Electronic Supplementary Information:

Comprehensive Integration of Homogeneous Bioassays via Centrifugo-Pneumatic Cascading

Table S1 contains a geometrical description of the main parts featured in the figures describing the pneumatic tool kit as well as the main parameters regarding the operation mode.

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<td>Loading volume: 50µL Pneumatic chamber total volume: 96µL</td>
<td>Air compression: 6000 rpm Air release: 150 rpm</td>
<td>Emptying (150rpm) 1st chamber: ~ 5 min Emptying 2nd chamber: ~ 3 min</td>
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<td>Figure 4a Mixing</td>
<td>Loading volume: 50 µL Pneumatic chamber total volume: 96 µL</td>
<td>Air compression: 6000 rpm Air release: 150 rpm</td>
<td>Emptying chambers (150rpm): ~5 min Mixing: ~1 min</td>
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<td>Figure 4b Particle sedimentation</td>
<td>Loading volume: 100 µL Pneumatic chamber total volume: 139 µL Sedimentation sieve: 48 µL</td>
<td>Air compression: 6000 rpm Air release: 150 rpm</td>
<td>Particles sedimentation (6000 rpm): ~5 s</td>
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<td>Figure 4c Blood separation</td>
<td>Loading volume: 100 µL Pneumatic chamber total volume: 139 µL Sedimentation sieve: 48 µL</td>
<td>Air compression: 6000 rpm Air release: 450 rpm</td>
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<td>Figure 4d Metering</td>
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<td>Air compression: 6000 rpm Air release: 450 rpm</td>
<td>Emptying chamber (150 rpm) ~4min</td>
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<td>Figure 4e Volumetric valving</td>
<td>Loading volume: 50 µL/10 µL Pneumatic chamber total volume: 96 µL</td>
<td>Air compression: 6000 rpm Air release: 150 rpm</td>
<td>Emptying (150 rpm) 1st chamber: ~ 5 min Emptying 2nd chamber: ~ 4 min</td>
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Operational Principle

Simplified Approach

We want to approximate the radial shift $\Delta r$ induced by a change in the angular spin rate from the filling phase at $\omega$ when the entrapped air of volume $V$ with the radial height $d$, i.e. $V = d \cdot A$, of to a lower rate $\omega_2 < \omega_1$ which leads to an expanded volume $V_2$ (Figure 1). $A_{\text{left}}$ and $A_{\text{right}}$ denote the cross sections of the left and the right hand chambers, respectively, and $\tilde{A} = A_{\text{left}} / A_{\text{right}}$ their ratio. The radial differences of the liquid levels at the first and second frequencies are represented by $h_1$ and $h_2$ and the radial spacing from the centre of rotation as $R_1$ and $R_2$. The liquid volume $\Delta V$ of density $\rho$ is displaced as the entrapped air volume expands, shifting the liquid levels by $\Delta r_{\text{left}}$ and $\Delta r_{\text{right}}$. The relation

$$A_r \Delta r \sim \Delta V_{\text{left}} \Delta A_r$$

results from continuity of mass (assuming the incompressibility of the liquid). We can then express the difference of the liquid levels at $\omega_2$

$$h_2 = h_1 + \Delta r + \Delta r_{\text{left}} = h_1 + (1 + \tilde{A}) \Delta r$$

in terms of the elevation of the meniscus in the right-hand branches $\Delta r$.

We first consider Boyle’s law for the entrapped gas volume

$$\frac{p_1}{p_2} = \frac{V_2}{V_1} = \frac{V_1 + \Delta V}{V_1} = 1 + \frac{\Delta V}{V_1} = 1 + \frac{\Delta r}{d_1}$$

(1)

assuming a constant cross sections $A_{\text{left}}$ of the compression chamber.

On the other hand, the hydrostatic pressure head in the centrifugally induced artificial gravity field $g_{\omega,i} = R_i \omega_i^2$ between the two vessels is obtained from

$$p_i = p_0 + \rho R_i \omega_i^2 h_i$$

with the environmental pressure $p_0$. For large spacing from the centre of rotation $R_i$ and small relative radial shifts of the menisci $\Delta r_{\text{left}} / R_i << 1$ and $\Delta r / R_i << 1$, i.e. $R_1 \approx R_2$, and we also assume $p_0 << p_i$. We then obtain a hydrostatic pressure ratio

$$\frac{p_1}{p_2} = \tilde{\omega}^2 \frac{h_1}{h_2} = \tilde{\omega}^2 \frac{h_1}{h_1 + \Delta r (1 + \tilde{A})} = \tilde{\omega}^2 \frac{1}{1 + \frac{\Delta r}{h_1} (1 + \tilde{A})} \propto \tilde{\omega}^2$$

(2)

with the spin rate ratio $\tilde{\omega} = \omega_1 / \omega_2$. Combining the pressure ratios from Boyle’s law (1) and the hydrostatic pressure heads in two spin phases (2) eventually yields the radial shift of the right-hand meniscus

$$\Delta r = d_1 \left[ 1 - \tilde{\omega}^2 \right]$$

(3)

which is responsible for triggering the right-hand siphon. So within the limitations of the coarse approximation (1) which assumes vanishing environmental pressure $p_0 << p_i$, constant chamber cross sections $A_i$ and small radial changes, the lift of the meniscus $\Delta r$ in the right chamber with $\omega_2 < \omega_1$ essentially scales with the radial height $d_1$ of the gas volume entrapped after the priming phase at $\omega_1$ and with the square of the ratio of the spinning frequencies $0 < \tilde{\omega} < 1$. 

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**Exact Calculation for Simplified Geometry**

For a more exact calculation, the gas volume \( V_0 \) measured at the environmental pressure \( p_0 \) would have to be known (it depends on the detailed course of the priming process) and we obtain \( p_0 \) \( V_0 = p_1 \) \( V_1 = (p_0 + \rho R_1 \omega_1^2 h_1) \) \( d_1 \) \( A_{\text{left}} \) using Boyle’s law (1) again which can be rewritten using \( R_1 = R_{\text{left}} + d_1 \) to express the initial split of the liquid levels

\[
h_1(\omega_1) = \left( \frac{V_0}{d_1 A_{\text{left}}} - 1 \right) \frac{p_0}{\rho (R_{\text{left}} + d_1) \omega_1^2}
\]

as a mere function of the initial spin rate \( \omega_1 \) and known values. So the more detailed calculation of the pressure ratio yields

\[
\frac{p_1}{p_2} = \frac{p_0 + \frac{R_1}{2} h_1(\omega_1)}{p_0 + \frac{R_2}{2} h_2} = \frac{p_0 + \frac{R_{\text{left}} + d_1}{2} h_1(\omega_1)}{p_0 + \frac{R_{\text{left}} + d_1 + \frac{r}{A}}{2} \left[ h_1(\omega_1) + (1 + \tilde{A}) \frac{r}{d_1} \right]} \quad (4)
\]

So equating (1) with (4) provides an equation for \( \Delta r \) which explicitly reads

\[
1 + \frac{r}{p_0 \left[ R_{\text{left}} + d_1 + \frac{r}{A} \right]} \left[ \left( \frac{\tilde{V} - 1}{\rho} \right) \frac{p_0}{(R_{\text{left}} + d_1)^2} \left( 1 + \tilde{A} \right) \frac{r}{d_1} \right] = 1 + \frac{r}{d_1}
\]

as a function and known experimental parameters, i.e. the entrapped gas volume \( V_0 \) measured at the environmental pressure \( p_0 \), the liquid density \( \rho \), the geometry and alignment if the disc-based structures characterized by the cross section of the compression chambers \( A_{\text{left}} \) and \( A_{\text{right}} = A_{\text{left}} / \tilde{A} \), the radial distance of the upper edge of the compression chamber from the centre of rotation \( R_{\text{left}} \), and the radial extension of the entrapped gas pocket \( d_1 \) measured at the angular frequency \( \omega_1 \), and the volume compression ratio \( \tilde{V} = V_0 / V_1 = p_1 / p_0 \). We can rewrite

\[
\left( \frac{1 + \frac{r}{d_1}}{p_0 \left[ R_{\text{left}} + d_1 + \frac{r}{A} \right]} \left[ \left( \frac{\tilde{V} - 1}{\rho} \right) \frac{p_0}{(R_{\text{left}} + d_1)^2} \left( 1 + \tilde{A} \right) \frac{r}{d_1} \right] \right) \tilde{V} = 0 \quad (5)
\]
so the exact (at least when assuming constant cross sections $A_{\text{left}}$ and $A_{\text{right}}$) radial shift $\Delta r(\omega_1, \omega_2)$ is the solution of this inhomogeneous cubic equation.