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.

Figure number	**Volumes**	**Operation frequencies**	**Operation times**
Figure 3 Pneumatic cascade Principle	Loading volume: 50μL Pneumatic chamber total volume: 96μL	Air compression: 6000 rpm Air release: 150 rpm	Emptying (150rpm) 1st chamber: ~ 5 min Emptying 2nd chamber: ~ 3 min
Figure 4a Mixing	Loading volume: 50 μL Pneumatic chamber total volume:96 μL	Air compression: 6000 rpm Air release: 150 rpm	Emptying chambers (150rpm): ~5 min Mixing: ~1 min
Figure 4b Particle sedimentation	Loading volume: 100 μL Pneumatic chamber total volume:139 μL Sedimentation sieve:48 μL	Air compression: 6000 rpm Air release: 150 rpm	Particles sedimentation (6000 rpm): ~5 s
Figure 4c Blood separation	Loading volume: 100 μL Pneumatic chamber total volume: 139 μL Sedimentation sieve:48μL	Air compression: 6000 rpm Air release: 450 rpm	Blood separation (6000 rpm): ~ 2min
Figure 4d Metering	Loading volume: 50 μL Pneumatic chamber total volume:121 μL Waste volume chamber:11 μL	Air compression: 6000 rpm Air release: 450 rpm	Emptying chamber (150 rpm): ~4min
Figure 4e Volumetric valving	Loading volume: 50 μL/10 μL Pneumatic chamber total volume:96 μL	Air compression: 6000 rpm Air release: 150 rpm	Emptying (150 rpm) 1st chamber: ~ 5 min Emptying 2nd chamber: ~ 4 min

Operational Principle

Simplified Approach

We want to approximate the radial shift Δr induce by a change in the angular spin rate from the filling phase at ω when the entrapped air of volume V with the radial height d, i.e. $V = d\,A$, of to a lower rate $\omega_2 < \omega_1$ which leads to an expanded volume V_2 (Figure 1). A_{left} and A_{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 ΔV of density ρ is displaced as the entrapped air volume expands, shifting the liquid levels by Δr_{left} and Δr. The relation

$$\Delta r = \Delta r_{\text{left}}\,\tilde{A}$$

results from continuity of mass (assuming the incompressibility of the liquid). We can then express the difference of the liquid levels at ω_2

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

in terms of the elevation of the meniscus in the right-hand branches Δr.
We first consider Boyle's law for the entrapped gas volume

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

assuming a constant cross sections A_{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

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

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

$$\Delta r = d_1 \cdot \left|1 - \tilde{\omega}^2\right| \qquad (3)$$

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 Δr 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 ω_1 and with the square of the ratio of the spinning frequencies $0 < \tilde{\omega} < 1$.

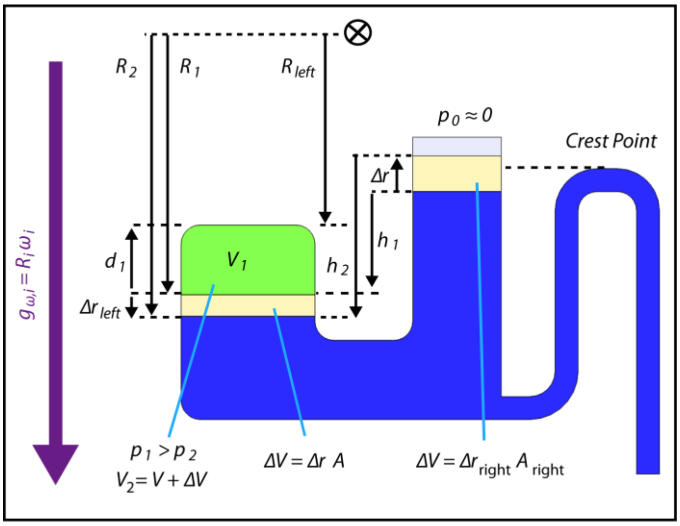

Figure S1. Schematic representation of one pneumatic unit as a core constituent of the pneumatic cascade.

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 $p_0\, V_0 = p_1\, V_1 = (p_0 + \rho\, R_1\, \omega_1{}^2\, h_1)\, d_1\, A_{\mathrm{left}}$ using Boyle's law (1) again which can be rewritten using $R_1 = R_{\mathrm{left}} + d_1$ to express the initial split of the liquid levels

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

as a mere function of the initial spin rate ω_1 and known values. So the more detailed calculation of the pressure ratio yields

$$\frac{p_1}{p_2} = \frac{p_0 + \rho\, R_1\, \omega_1^2 h_1(\omega_1)}{p_0 + \rho\, R_2\, \omega_2^2 h_2} = \frac{p_0 + \rho \left(R_{\mathrm{left}} + d_1\right) \omega_1^2 h_1(\omega_1)}{p_0 + \rho \left[R_{\mathrm{left}} + d_1 + \frac{\Delta r}{\tilde{A}} \right] \omega_2^2 \left[h_1(\omega_1) + \left(1 + \tilde{A}\right) \Delta r \right]} \qquad (4)$$

So equating (1) with (4) provides an equation for Δr which explicitly reads

$$\frac{\tilde{V}}{1 + \frac{\rho}{p_0} \left[R_{\mathrm{left}} + d_1 + \frac{\Delta r}{\tilde{A}} \right] \omega_2^2 \left[\left(\tilde{V} - 1\right) \frac{p_0}{\rho \left(R_{\mathrm{left}} + d_1\right) \omega_1^2} + \left(1 + \tilde{A}\right) \Delta r \right]} = 1 + \frac{\Delta r}{d_1}$$

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 ρ, the geometry and alignment if the disc-based structures characterized by the cross section of the compression chambers A_{left}, and $A_{\mathrm{right}} = A_{\mathrm{left}} / \tilde{A}$, the radial distance of the upper edge of the compression chamber from the centre of rotation R_{left}, and the radial extension of the entrapped gas pocket d_1 measured at the angular frequency ω_1, and the volume compression ratio $\tilde{V} = V_0 / V_1 = p_1 / p_0$. We can rewrite

$$\left(1 + \frac{\Delta r}{d_1}\right) \left\{ 1 + \frac{\rho}{p_0} \left[R_{\mathrm{left}} + d_1 + \frac{\Delta r}{\tilde{A}} \right] \omega_2^2 \left[\left(\tilde{V} - 1\right) \frac{p_0}{\rho \left(R_{\mathrm{left}} + d_1\right) \omega_1^2} + \left(1 + \tilde{A}\right) \Delta r \right] \right\} - \tilde{V} = 0 \qquad (5)$$

so the exact (at least when assuming constant cross sections A_{left} and A_{right}) radial shift $\Delta r(\omega_{1,}\ \omega_2)$ is the solution of this inhomogeneous cubic equation.