Electronic Supplementary Information

# Bubble-free on-chip continuous-flow polymerase chain reaction: Concept and application

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To examine factors affecting bubble formation and elimination, the three forces – $F_I$, $F_O$, and $F_S$ – introduced in the Principle section are expressed in the following three equations.

1. When the gas phase is considered ideal, force from within the bubble, $F_I$, can be derived as follows.

$$F_I = P_0\left[\exp\frac{-\Delta_{vap}H_{T,m}}{R}\left(\frac{1}{T_z}-\frac{1}{T_0}\right)\right]$$

$$\left\{\exp\frac{V_{l,m}\left[2.793\times10^{9}\times(z_{outlet}-z)\mu\bar{u}/m^{2}+P_{atm}-P_0\exp\frac{-\Delta_{vap}H_{T,m}}{R}\left(\frac{1}{T_z}-\frac{1}{T_0}\right)\right]}{RT_z}\right\}$$

$$+\,k_{c,z}\bullet C_{air,inlet}$$

2. Force from the outer surroundings, $F_O$, can be derived as follows, taking into account a channel width and height of 200 μm and 75 μm, respectively.

$$F_O = 2.793\times10^{9}\times(z_{outlet}-z)\mu\bar{u}/m^{2}+P_{atm}$$

3. The surface tension of the surrounding liquid medium, $F_S$, on each surface point can be expressed using the principal radii of curvature shown below.

$$F_S = \gamma\left(\frac{1}{R'_{1,z}}+\frac{1}{R'_{2,z}}\right)$$

Detailed mathematical derivations are introduced in this section to carefully reveal how physical factors such as liquid property, channel dimension, and operation parameters are related with three forces – $F_I$, $F_O$, and $F_S$ – mentioned in the text.

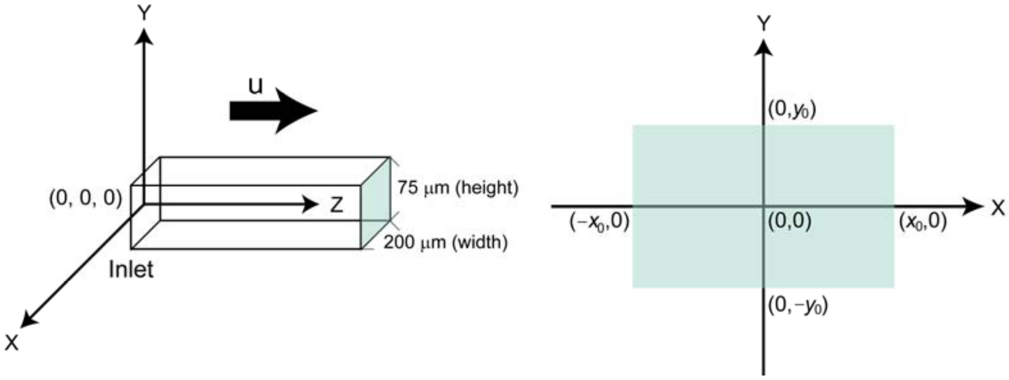


Figure S1. Channel size and coordinate system

## I. Discussion on $F_O$ ($P_{l,z}$) and $F_S$ ($P_s$)

$P_l$ can be calculated by Navier–Stokes equation. For incompressible continuous liquid, Navier–Stokes equation with formation of $\frac{Du}{D\theta} = f_B - \frac{1}{\rho}\nabla P + \nu\nabla^2 u$ (1.1) can be derived to three equations for the special chip structure with steady state flow assumption.

$$-\frac{1}{\rho}\frac{\partial P}{\partial z} + \nu\left(\frac{\partial^2 u_Z}{\partial y^2} + \frac{\partial^2 u_Z}{\partial x^2}\right) = 0 \quad 1.2$$

$$\rho g = -\frac{\partial P}{\partial y} \approx 0 \quad 1.3$$

$$0 = \frac{\partial P}{\partial x} \quad 1.4$$

Adding boundary conditions below:

$$x = \pm x_0, u_Z = 0 \quad 1.5$$

$$y = \pm y_0, u_Z = 0 \qquad 1.6$$

By integrating above three equations through variable separation approach, velocity distribution across the channel cross section can be represented as Eq. 1.7.

$$\bar{u} = \frac{{y_0}^2}{3\mu}\left(-\frac{dp}{dz}\right)\left[1 - \frac{192 y_0}{\pi^5 x_0}\sum_{\mathrm{n=0}}^{\infty}\frac{tgh\left(\frac{2n+1}{2y_0}\pi x_0\right)}{(2n+1)^5}\right] \qquad 1.7$$

Since

$$-\frac{dp_l}{dz} = \frac{\lambda\rho\bar{u}^2}{2d_h} = \frac{C\mu\bar{u}}{2d_h^2} \qquad 1.8$$

Therefore,

$$C = \frac{6d_h^2}{{y_0}^2} / \left[1 - \frac{192 y_0}{\pi^5 x_0}\sum_{\mathrm{n=0}}^{\infty}\frac{tgh\left(\frac{2n+1}{2y_0}\pi x_0\right)}{(2n+1)^5}\right] \qquad 1.9$$

The microchannel is 200 μm wide and 75 μm high, whose $x_0$, $y_0$, and $d_h$ are 37.5 μm, 100 μm, and 109.1 μm, respectively. In this case, *C* is calculated to be 66.482 using Eclipse software.

Computing value error of infinite series based on various n, difference can be seen as follows:

n = 5: 1.0040518348532015
n = 15: 1.0040637834523038
n = 45: 1.0040639353004046

The microchip channel dimensions used here are 37.5 μm, 100 μm, and 109.1 μm. Since pressure drop is linear shape all throughout the whole channel, the function of channel point to the pressure can be finally represented as in Eq. 1.10.

$$P_{l,z} = 2.793\times10^9\times(z_{outlet} - z)\mu\bar{u} / m^2 + P_{atm} \longrightarrow \mathrm{F_O} \qquad 1.10$$

Also, the surface tension of initial bubble, $F_S$, can be expressed as Eq. 1.11.

$$P_s = \gamma\left(\frac{1}{R'_{1,z}} + \frac{1}{R'_{2,z}}\right) \longrightarrow F_S \qquad 1.11$$

$R'_{1,z}$ and $R'_{2,z}$ are the principal radii of curvature for all the surface points on the bubble.

## II. Discussion on $F_I$ ($P_{g,z} + P_{air,z}$)

When a bubble forms and grows, the increase in the number of gas phase molecules inside the bubble comes from the evaporation of the liquid medium and air molecules dissolved in liquid. Therefore, bubble stability is mainly based on these two factors. For material equilibrium between liquid and gas phases, the following equations can be constructed as shown in Eq. 2.1.

$$-S_g dT + V_g dP_g = -S_l dT + V_l dP_l \qquad 2.1$$

Since $V_g \gg V_l$ 2.2

Then, $T(S_g - S_l) = \Delta_{vap} H_T$ 2.3

If the gas is considered to be an ideal gas, which means

$$V_g = \frac{nRT}{P_g} \qquad 2.4$$

Then, the Eq. 2.1 can be modified as Eq. 2.5.

$$\frac{\Delta_{vap} H_{T,m}}{T^2} = \frac{dInP}{dT} \qquad 2.5$$

If $\Delta_{vap} H_{T,m}$ is considered to be a constant, then Eq. 2.5 can be integrated to obtain Eq. 2.6.

$$\frac{-\Delta_{vap}H_{T,m}}{R}(\frac{1}{T_z}-\frac{1}{T_0})=In\frac{P}{P_0} \qquad 2.6$$

If temperature is fixed, then Eq. 2.7 can be derived from Eq. 2.1.

$$V_g dP_g = V_l dP_l \qquad 2.7$$

By integrating Eq. 2.7, the following Eq. 2.8 can be obtained.

$$RTIn\frac{P_{g,z}}{P}=V_{l,m}(P_{l,z}-P) \qquad 2.8$$

The final gas phase pressure interacting with the liquid medium at each point along the channel at equilibrium can be obtained by multiplying Eq. 2.6 and 2.8 as shown in Eq. 2.9.

$$P_{g,z}=P_0\left[\exp\frac{-\Delta_{vap}H_{T,m}}{R}(\frac{1}{T_z}-\frac{1}{T_0})\right]\left\{\exp\frac{V_{l,m}\left[P_z-P_0\exp\frac{-\Delta_{vap}H_{T,m}}{R}(\frac{1}{T_z}-\frac{1}{T_0})\right]}{RT_z}\right\} \qquad 2.9$$

Considering the low solubility of air into the liquid, Henry's law can be expressed as Eq. 2.10, where $k_c$ is the function of temperature and pressure of the liquid.

$$P_{air}=k_c \bullet C_{air} \qquad 2.10$$

Inside a microchannel, however, the temperature and the pressure are fixed on each point along the Z-axis. Therefore, the air pressure at each point on Z-axis should be expressed as Eq. 2.11.

$$P_{air,z}=k_{c,z} \bullet C_{air,z} \qquad 2.11$$

In the first step of liquid medium introduction, flow is continuous and stable, and leakage of air molecules does not take place from the liquid through the sealed channel structure. In this sense, $P_{air,z}$ can also be a function of known air concentration in the inlet liquid.

$$P_{air,z} = \varphi(\bar{u}) \bullet k_{c,z} \bullet C_{air,inlet} \qquad (\varphi(\bar{u}) \leq 1) \tag{2.12}$$

$\varphi(\bar{u})$ is the function of liquid velocity and dynamic motion between the gas and liquid phases. In order to ensure bubble elimination at each point throughout the whole channel, the value is selected to be 1 in the calculation process which implies that air molecules in liquid and gas phases are totally satisfied to each other, and can be expressed as Eq. 2.13.

$$P_{air,z} = k_{c,z} \bullet C_{air,inlet} \tag{2.13}$$

Therefore, the total inner pressure, $F_I$, can be expressed as Eq. 2.14.

$$P_I = P_{g,z} + P_{air,z} \longrightarrow F_I \tag{2.14}$$

# 3. Discussion on bubble elimination condition

The condition for bubble elimination is like below.

$$P_{g,z} + P_{air,z} \leq P_{l,z} + P_s \tag{3.1}$$

By substituting each value, the final equation for bubble elimination can be derived as Eq. 3.2.

$$P_0\left[\exp\frac{-\Delta_{vap}H_{T,m}}{R}(\frac{1}{T_z}-\frac{1}{T_0})\right]$$

$$\left\{\exp\frac{V_{l,m}\left[2.793\times10^9\times(z_{outlet}-z)\mu\bar{u}/m^2+P_{atm}-P_0\exp\frac{-\Delta_{vap}H_{T,m}}{R}(\frac{1}{T_z}-\frac{1}{T_0})\right]}{RT_z}\right\} \quad 3.2$$

$$+k_{c,z}\cdot C_{air,inlet}\leq 2.793\times10^9\times(z_{outlet}-z)\mu\bar{u}/m^2+P_{atm}+\gamma(\frac{1}{R_{1,z}^{'}}+\frac{1}{R_{2,z}^{'}})$$

Based on the above derivation, in order to eliminate bubbles, $F_I$ should be decreased and $F_O$ and $F_S$ should be increased.

In summary, for bubble elimination, it is necessary to increase $F_S$ and $F_O$ but decrease $F_I$, and the three equations above can provide direction on how to eliminate bubble. Based on the above three equations, it is apparent that to decrease $F_I$, the introduced liquid should have a high boiling point, small variations in air solubility under varying temperatures and pressure conditions, and low evaporation enthalpy. To increase $F_O$, the introduced liquid should possess high viscosity and high velocity. To increase $F_S$, the surface of the internal microchannel should be smooth, and the introduced liquid should possess a high surface tension. Taking all these parameters into consideration, bubble elimination can be controlled in a more precise and accurate manner.

## Note

In order to simplify and give general illustration, the above derivation mostly bases on ideal condition. But for real case, above equation should be modified based on real situation. For example, state equation of ideal gas (Eq. 2.4) can be modified by Benedict-Webb-Rubin equation for hydrocarbon or Martin-Hou equation for polar material to get more accurate results. General compressibility factor diagram of real gas also can be used if necessary.

## Glossary

| | |
|---|---|
| $\theta$ | time |
| $v$ | dynamic viscosity |
| $u$ | liquid velocity |
| $\bar{u}$ | average velocity of liquid |
| $\rho$ | liquid density |
| $-\Delta_{vap}H_{T,m}$ | molar evaporation enthalpy at temperature $T$ |
| $m$ | meter (the length unit) |
| $Re$ | Reynolds number |
| $S$ | entropy |
| $R$ | Avogadro's constant |
| $V_{l,\ m}$ | molar volume of liquid |
| $R'_{1,z}$ , $R'_{2,z}$ | principal radii of curvature at point $z$ on a bubble |
| $\gamma$ | surface tension |
| $f_{\mathrm{B}}$ | body force per unit volume acting on the liquid |
| $C$ | dimensionless constant, equal to $\lambda \cdot \mathrm{Re}$ |
| $\lambda$ | frictional coefficient of viscous flow |
| $\mu$ | viscosity |
| $P$ | pressure |
| $d_h$ | equivalent diameter |
| $z$ | ordinate value on $Z$ axis of channel at any point |
| $z_{outlet}$ | ordinate value on $Z$ axis at channel outlet |
| $P_{atm}$ | atmospheric pressure |
| $P_{\mathrm{air,\ z}}$ | air pressure at point $z$ |
| $P_{\mathrm{s}}$ | pressure of surface tension |
| $k_{\mathrm{c,\ z}}$ | Henry's constant based on molar concentration |
| $C_{air,\ inlet}$ | concentration of dissolved air molecules in the channel inlet |
| $C_{air,\ z}$ | concentration of dissolved air molecules at point $z$ |
| $T$ | temperature |
| $P_0$ | any known evaporation pressure at temperature $T_0$ |
| $T_z$ | temperature at point $z$ of the channel |
| $V_g$ | volume of gas |
| $P_{l,\ z}$ | internal pressure of liquid medium at point $z$ |

| | |
|---|---|
| $P_{g,z}$ | evaporation pressure at point $z$ |
| $S_l$ | entropy of liquid phase |
| $S_g$ | entropy of gas phase |
| $x_0$ | half of channel width |
| $y_0$ | half of channel height |