Supporting Information

Supplemental Material S1. Programming codes for IM-dPCR system

The Python code and Android studio application to control the IM-dPCR system can be downloaded from Github under the following link: https://github.com/cDNAg1/dPCR-Device.git. All variables required by the code as well as experimental data acquired by the device during a dPCR run is saved in an SQL database (IoT dPCR.db) that also can be found on the Github. For thermal cycling, the device measures the resistance of the gold wire structure by reading the value of the ADC. The ADC readout is then converted into a temperature using the parameters of the database. An error is calculated from the difference to the set temperature and the error used to calculate a response from a PID controller. Afterwards, the PID response is converted into a pulse width modulation, which is passed to the LEDs. During cycling, all measured values (ADC value, voltages, measured temperatures) and output values (PID value, PWM) are saved to a database for later extraction by the user, if so desired.

Table S1. List of parts required for IM-dPCR system.

Function	Part	Model	Supplier	Amount
Firmware	Controller	Raspberry Pi 4 – 4GB	Adafruit, USA	1
	Li-ion Battery	PB-PD20	XR-Smart Tech Industry, China	1
Thermal cycling	Analog-to-digital converter	MCP3304	Microchip Technology, USA	1
	MOSFET	SK C3851 67 Y	SK Hynix, Korea	2
	LED (for heating)	LXML-PR02-1000	Lumileds, USA	4
	Heatsink	BGA35X35X10	Fischer, Germany	1
	Resistors	100 / 200 / 750 / 1000 / 10000 Ω	ICBanq, Korea	9
	Stacking Header	2x20 Extra Tall Header	Adafruit, USA	2
	Cooling fan	Pi-FAN LD3007MS	ICBanq, Korea	1
Fluorescence imaging	Board to Wire connector	5046-02	Molex, USA	2
	CMOS camera	IMX477	Sony, Japan	1
	Multi-color LED (for excitation)	LZ4-20MA00	Lumileds, USA	1
	Multi band pass filter (excitation)	89402x	Chroma, USA	1
	Multi band pass filter (emission)	89402m	Chroma, USA	1
	Lens	LN049	Arducam, USA	1

Table S2. Sequence of the forward and reverse primers as well as the probe used for 9-plex RT-dPCR experiments

Virus	Name	Sequence 5'-3'
H1N1	Forward primer	GGCGATCTACTCAACTGTCG
	Reverse primer	ACATCCAGAAGCTGATTGCC
	Probe	[FAM] CACTAGTGCTTCTGGTCTCCCTGGG [BHQ1]
H3N2	Forward primer	AGATTGCTGACTCCCAGCAT
	Reverse primer	CTCGATCCAGCCATTTGCTC
	Probe	[TAMRA] TGGTTCTGGCCAGCACTACAGCT [BHQ2]
IFZ B	Forward primer	GCAACAAAGAAGAAAGGCCTAA
	Reverse primer	GCACAGAGCGTTCCTAGTTT
	Probe	[Cyanine 5] AAGGCCACGAAAGCTCAGCA [BHQ3]
DENV2	Forward primer	TACTGTGTGCTACGTGCTGA
	Reverse primer	TCTGCCTGGTCTTCCCATTT
	Probe	[FAM] ACGTCGGCAGCTCTCTCCAATTCCA [BHQ1]
DENV3	Forward primer	GTGGTTTGTTTGGCAAGGGA
	Reverse primer	TCTCCTGTGTGCACTGTGAT
	Probe	[TAMRA] TGAAATTTCGCGCATGTCACCAAGCT [BHQ2]
DENV4	Forward primer	GACACCAGAACACCACAACC
	Reverse primer	CAGCTTCACTGGCTGATGTC
	Probe	[Cyanine 5] CCCGAGGAGAGCCCACAGCCA [BHQ3]
OC43	Forward primer	CCCAAGTAGCGATGAGGCTA
	Reverse primer	GTAACCCTGAGGGAGTACCG
	Probe	[FAM] CCGACTAGGTTTCCGCCTGGCA [BHQ1]
229E	Forward primer	CAACAAGCTCCAACAGGCAT
	Reverse primer	GCACGGCAACTGTCATGTAT
	Probe	[TAMRA] AGCACGCCGCTCAGCAAGGT[BHQ2]
NL63	Forward primer	GTTGCTGCTGTTACTTTGGC
	Reverse primer	CTCTCTGGTAGGAACACGCT
	Probe	[Cyanine 5] AGCCTCTTTCTCAACCCAGGGCT [BHQ2]

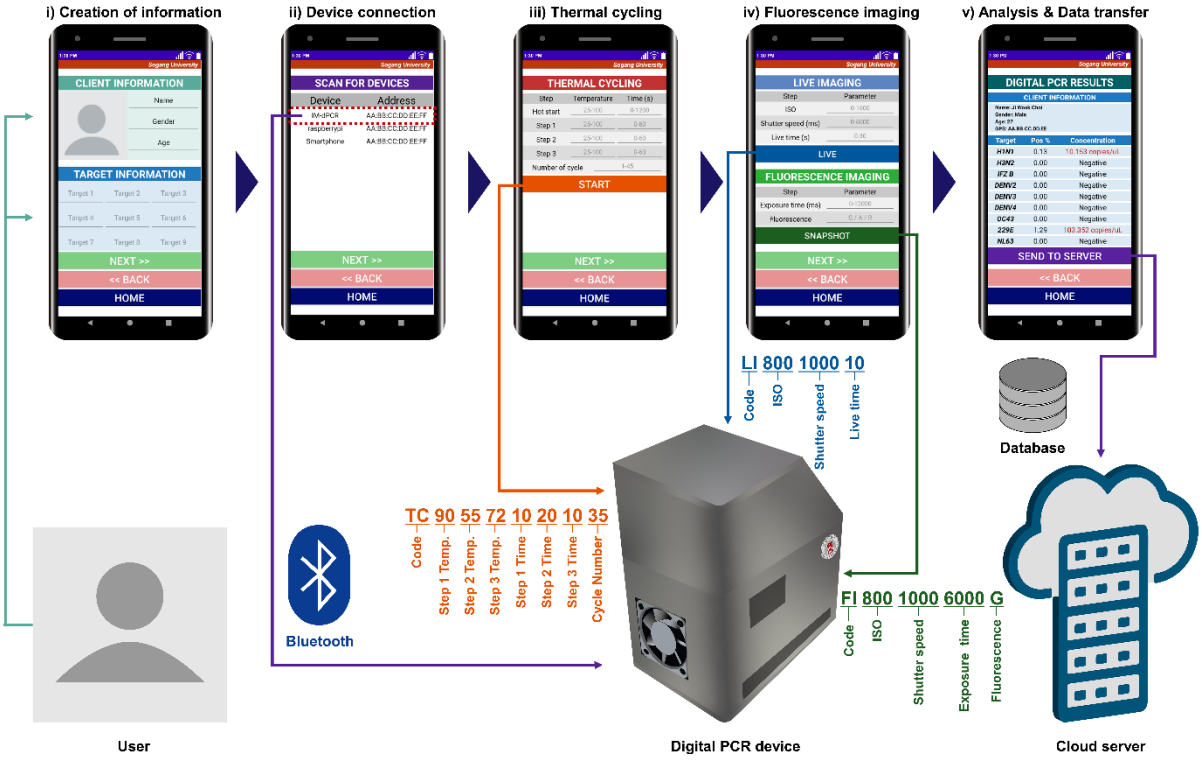

Fig. S1 Workflow of the smartphone application. The application consisted of five activities: i) Creation of information: the information of the client and target RNA is input into the application, ii) Device connection: the smartphone and the dPCR device are connected via Bluetooth communication, iii) Thermal cycling: parameters for PCR operation are entered and transmitted to the dPCR device, iv) Fluorescence imaging: parameters for fluorescence imaging are input and transmitted to the dPCR device, v) Analysis & Data transfer: the concentration of target RNA is quantified and the database files including dPCR results, client information, and GPS are transmitted to the server via Wi-fi or 5G connection.

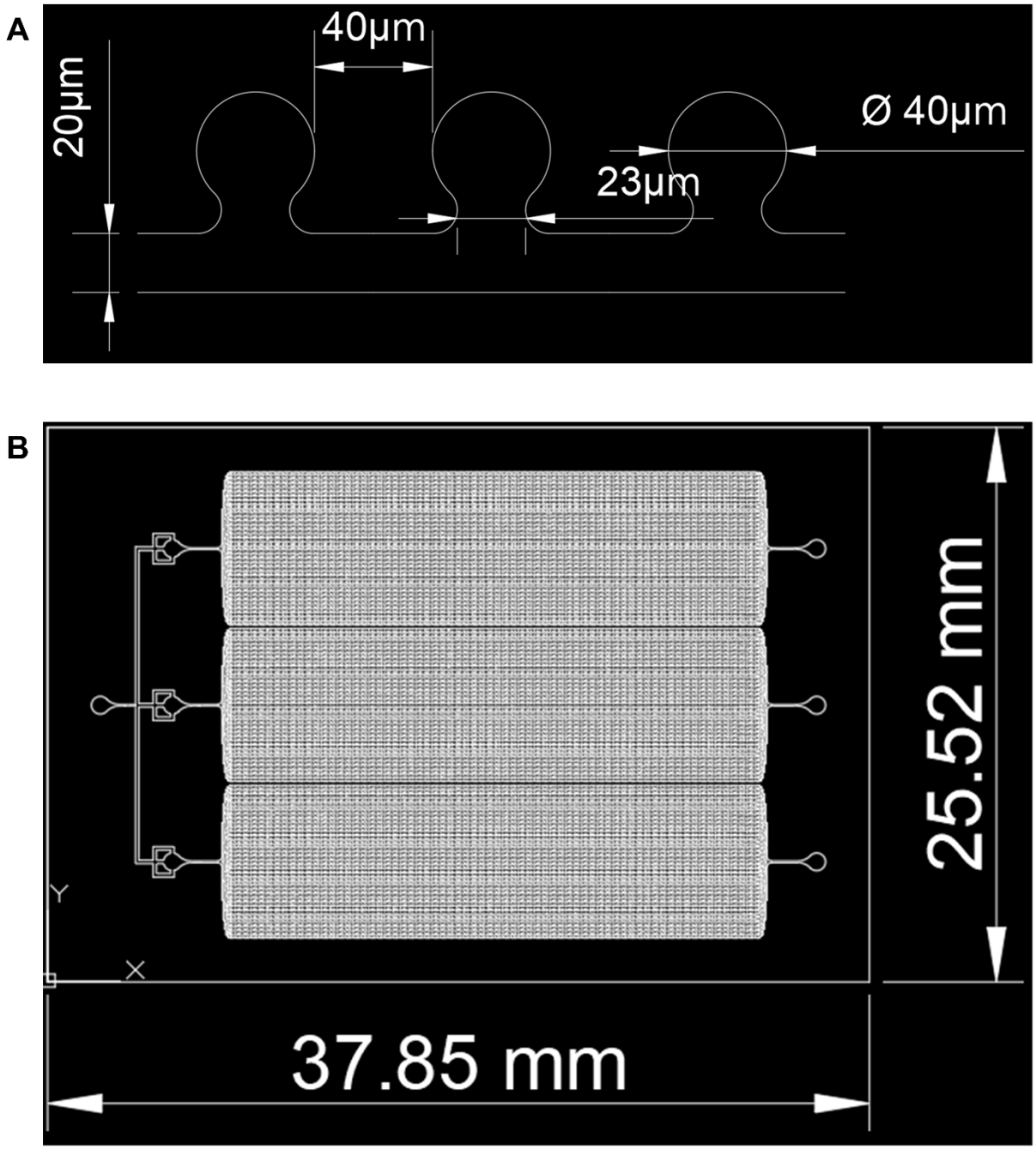

Fig. S2 CAD drawings and dimensions of the SPM chip. CAD drawing of single channel consisting of microwells (diameter: 40 µm) and a straight channel (width: 50 µm) (A). CAD drawing of the SPM chip consisting of 3 chambers with 21,000 microwells (B).

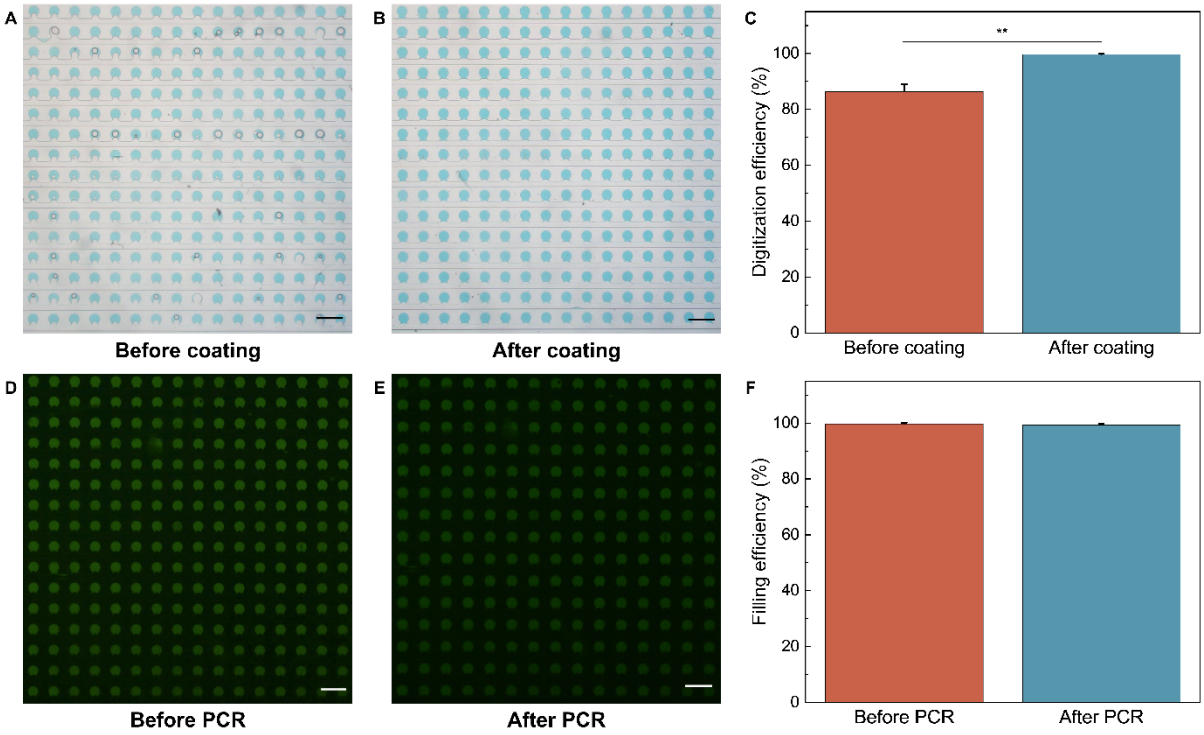

Fig. S3 Characterization of the SPM chip. Microscopy images showing the self-priming results before coating (A) and after coating (B). Graph representing the digitization efficiency of the SPM chip (**p<0.01) (C). Fluorescence images of the SPM chip before PCR (D) and after PCR (E). Graph of filling and evaporation in the SPM chip (F). The scale bars are 100 μm.

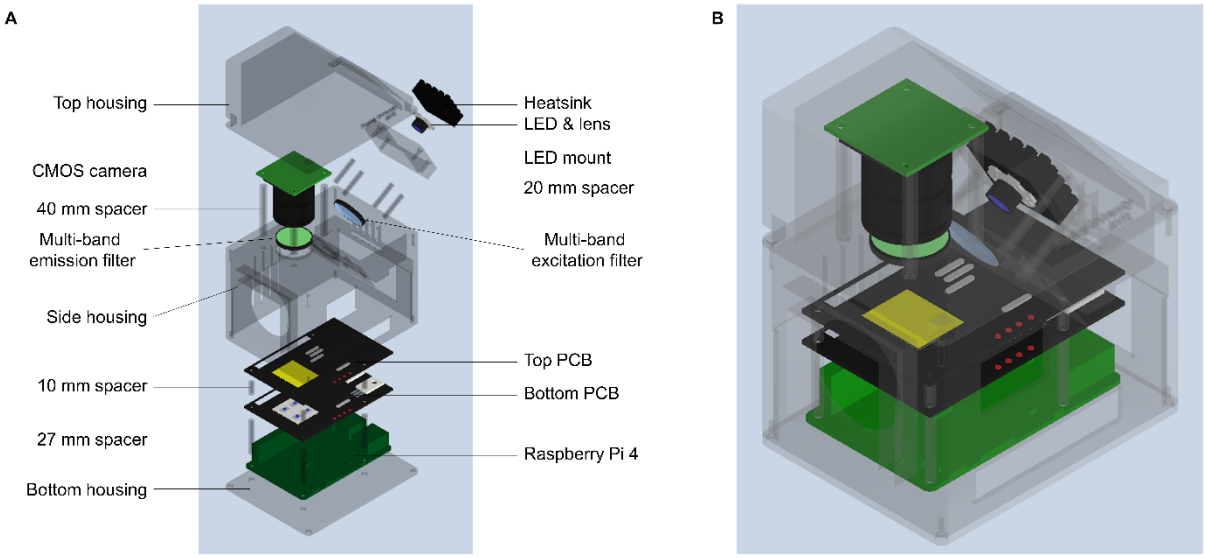

Fig. S4 3D CAD drawing of the dPCR device. Exploded view of the device showing the plasmonic heating-based thermal cycler, multicolor fluorescence imaging setup, single board computer, and 3D printed frame (A). CAD drawing of the assembled dPCR device (B).

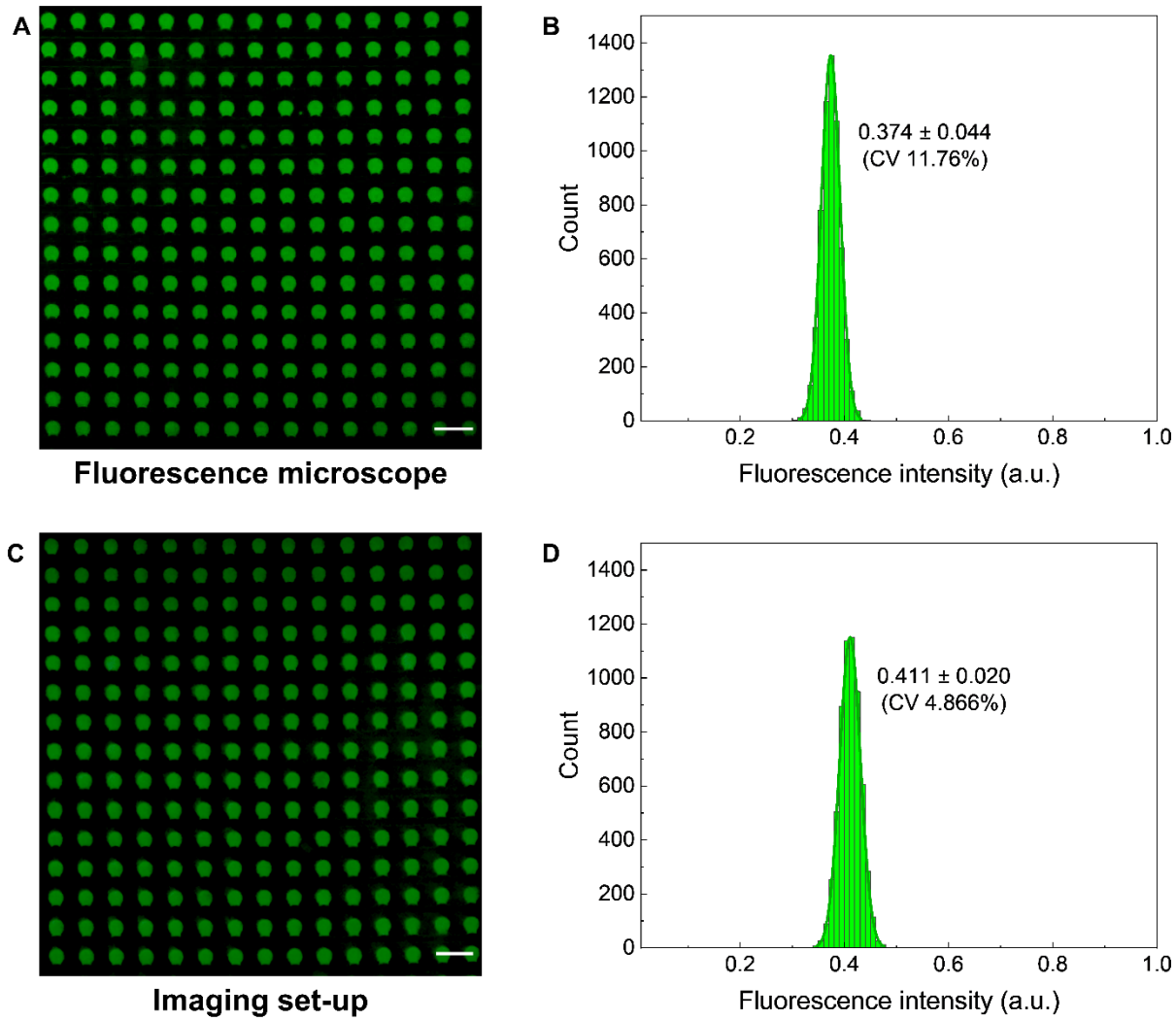

Fig. S5 Comparison of fluorescence imaging qualities between the fluorescence microscope and the imaging set-up. Fluorescence images of the SPM chip taken by the fluorescence microscope (A) and imaging set-up (C). Histograms representing the distribution of fluorescence intensities (B/D). The scale bars are 100 μm.

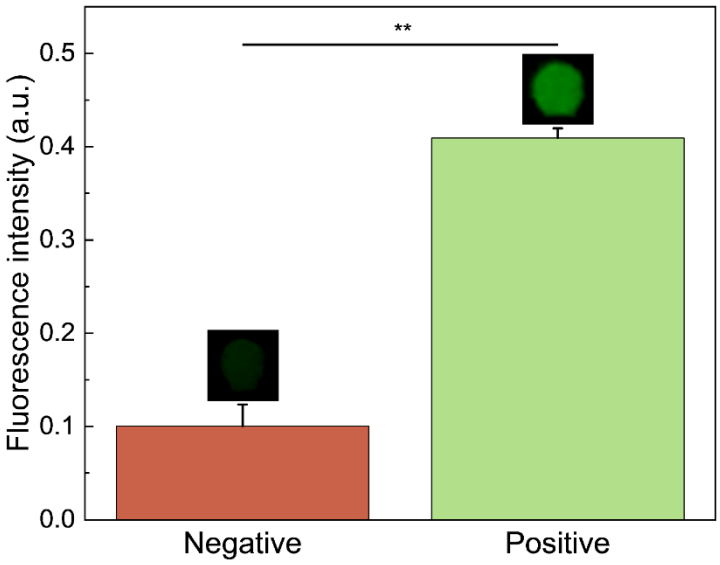

Fig. S6 Graph of fluorescence intensity of the negative wells and the positive wells (**p<0.01).

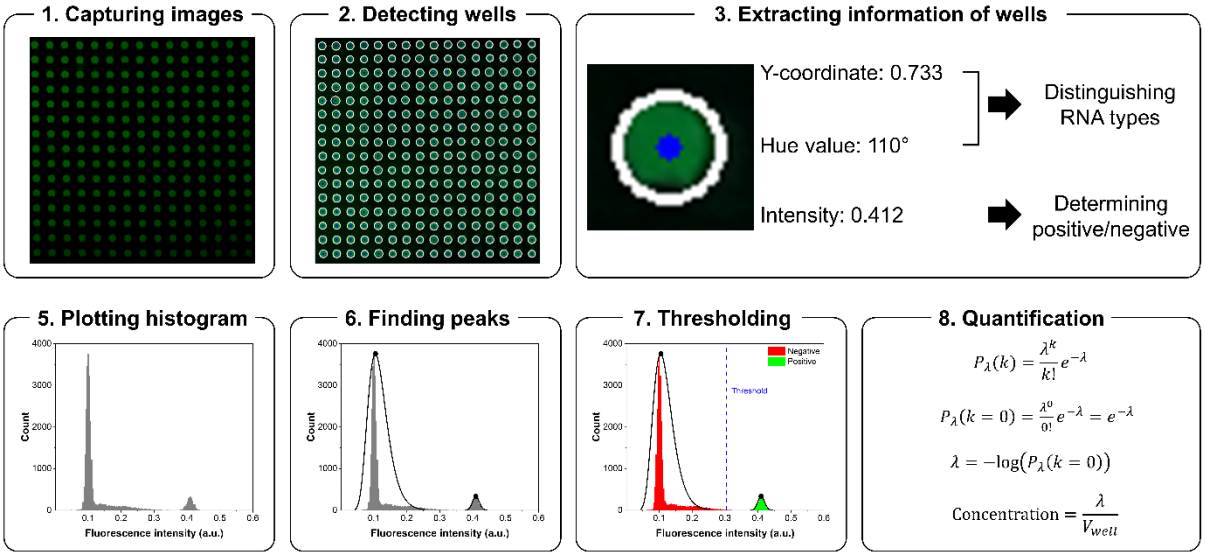

Fig. S7 Workflow of the image analysis algorithm. The fluorescence images were captured by the imaging set-up, and location of wells was detected. The information of wells containing normalized y-distance, Hue value, and intensity was extracted. The ratio of the positive wells was analyzed by auto thresholding process: i) plotting histogram, ii) finding peaks, iii) thresholding. The concentration of target RNA was quantified by Poisson's distribution.

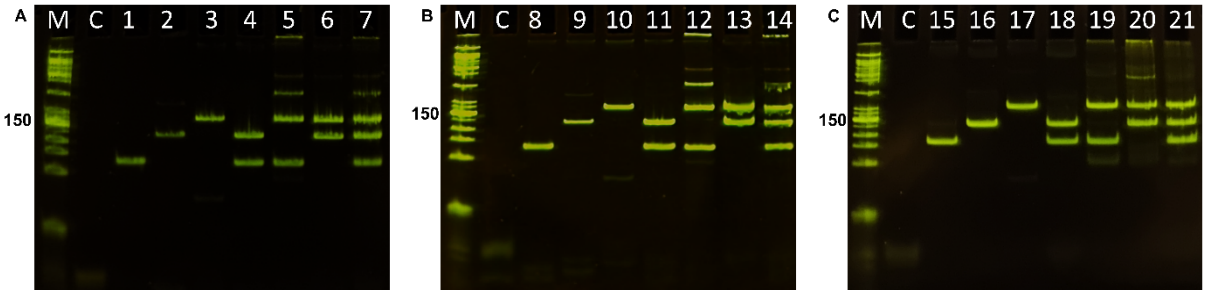

Fig. S8 12% Polyacrylamide gel (PAGE) images of 9-plex RT-PCR assay. Each lane represents the amplification result of single, duplex, triplex RT-PCR of influenza virus (A), dengue virus (B) and human coronavirus (C) with different primer pairs. M. 25 bp DNA Ladder; C. negative control without target; lanes 1 to 21, amplicons by primer pairs H1N1, H3N2, IFZ B, H1N1/H3N2, H1N1/IFZ B, H3N2/IFZ B, H1N1/H3N2/IFZ B, DENV2, DENV3, DENV4, DENV2/ DENV3, DENV2/ DENV4, DENV3/ DENV4, DENV2/ DENV3/ DENV4, OC43, 229E, NL63, OC43/229E, OC43/NL63, 229E/NL63, OC43/229E/NL63, respectively.

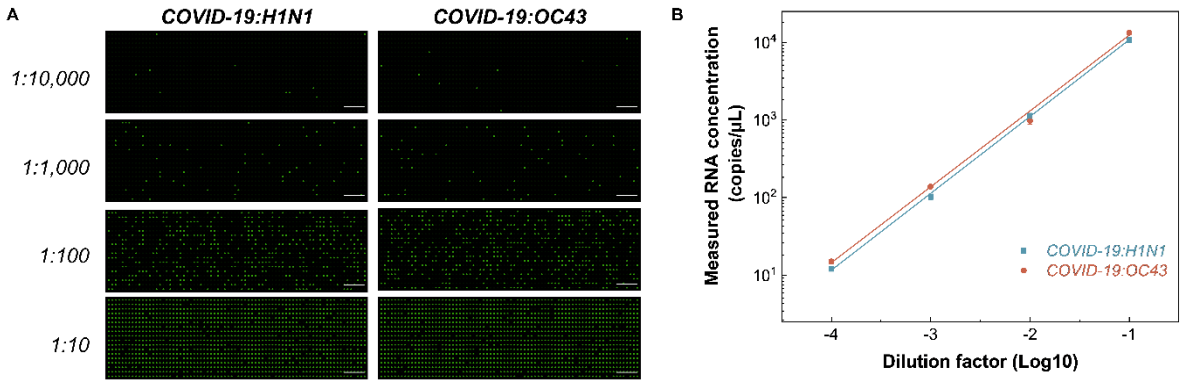

Fig. S9 Quantification analysis of COVID-19 using mock samples. Fluorescence images representing dPCR analysis with a serial dilution of the target RNA ranging from 1:10,000 to 1:10 (A). The mock samples contained influenza virus (H1N1) and human coronavirus (OC43). Linear regression curve showing dPCR results using mock samples. The scale bar are 500 μm.