

Support Information:

1. Concentration dependent fluorescence signal correlation:

$$\text{Beer Lambert's law: } \varepsilon lc = \ln \frac{P_0}{P_T} \quad (1)$$

P_0 is the intensity/power of the incident light; P_T is the intensity/power of the transmitted light and ε is the molar absorption coefficient under wavelength λ .

$$\text{The absorbed light: } P_A = P_0 - P_T = P_0(1 - e^{-\varepsilon lc}) \quad (2)$$

$$\text{The emitted fluorescence light: } P_F \propto \varphi \cdot P_A \propto \varphi \cdot P_0(1 - e^{-\varepsilon lc}) \quad (3)$$

φ is the quantum yield of fluorescence dye.

Thus, the emitted fluorescence light:

$$P_F = \varphi \cdot P_0 \cdot k \cdot (1 - e^{-\varepsilon lc}) = k'(1 - e^{-\varepsilon lc}) \quad (4)$$

k' is lumped parameter that depends on input power (P_0), quantum yield (φ) of the fluorescence dye and the collection efficiency of the optic system (k).

Signal intensity contributed from the emitted fluorescence light:

$$I(c) = I_s - I_b = \varphi \cdot P_0 \cdot k \cdot R \cdot (1 - e^{-\varepsilon lc}) \quad (5)$$

I_s is the obtained signal from the camera, I_b is background noise. R is the responsibility of the camera. For the selected fluorescence dye - Alexa Fluor 488, the quantum yield φ is 0.92 from reference (Molecular probes). The molar absorption coefficient ε is 71,000, which was also obtained from Molecular Probes. The camera responsibility and collection efficiency are obtained from curve fittings below.

For different concentration of fluorescence dye, the ratio of the signal intensity J :

$$J = \frac{I_{C2}}{I_{C1}} = \frac{\varphi \cdot P_0 \cdot k \cdot R \cdot (1 - e^{-\varepsilon lc1})}{\varphi \cdot P_0 \cdot k \cdot R \cdot (1 - e^{-\varepsilon lc2})} = \frac{(1 - e^{-\varepsilon lc1})}{(1 - e^{-\varepsilon lc2})} \quad (6)$$

Both $C1$ and $C2$ are the concentrations of fluorescence dye, where $C2$ is fixed. By comparing J obtained from equation (6) and experimental results, a good fitting is achieved with a deviation ~4%. This indicates the experimental results are well fitted into equation (5).

Thus, theoretically values of signal intensity are obtained by curve fittings to experimental results and I(c) as a function of dye concentration can be obtained.

2. Flow rate determination based on the concentration-dependent fluorescence signal change:

Microchannel 1 served as inlet channel. Microchannel 2 served as outlets and downstream that was connected to a small liquid reservoir. The concentration of fluorescence dye in microchannel was maintained at C_1 while the initial concentration of dye in microchannel 2 was C_2 ($C_1 \gg C_2$). While solution in microchannel 1 was transport into microchannel 2 through the nanochannels, the concentration of dye in microchannel 2 will gradually increase to C_2' after certain time. According to mass balance rule,

$$C_2' \cdot V' = C_2V + C_1n\bar{q}t \quad (7)$$

Where V is the initial volume of liquid in microchannel C_2 . V' is the final volume of liquid in microchannel C_2 . \bar{q} is the average flow rate of liquid in each nanochannel and n is the number of nanochannels.

$$\text{Since } V' = V + n\bar{q}t \quad (8)$$

$$C_2' \cdot (V + n\bar{q}t) = C_2V + C_1n\bar{q}t \quad (9)$$

The average flow rate of liquid in each nanochannel:

$$\bar{q} = \frac{V(C_2' - C_2)}{nt(C_1 - C_2')} \quad (10)$$