Fabrication of the through-hole microwell chips (TMC). The silicon wafer (thickness of 500 μm and diameter of 6 in) was ultrasonically cleaned with acetone, anhydrous ethanol and deionized water in turn and dried with nitrogen. Chromium was coated on the silicon wafer by a magnetron sputtering machine (ANELVA SPF-430H, Japan). The thickness of the Cr film was approximately 100 nm, which was obtained via a sputtering rate of 14 $nm \cdot min^{-1}$. The silicon wafer was placed on a hot plate at 140°C for 10 min followed by the spin coating process. To obtain an approximately 5-μm photoresist layer, the spin coating process was divided into two stages. In the first stage, the spin speed was 1000 $r \cdot min^{-1}$ and the time was 30 s. In the second stage, the spin speed was 4000 $r \cdot min^{-1}$ and the time was 60 s. The silicon wafer coated with photoresist was baked at 100°C with a hot plate for 5 min followed by the lithography and developing steps. The exposed Cr under photoresist was removed by immersing the substrate in concentrated hydrochloric acid solution (30 wt%) at a temperature of 80°C for 20 min. Finally, the through-hole capillary microarray was formed via inductive coupled plasma (ICP) etching, photoresist cleaning and diamond wheel grinding processes.

Image processing and data analysis procedures. Three channel fluorescence images of TMC (fluorescence channels for FAM™, VIC™ and ROX™ dyes was named as channel 1, channel 2 and channel 3, respectively) were collected using the self-developed fluorescence imaging system. Images for channel 2 and channel 3 were aligned to channel 1 using image registration algorithm based on mutual information.

The grey value in image of channel 1 was binarized to obtain isolated connected domains. The center coordinates of each connected domain was extracted and region of interest (ROI) was defined. The number of ROI was counted and defined as the number of the units filled with sample (^{N_f}). The sum of the gray values of all points in the ROI was defined as the grey value of the microwell. The grey values of the microwell on all fluorescence channels were calculated and four parameters (center coordinates, gray value of channel 1, gray value of channel 2, gray value of channel 3) were outputted for each microwell. Quality control (QC) was carried out with gray value of channel 3. Microwells with too large or too small gray values would be eliminated. The fluorescence intensity of FAM™ and VIC™ channels for the remaining microwells was obtained by fluorescence compensation. The scatter diagram was drawn using the fluorescence intensity of FAM™ channel as X-axis and that of VIC™ channel as Y-axis. Four clusters of data (negative, positive of FAM™ labelled gene, positive of VIC™ labelled gene and positive of both FAM™ labelled gene and VIC™ labelled gene) were obtained by the modified k-means algorithm. The numbers of negative microwells and positive microwells for FAM™ channel and VIC™ channel were counted and the quantitative results were calculated using Poisson statistics.

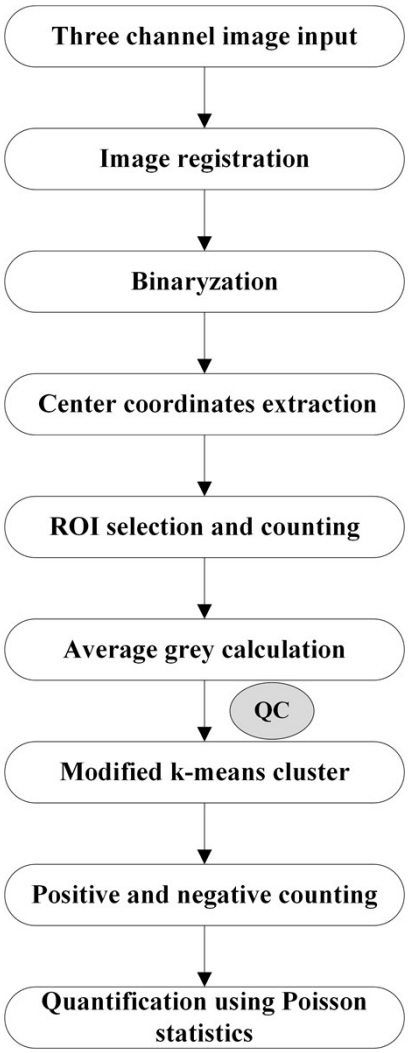

Figure S1. Workflow of image processing and data analysis. ROI stands for region of interest, QC stands for quality control.

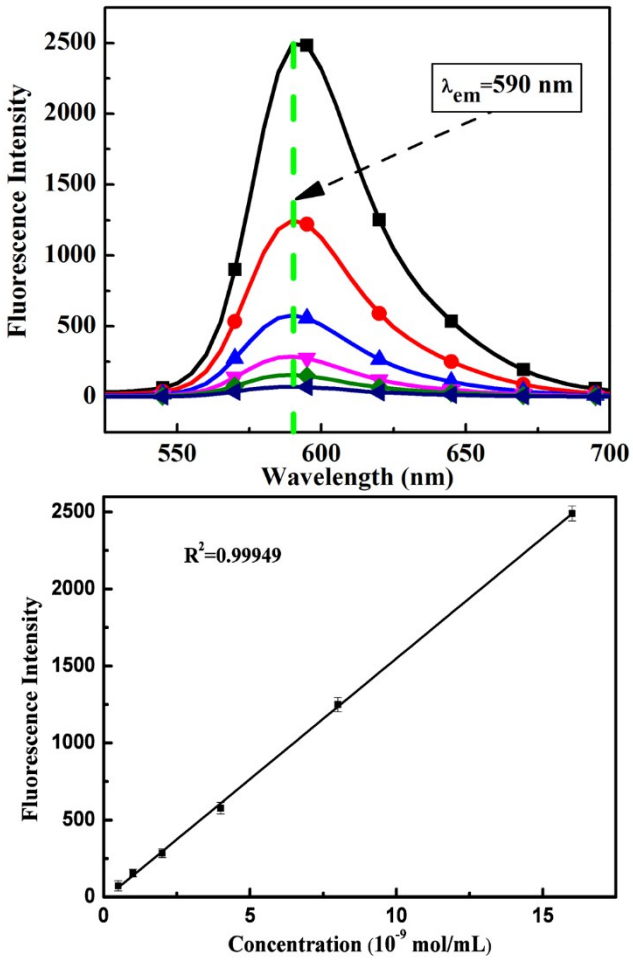

Figure S2. Fluorescence spectra (up) and standard curve (down) of Rhodamine aqueous solution at different concentrations. The standard curve was obtained at the emission wavelength of 590 nm. The slope and intercept of the standard curve are 156.68 and 17.1, respectively.

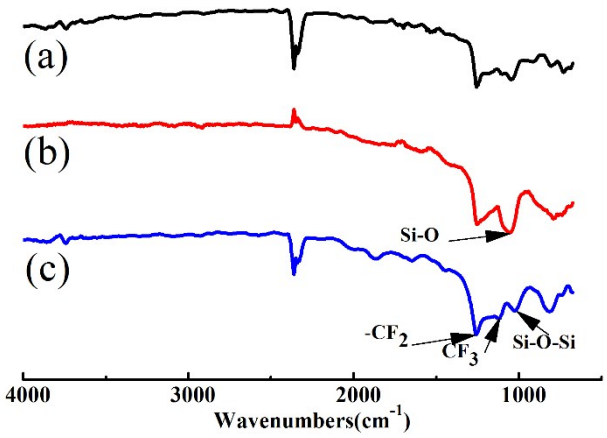

Figure S3. Attenuated total reflection infrared spectroscopy results of an unmodified TMC (a), hydrophilic TMC (b) and TMC with a hydrophobic exterior surface and hydrophilic interior surface (c).

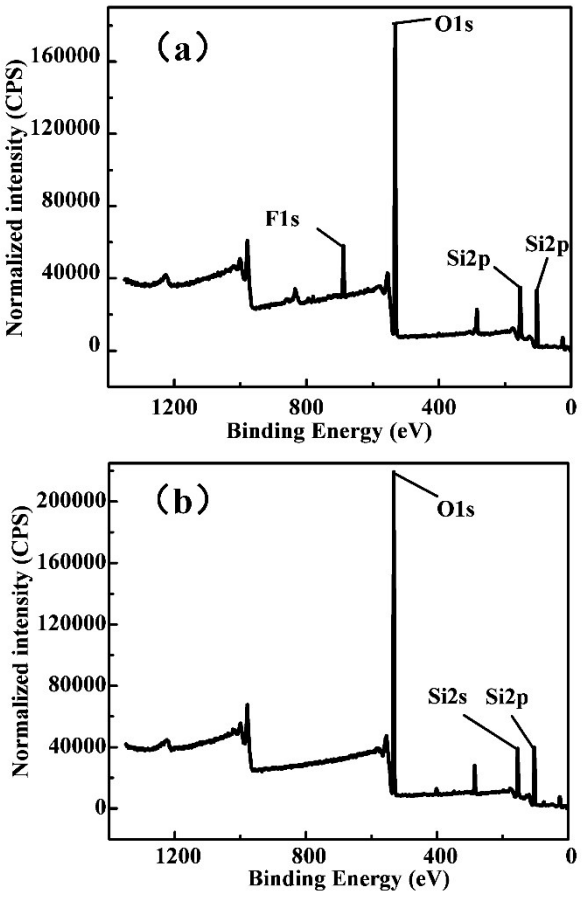

Figure S4. X-ray photoelectron spectroscopy results of the TMC with a hydrophobic exterior surface (a) and hydrophilic interior surface (b).

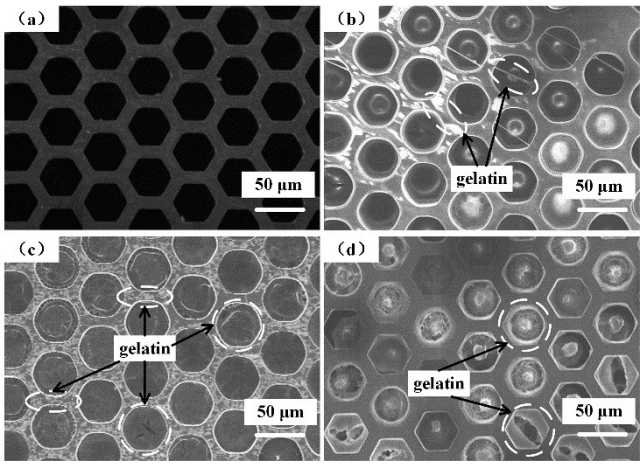

Figure S5. SEM images of a blank TMC (a) and TMC filled with gelatin. An unmodified TMC (b), hydrophilic TMC (c) and TMC with a hydrophobic exterior surface and hydrophilic interior surface (d) were used.

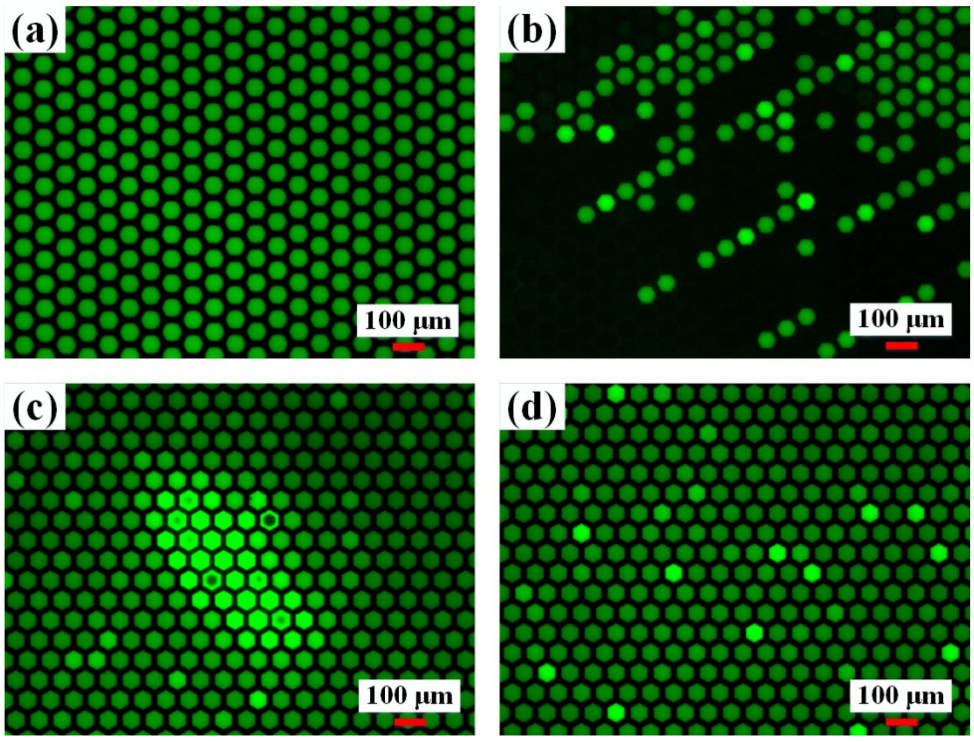

Figure S6. Fluorescence microscope image of different through-hole microwell chips after dPCR. (a) Negative control on TMC with hydrophobic exterior surface and hydrophilic interior surface; (b) dPCR on unmodified TMC; (c) dPCR on hydrophilic TMC; (d) dPCR on TMC with hydrophobic exterior surface and hydrophilic interior surface.

Table S1. Detailed data for unit filling rate (UFR) and sample residual rate (SRR) of TMC s with different surface properties.

contact time (s)	No.	numbers of the unit filled	UFR (%)	average UFR (%)	grey value	SRR (%)	Average SSR (%)
	1	99082	97.14		2175.6	90.5	
0	2	100008	98.05	97.7±0.4	2188.9	99.9	95.1±3.9
	3	99957	98.00		2182.3	94.8	
	1	95854	93.97		2021.8	19.9	
5	2	92132	90.33	93.4±2.3	2061.9	55.7	38.6±14.7
	3	97805	95.89		2088.7	40.3	
	1	90885	89.10		1975	31.5	
10	2	96814	94.92	92.9±2.7	2021.8	11.4	24.3±9.2
	3	96660	94.76		2055.2	30	
	1	90084	88.32		1888.1	8.3	
15	2	95558	93.68	91.5±2.3	1981.7	3.4	6.9±2.4
	3	94250	92.40		1968.3	8.8	
	1	89755	88.00		1868.1	4.4	
20	2	94447	92.60	91.1±2.2	1968.3	7	4.9±1.5
	3	94558	92.70		1961.7	3.4	
	1	51668	50.65		1085.9	2.3	
25	2	38887	38.12	41.0±7.0	805.2	0.9	1.2±0.8
	3	35004	34.32		718.3	0.4	