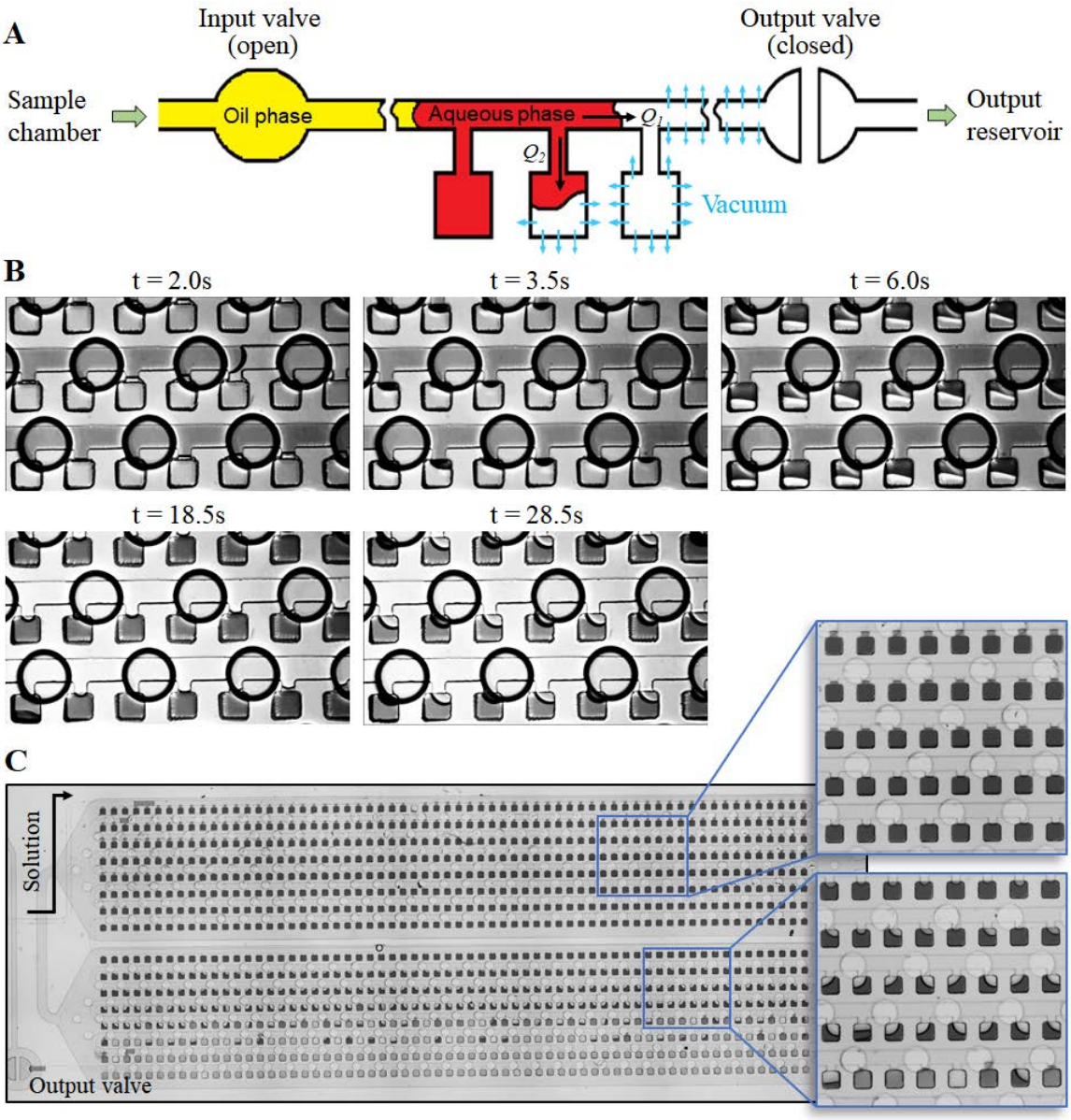

Figure S2. Dead-end filling of a limited sample volume on the μAPPAD chips. (**A**) Schematic of the dead-end microwell loading driven by the vacuum applied to the pneumatic chamber above the microwell array. The inlet valve is kept open and 2.5 μL aqueous solution held in the sample chamber is drawn through the channel and into the microwells at a volumetric flow rate of Q_1 and Q_2, respectively. The microwells are isolated by an oil phase immediately following the solution plug in the main channel. (**B**) Time-lapse images showing the process of incomplete microwell filling in a parallel-channel chip. The vacuum-driven flow of a dye solution in the main channels was seen to be too fast to completely fill the microwells before they were sealed by the oil plug. (**C**) A representative image of a tandem-channel chip after the sample digitization. The zoom-in images highlight the volume of solution loaded in the microwells decreases along the channel and the poor filling quality for the microwells close to the dead end of the channel.

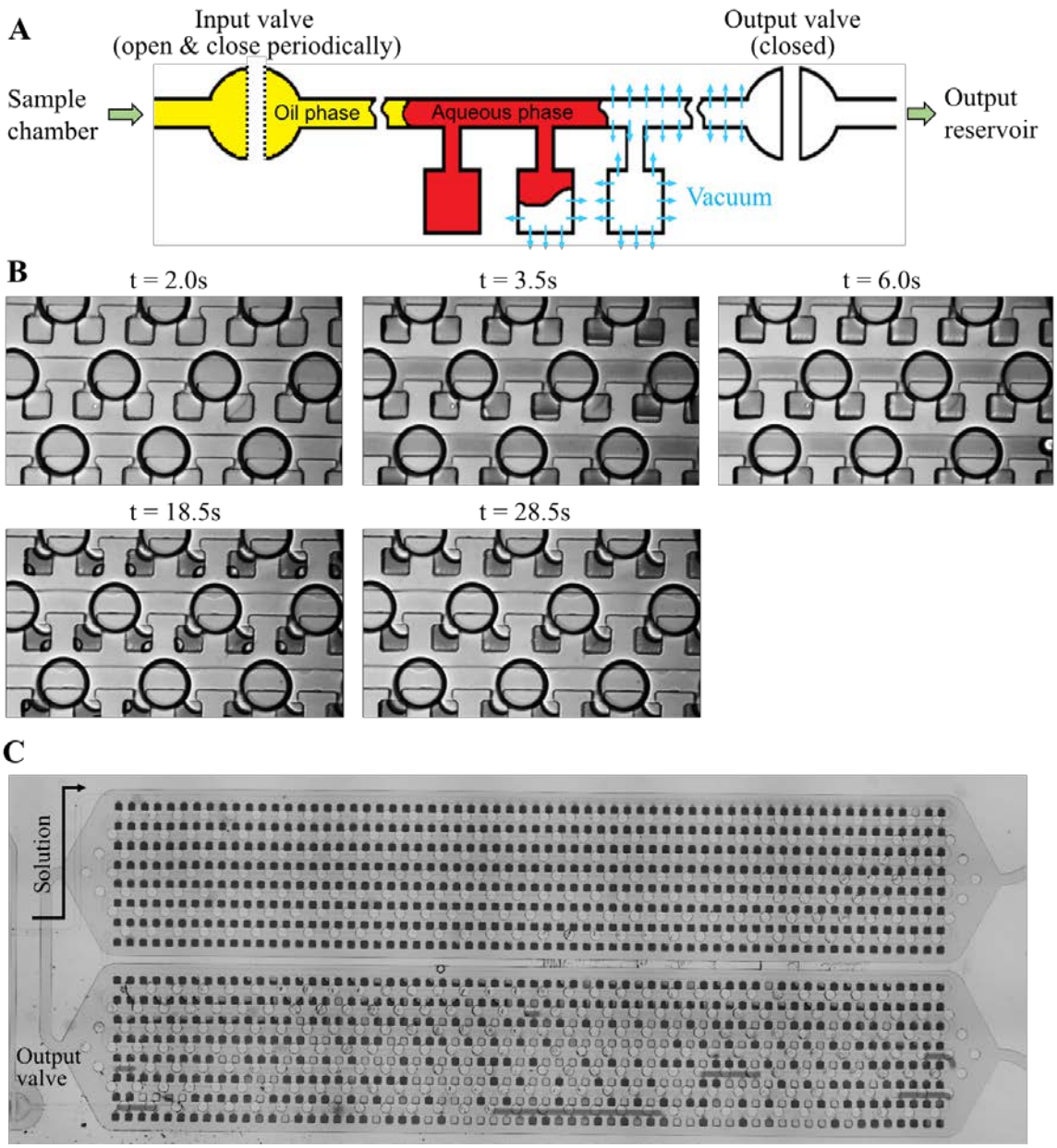

Figure S3. Pulsatile dead-end filling of microwells on the µAPPAD chips. (**A**) Schematic of the Pulsatile dead-end microwell loading. The inlet valve is periodically closed to pause the solution flow in the main channel. (**B**) Time-lapse images showing incomplete microwell filling in a parallel-channel chip. Closing the inlet valve periodically slowed the vacuum-driven flow of a dye solution in the main channels, but cannot completely fill the microwells before they were sealed by the oil plug. The time periods for closing and opening the inlet valve were set to 2 sec each in one cycle while keeping the outlet valve closed all the time. (**C**) A representative image of a tandem-channel chip after the sample digitization using the same valve actuation settings as in (**B**). Similar to the dead-end filling method, this modified dead-end filling approach resulted in decreasing solution volume loaded into the microwells along the channel and very poor sample digitization close to the dead end of the channel.

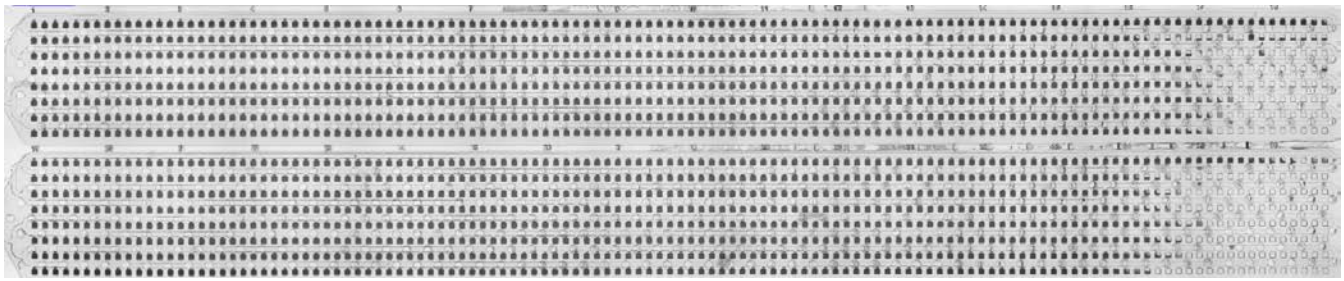

Figure S4. **Sample digitization in a parallel-channel chip**. Representative image of a parallel-channel chip after the sample digitization using the μAPPAD method shows the complete filling of all the microwells exposed to the dye solution except for the last few ones in each channel. The time periods for closing and opening the outlet valve were set to 2 sec and 3 sec, respectively, while keeping the inlet valve open.

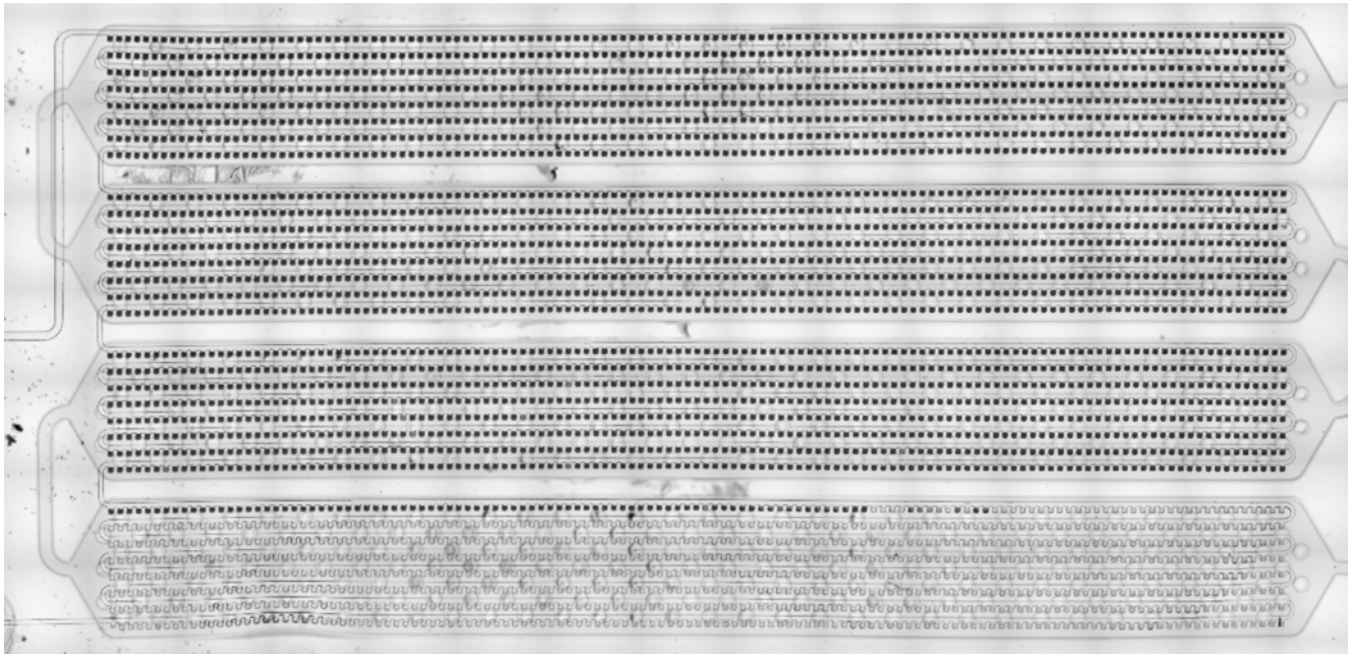

Figure S5. **Robust and uniform sample digitization in a chip of different microwell geometries**. The tandem-channel chip contains 4096 square microwells and each microwell is 60 μm in width and 100 μm in height. The main channel is 100 μm wide and 50 μm high. In this case, the μAPPAD process was conducted by closing and opening the outlet valve for 3 sec and 0.5 sec in each cycle, respectively.