Supplementary Information

A method of sequential liquid dispensing for the multiplexed genetic diagnosis of viral infections in a microfluidic device

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S1. A comparison between the performance of the lateral-type and the vertical-type phaseguides

In our previous study, we explored sequential sample dispensing into five microchambers (volume = 3 µL) by controlling the burst pressures in three types of phaseguide ridge structures (hereinafter referred to as lateral-type phaseguides) with different inclined angles against the flow direction. A subsequent study revealed that a set of only two phaseguides of the three ones is sufficient for our microfluidic diagnostic device (Fig. S1a); that is, a backflow of liquid from the microchannel did not occur when the LAMP assay was performed without one of the phaseguides, which was located near the entrance to the chamber. This is probably because the attractive force toward the central region of the microchannel acted on water molecules owing to their cohesive nature.

Moreover, lateral-type phaseguides can act as pressure barriers owing to the meniscus pinning effect, as illustrated in Fig. S1a. In theory, the burst pressure can be derived from the Young–Laplace equation as

\[ \Delta P = \gamma (1/R_1 + 1/R_2), \]

where \( \Delta P \) is the pressure difference across the fluid interface, \( \gamma \) is the surface tension of water, and \( R_1 \) and \( R_2 \) are the principal radii of the surface curvature. For a rectangular microchannel with a phaseguide ridge and different inclination angles \( \alpha \) (°), the burst pressure \( P(\alpha) \) (Pa) can be expressed as follows:

\[
P(\alpha) = \gamma \left( \frac{2 \cos \theta_m \cos \alpha}{W} + \frac{\cos \left( \min(\theta_m + 90°, 180°) \right)}{H-h} \right)(S1)
\]

where \( W \) and \( H \) are the width and height of the rectangular microchannel, respectively; \( h \) is the height of the phaseguide ridge; \( \theta_m \) is the water contact angle for the sidewall surfaces of the microchannel and the top surface of the phaseguide ridge (i.e., PDMS), and \( \theta_b \) is the water contact angle for the bottom surface of the microchannel (i.e., the silicone-based adhesive tape). The inclination angle \( \alpha \) is defined as the angle between the phaseguide ridge and sidewall of the microchannel. Moreover, \( \theta_m + 90° \leq 180° \) in the second term on the right side of Eq. (S1) is defined as the water contact angle at the back edge of the phaseguide ridge.

For example, the burst pressures in a microchannel (\( H = 50 \mu m, \) and \( W = 200 \mu m \) for phaseguide \( S_1 \) and \( W = 100 \mu m \) for phaseguide \( S_2 \), respectively) integrated with the phaseguide ridge (\( h = 25 \mu m \)) were \( P_1 = 3.50 \) and \( P_2 = 3.73 \) kPa for the inclination angles \( \alpha = 15 \) and 0°, respectively, where the surface tension of water was 0.073 N/m and the water contact angles \( \theta_m \) and \( \theta_b \) were 108 and 97°, respectively (Table S1). However, lateral-type phaseguide (Fig. S1a) has some limitations; that is, the burst pressure differences between each phaseguide could not be designed to make it large (e.g., \( P_2/P_1 = 1.07 \) in the case of the abovementioned geometrical dimensions), and a high burst pressure of \( P_2 \) could not be designed while maintaining the low burst pressure of \( P_1 \) because they both increase at the same time by increasing the height of the phaseguide ridge (\( h \)) for the stop valves \( S_1 \) and \( S_2 \). In addition, a complicated two-step photolithography process was required to integrate such ridge structures into a PDMS microchannel, resulting in a long processing time (~3 h), and modest repeatability and accuracy in the heights of the phaseguide ridge (\( h \)) and microchannel (\( H \)).

Therefore, we proposed a vertical-type phaseguide structure, as shown in Fig. S1b. This simple geometric design allows the use of a one-step photolithography process, thereby reducing the processing time (~1 h). Moreover, the burst pressure can be controlled by the gap distance \( g \) between the vertical sidewalls of the phaseguide and microchannel. This improves the dimensional accuracy and reproducibility of the phaseguide structures because the lateral dimensions are consistently better than the vertical ones in the photolithography process. In addition, this improves the pressure differences between each phaseguide (\( P_1 \) vs. \( P_2 \)), and allows the design of a higher burst pressure \( P_2 \) for \( S_2 \), while maintaining a relatively low burst pressure \( P_1 \) for \( S_1 \). For example, the theoretical burst pressures of the vertical-type phaseguides were \( P_1 = 3.02 \) and \( P_2 = 5.41 \) kPa for the gap distances of \( g = 40 \) and 20 µm, respectively, resulting in \( P_2/P_1 = 1.79 \) (Table S2). The burst pressure of the vertical-type phaseguide \( P(g) \) (Pa) can be derived as follows:

\[
P(g) = \gamma \left( \frac{\cos \left( \min(\theta_m + 90°, 180°) \right)}{g} + \frac{\cos \theta_m + \cos \alpha}{H} \right)(S2)
\]
where the angle $\theta_m + 90^\circ$ (≤180°) in the first term on the right side of Eq. (S2) is defined as the water contact angle at the back edge of the vertical-type phaseguide structure.

**Table S1** Theoretical burst pressures for the lateral-type phaseguide structures.

| $W$ (µm) | $H$ (µm) | $H'$ (µm) | $A$ (deg.) | Burst pressure (kPa) | Ratio ($P_2/P_1$) |
|----------|----------|-----------|------------|---------------------|------------------|
| $P_1$    | 200      | 50        | 25         | 3.50                | 1.00             |
| $P_2$    | 100      | 50        | 25         | 3.73                | 1.07             |

**Table S2** Theoretical burst pressures for the vertical-type phaseguide structures.

| $W$ (µm) | $H$ (µm) | $g$ (µm) | Burst pressure (kPa) | Ratio ($P_2/P_1$) |
|----------|----------|----------|---------------------|------------------|
| $P_1$    | 200      | 50       | 3.02                | 1.00             |
| $P_2$    | 100      | 50       | 5.41                | 1.79             |
Table S3  Theoretical calculations for estimating the dispensing number $N_{th}$ for microfluidic devices in the single-row format ($L_1 = 5.0$ mm, $L_2 = 1.25$ mm, and $L_3 = 0.2$ mm) as a function of the flow rate. The burst pressures of the temporary stop valve $S_1$ ($g = 36.5$ µm) and permanent stop valve $S_2$ ($g = 17.9$ µm) were designed to be $P_1 = 3.23$ and $P_2 = 5.95$ kPa, respectively. The theoretical dispensing numbers $N_{th}$ were determined assuming that the total pressure calculated using Eqs. (1) and (2) must be smaller than the burst pressure $P_2$ of the permanent stop valve $S_2$.

| Flow rate (µL/min) | 2.5 | 5.0 | 10  | 15  | 20 |
|-------------------|-----|-----|-----|-----|----|
| $P_1$ (kPa)       |     |     |     |     |    |
| $P_2$ (kPa)       |     | 5.95|     |     |    |
| $\Delta P (L_1)$ (kPa) | 0.233 | 0.349 | 0.465 | 0.698 | 0.931 |
| $\Delta P (L_2)$ (kPa) | 0.058 | 0.087 | 0.116 | 0.175 | 0.233 |
| $\Delta P (L_3)$ (kPa) | 0.024 | 0.036 | 0.048 | 0.072 | 0.096 |

| Filled chamber No. | Theoretical pressure values applied to permanent stop valve $S_2$ (kPa) |
|--------------------|---------------------------------------------------------------------|
| 1                  | 3.23 3.23 3.23 3.23 3.23                                           |
| 2                  | 3.55 3.70 3.86 4.18 4.49                                           |
| 3                  | 3.78 4.05 4.32 4.87 5.42                                           |
| 4                  | 4.01 4.40 4.79 5.57 6.35                                           |
| 5                  | 4.24 4.75 5.25 6.27 7.28                                           |
| 6                  | 4.48 5.10 5.75 6.97 8.21                                           |
| 7                  | 4.71 5.45 6.18 7.67 9.15                                           |
| 8                  | 4.94 5.80 6.65 8.36 10.08                                          |
| 9                  | 5.17 6.15 7.11 9.06 11.01                                          |
| 10                 | 5.41 6.49 7.58 9.76 11.94                                          |
Table S4: Theoretical calculations for estimating the dispensing number $N_{th}$ for microfluidic devices in the staggered two-row format ($L_1 = 2.5 \text{ mm}$, $L_2 = 1.00 \text{ mm}$, and $L_3 = 0.2 \text{ mm}$) as a function of the flow rate.

| Flow rate (µL/min) | 5.0 | 10 | 15 | 20 | 30 |
|--------------------|-----|----|----|----|----|
| $P_1$ (kPa)        | 3.23| 3.23| 3.23| 3.23| 3.23|
| $P_2$ (kPa)        | 5.95|    |    |    |    |
| $\Delta P (L_1)$ (kPa) | 0.116| 0.233| 0.349| 0.465| 0.698|
| $\Delta P (L_2)$ (kPa) | 0.047| 0.093| 0.140| 0.186| 0.279|
| $\Delta P (L_3)$ (kPa) | 0.024| 0.048| 0.072| 0.096| 0.144|

| Filled chamber No. | Theoretical pressure values applied to permanent stop valve $S_2$ (kPa) |
|--------------------|-------------------------------------------------|
| 1                  | 3.23, 3.23, 3.23, 3.23, 3.23                   |
| 2                  | 3.42, 3.60, 3.79, 3.98, 4.35                   |
| 3                  | 3.53, 3.84, 4.14, 4.44, 5.05                   |
| 4                  | 3.65, 4.07, 4.49, 4.91, 5.75                   |
| 5                  | 3.77, 4.30, 4.84, 5.37, 6.45                   |
| 6                  | 3.88, 4.54, 5.19, 5.84, 7.14                   |
| 7                  | 4.00, 4.77, 5.54, 6.30, 7.84                   |
| 8                  | 4.11, 5.00, 5.89, 6.77, 8.54                   |
| 9                  | 4.23, 5.24, 6.23, 7.23, 9.24                   |
| 10                 | 4.35, 5.47, 6.58, 7.70, 9.94                   |
S2. Effect of the corner radius of the vertical-type phaseguide structure on the burst pressure

A theoretical model for describing the burst pressure considering the corner radius of the vertical-type phaseguide structure is shown in Fig. S2. Fig. S3a shows the calculated burst pressure $P_1$ for phaseguide $S_1$ (e.g., $g = 40 \, \mu\text{m}$, $W = 200 \, \mu\text{m}$, $H = 50 \, \mu\text{m}$) and $P_2$ for phaseguide $S_2$ (e.g., $g = 20 \, \mu\text{m}$, $W = 100 \, \mu\text{m}$, $H = 50 \, \mu\text{m}$) as a function of the corner radius $r$ of the phaseguides. Moreover, the maximal burst pressure for each corner radius $r$ was plotted among all the values calculated by Eq. (4), as described in the manuscript, which depends on angle $\beta$. Both burst pressures decreased with an increase in corner radius $r$. The normalized burst pressures (a maximum of 1 at $r = 0 \, \mu\text{m}$) are shown in Fig. 5b as a function of the ratio of the corner radius to the gap distance ($r/g$). The two sets of data approximately overlap by making the results dimensionless. The results indicate that the burst pressure decreases to approximately 90% at $r/g = 0.20$. Considering the dimensional accuracy of negative thick photoresist (SU-8) patterns in a typical photolithography process, it is desirable to maintain the corner radius of the phaseguide below at least the ratio $r/g = 0.40$, resulting in an ideal burst pressure of 85% or higher.

![Fig. S2 Theoretical model for describing the burst pressure considering the corner radius of the vertical-type phaseguide structure.](image)

![Fig. S3 (a) Burst pressure as a function of the corner radius ($r$). (b) Normalized burst pressure as a function of the ratio of the corner radius to the gap distance ($r/g$).](image)
S3. Portable LED lighting device and smartphone web app for on-site diagnostics

A smartphone web application for image acquisition and data analysis was custom-built by OptTech LLC., Aichi, Japan, to enable on-site diagnostics, as shown in Fig. S4. Test results that are either positive or negative for the simultaneous detection of four different types of infectious diseases could be automatically determined from an image acquired using a smartphone camera. An example of the LAMP assay is shown in Fig. S4, which demonstrates a positive COVID-19 test result displayed on the smartphone screen.

Here, a custom-built portable lighting device (130 mm (W) × 90 mm (D) × 85 mm (H)) was developed to guarantee reproducibility and reliability for diagnostic testing by maintaining the same lighting conditions when taking pictures. The polymer-based body structure of the lighting device was fabricated using 3D printing technology, and integrated with a multicolor light-emitting diode (LED) array straight in multiple rows on a part of the floor of the device as a light source. After the LAMP assay was performed, the microfluidic device was placed inside the lighting box, and subsequently illuminated with the LED light, which was reflected by a concave white rubber plate as a reflective mirror on the ceiling of the device. Next, the smartphone was placed on the top surface of the device, and a picture of an array of microchambers was captured through a small window on the device ceiling. Finally, the chromaticity of the microchambers was automatically analyzed using the developed smartphone application. Moreover, the spectral power distribution of the LED light source was optimized to enhance the color discrimination between the positive (sky blue) and negative (violet) reactions while maintaining the arbitrary illuminant color based on the spectral characteristics of the positive and negative chambers. Additionally, the diagnostic accuracy (the color identification accuracy) of the smartphone application has reached approximately 100% by employing machine learning algorithms.

Fig. S4 Portable LED lighting device and smartphone app screen for on-site diagnostics. As an example, a positive COVID-19 test result was displayed on the screen of the smartphone.
References

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