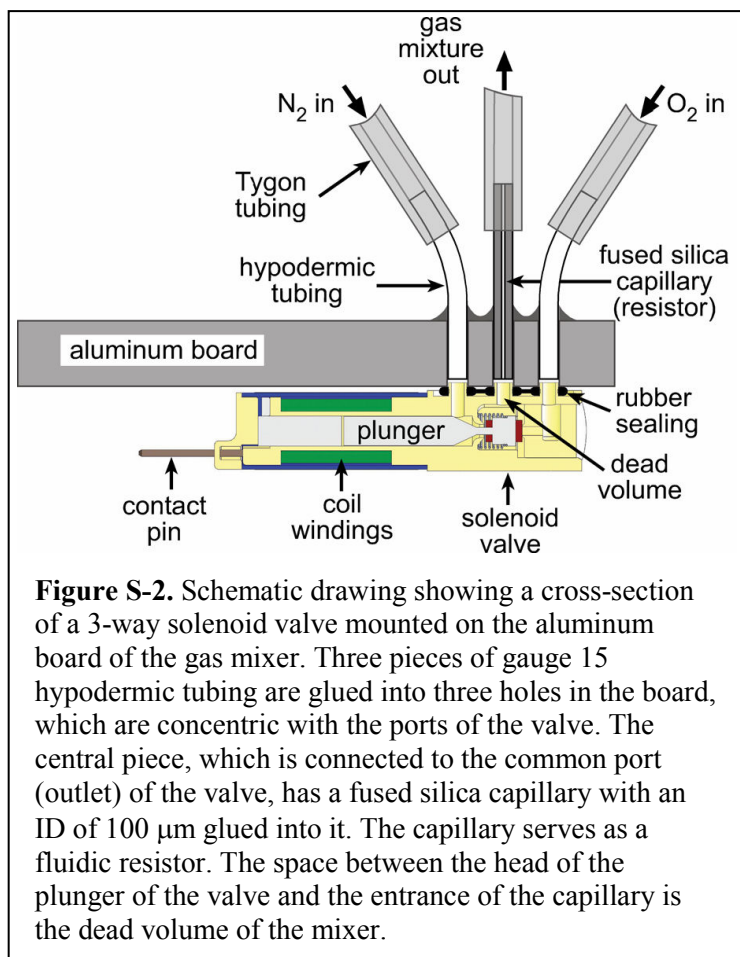


The multi-channel gas mixer (Fig. S-1) is designed to mix two gases fed to its inlets in desired proportions and to supply the gas mixtures to microfluidic devices at volumetric flow rates, Q , of 0.1 – 0.3 mL/s. The mixer has 10 identical channels (only 9 channels are used in the present work), each consisting of three essential elements: a miniature 3-way solenoid valve (LHDA 1223111H by The Lee Company), which has two inlets and one outlet and is periodically switched with a controlled duty cycle; a fluidic resistor connected to the common port (outlet) of the valve to control the flow rate through the valve by setting the pressure at its inlets; and a mixer tube downstream of the resistor to homogenize the gas mixture generated by the valve (Fig. S-1 and S-2). The 10 valves are face-mounted on a polished aluminum board with 3 rows of holes in it and 10 holes in each row (Fig. S-1 and S-2). The holes in the two outer rows are concentric with the valve inlets, and the holes in the central row are concentric with the valve outlets. 20 mm long pieces of stainless steel hypodermic tubing (gauge 15) are glued into the holes (Fig. S-2). The steel tubing pieces in the outer rows are connected through short segments of Tygon tubing (with the internal diameter, ID, of 1/16") to two separate gas distributors (made of modified 10 cc plastic syringes), one for all normally-opened valve inlets and one for all normally-closed valve inlets. The former gas distributor is connected to a



μm and serve as fluidic resistors immediately adjacent to the valve outlets. Air flow rates of 0.1 and 0.2 mL/s through a valve (as measured by the displacement of water from a graduated cylinder) require pressures of 2 psi (14 kPa) and 4 psi (28 kPa), respectively, at the valve inlets. The pieces of hypodermic tubing in the central row are connected to the gas inlets of the microfluidic device through the gas mixer tubes, which are 10 cm long segments of Tygon tubing with ID = 3/16", and 12 cm long segments of Tygon tubing with ID = 0.75 mm (with some connectors in between; Fig. S-1 and S-2).

The solenoid valves are switched on and off periodically. The period, T , is normally 2 s. The duty cycle of the valve, which is defined as the ratio between the time when the valve is powered (ON time), t_{ON} , and the period, T , determines the fractions of time that the valve is opened to the flow of O_2 (or air) and to the flow of N_2 . At a volumetric flow rate $Q = 0.1$ mL/s in both ON and OFF states, the gas flow in a mixer tube has a mean

source of filtered, pressure-regulated nitrogen, N_2 (99.995% purity), and the latter distributor is connected to filtered, pressure-regulated oxygen (O_2) or air.

The pieces of hypodermic tubing in the central row, which are connected to the outlets of the solenoid valves, have 20 mm long segments of polyimide-coated fused-silica capillaries (TSP100200 by Polymicro Technologies) glued into them, with the rest of the tubing interior filled with epoxy (Fig. S-2). The capillaries have an ID of 100

velocity $\bar{v} = 0.56$ cm/s and one period of the valve corresponds to an ~ 1.1 cm long segment of gas injected into the mixer tube, with a total of 9 segments in the mixer tube.

To explore the distribution of oxygen concentration, $[O_2]$, in a mixer tube, we used a code in MatLab to perform one-dimensional (1D) numerical simulations of the $[O_2]$ profile along the tube, accounting for the flow of gas and diffusion of O_2 , but disregarding the non-uniformity of both flow velocity and $[O_2]$ over cross-section of the tube. (The actual velocity profile was expected to be parabolic, leading to some non-uniformity of the $[O_2]$ profile across the tube.) The value of $[O_2]$ at the inlet (left edge) changed between 0 (N_2) and 100% (O_2) with the period $T = 2$ s and a duty cycle t_{ON} / T ; O_2 diffused along the 10 cm computational domain with a diffusion coefficient, $D_{gas} = 0.2$ cm²/s, and the entire O_2 distribution uniformly moved towards the outlet (right edge) with velocity \bar{v} . The initial condition was $[O_2] = 0$ everywhere.

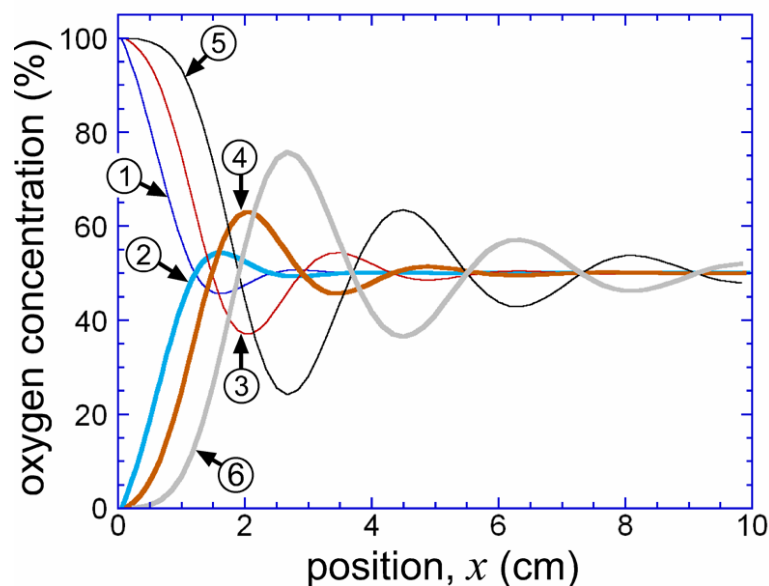
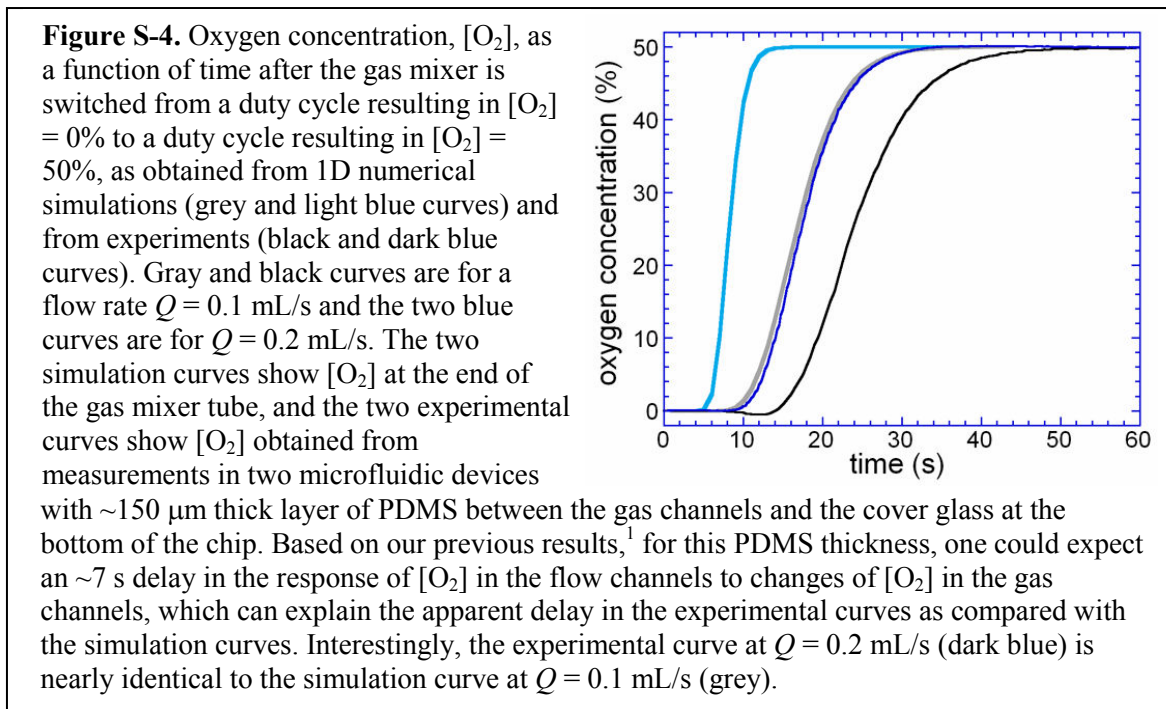


Figure S-3. Distributions of oxygen concentration, $[O_2]$ (in %), along the 10 cm long gas mixer tube after 20 cycles of the solenoid valve starting from $[O_2] = 0\%$ (when steady states are reached) from 1D numerical simulations in MatLab. The simulations were performed for oxygen diffusion coefficient $D_{gas} = 0.2$ cm²/s, a period $T = 2$ s, and a duty cycle $t_{ON} / T = 50\%$. (The condition at the left boundary, $x = 0$, was changing periodically between $[O_2] = 100\%$ for 1 s and 0% for 1 s.) The gas flow velocities (from left to right) were $\bar{v} = 0.56$ cm/s (curves 1 and 2), 1.12 cm/s (curves 3 and 4), and 1.68 cm/s (curves 5 and 6) corresponding to the volumetric flow rates $Q = 0.1$, 0.2 , and 0.3 mL/s, respectively. The odd- and even-numbered curves correspond to moments at the ends of the phases with $[O_2] = 100\%$ (end of O_2 injection) and $[O_2] = 0\%$ (end of N_2 injection) at $x = 0$.



The simulations were performed for duty cycles of 0.5 (Fig. S-3) and 0.1 (not shown), which were representative of proportional and disproportional mixing. The simulations at $\bar{v} = 0.56$ cm/s showed that when a steady state is reached after ~ 20 periods, $[O_2]$ at the outlet becomes equal to $t_{ON} / T \times 100\%$ with both fractional error and peak-to-peak variations (within one period) of < 0.001 of the mean concentration for both duty cycles. The peak-to-peak variations remained at < 0.001 of $t_{ON} / T \times 100\%$ also at $\bar{v} = 1.12$ cm/s (corresponding to $Q = 0.2$ mL/s) and reached an appreciable level of ~ 0.08 of the mean only at $\bar{v} = 1.68$ cm/s (Fig. S-3; with the mean value still within 0.001 of $t_{ON} / T \times 100\%$).

The simulations indicate that the mixing of the gas in the mixer tube is practically perfect with the steady state $[O_2]$ matching to the duty cycle up to $Q = 0.2$ mL/s. Moreover, according to the simulations, the steady states are established relatively quickly, with only 25 s at $Q = 0.1$ mL/s and only 11 s at $Q = 0.2$ mL/s required to reach 9/10 of the ultimate steady-states concentration (Fig. S-4). The transition times between 1/10 and 9/10 of the steady-state concentration were even shorter, at 13 and 5 s for $Q = 0.1$ mL/s and 0.2 mL/s, respectively.

To test the switching time of the gas mixer experimentally, we measured $[O_2]$ in the test channel of the device using a solution of the oxygen-sensitive fluorescent dye,

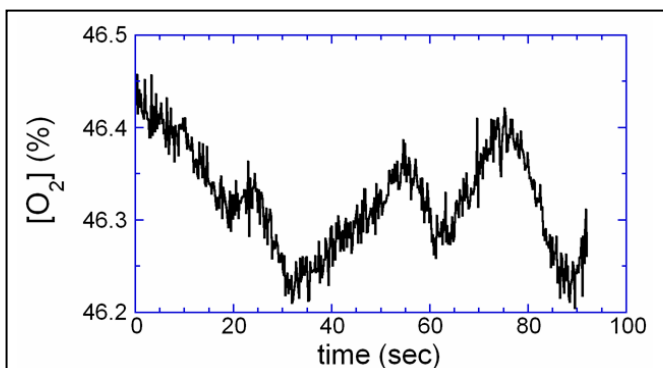


Figure S-5. Time series of oxygen concentration, $[O_2]$, measured in the test channel of the microfluidic device. The gas mixer was fed with O_2 and N_2 and all its channels operated at a flow rate $Q = 0.2$ mL/s, with a period $T = 2$ s and a duty cycle of ~ 0.5 . The measurements were done using oxygen-sensitive fluorescence of RTDP at a sampling rate of 5 times per second.

RTDP. The fluorescence was measured at a function of time after all 9 channels of the mixer operating at $Q = 0.2$ mL/s were switched from a cycle generating $[O_2] = 0\%$ to a cycle generating $[O_2] = 50\%$ (dark blue curve in Fig. S-4). Fluorescence micrographs of the test channel were taken every 0.5 s, and the intensity of fluorescence in the test channel, I , was converted into $[O_2]$ using the equation $[O_2] = (I_0 / I - 1) / K_q$ (with I_0 and K_q measured separately), as

explained in the main text. The transition was 9/10 complete after 24 s, and transition time from $[O_2] = 5\%$ to $[O_2] = 45\%$ (1/10 – 9/10 through the transition) was 12 s. In a separate experiment, all 9 channels of the mixer were switched from a cycle generating $[O_2] = 25\%$ to a cycle generating $[O_2] = 75\%$ and a nearly identical transition curve was measured (not shown). The test was repeated at $Q = 0.1$ mL/s in a similar two-layer device that had the same thickness of the PDMS layer between the gas channels and cover glass (150 μm , similar to the device used in the study), and the transition was 9/10 complete after 34 s with 16 s between 1/10 and 9/10 through the transition. Somewhat longer transition times in the experiments, as compared to the simulations, were likely due to a substantial delay (estimated at ~ 7 s) in the response of $[O_2]$ in the flow channels (measured by the fluorescent dye) to changes of $[O_2]$ in the gas channels due to a relatively thick layer of PDMS between the two channel layers.¹

To test for temporal variations of $[O_2]$ in the test channel of the device, in particular on the time scale of the period of the valve switching, $T = 2$ s, we acquired a 90 s time series of the intensity of fluorescence of RTDP in the test channel, I , taking 5 frames per second, and calculated $[O_2] = (I_0 / I - 1) / K_q$ for each frame. The mixer was fed with N_2 and O_2 at 4 psi and all of its channels operated at $t_{ON} / T \approx 0.5$ (with synchronous

switching) and $Q = 0.2$ mL/s. The resulting 90 s time series (Fig. S-5) showed that $[O_2]$ in the test channel remained nearly constant, varying with a standard deviation, SD, of only 0.055% from its 46.3% mean value. This value of SD corresponded to a coefficient of variation, CV, of ~ 0.001 , which was comparable to instrumental errors due to fluctuations in the power of fluorescence illumination and due to the vibration and drift of mechanical parts of the microscope. Moreover, Fourier analysis of the time series showed practically no component with the valve switching period of 2 s (it had an amplitude of 0.022% or 0.0005 of the mean value), which was an experimental evidence for the efficiency of the gas mixer tube. (We note, however, that because of the estimated ~ 7 s delay between variations of $[O_2]$ in the gas channels and the test channel, the amplitude of the 2 s variations of $[O_2]$ in the test channel was expected to be reduced by a factor of ~ 20 compared with that in the gas channels.)

It is worth noting that, according to the simulations, in spite of the extreme simplicity of its construction, the gas mixer tube achieves two important goals, which are generally difficult to combine: very low variations of $[O_2]$ at the outlet during a cycle of the valve ($CV < 0.001$ at $Q = 0.2$ mL/s) and a short transition time (only 5.5 cycles at $Q = 0.2$ mL/s). The combination of these useful features depends on the exact repetition of the valve cycles, including their period, T , and the ON time, t_{ON} . An important practical requirement for appropriate operation of the gas mixer is that the ratio between the volume of O_2 , V_O , and the total volume of gas, V_t , passing through the solenoid valve during one cycle closely matches the desired value of $[O_2]$ in the gas mixture.

The solenoid valves in the mixer had a short response time of ~ 4 ms, and from our previous work,² the repeatability of their switching was estimated as better than 1 ms. The valves were controlled using a home-built driver, which was interfaced with a dedicated computer through a National Instruments PCI-6503 card. The card was programmed using a code in LabView, and the timing of the signals sent to the valves was expected to be accurate within 1 ms as well. The error of 1 ms was very small compared with the 2 s period of the valve cycles and was thus expected to lead to negligible errors in the definition of both T and t_{ON} . A much more significant source of error in the definition of $[O_2]$ was the relation between the actual volume ratio V_O/V_t and

the electronically defined duty cycle, t_{ON}/T . Among the factors contributing to the lack of correspondence between the two ratios were differences in the pressures of O₂ (or air) and N₂ at the two valve inlets, difference between the viscosities of O₂ (or air) and N₂, and the presence of a dead volume between the plunger of the valve and the fluidic resistor (capillary; Fig. S-2).

To minimize differences between the pressures of O₂ and N₂, both pressures were carefully adjusted and equilibrated using two sensitive regulators (8310ANBF5 by Porter Instruments) and a single high-accuracy pressure gauge (digital gauge by Heise with an accuracy of 0.015 psi), which was alternatively connected to the two regulators. The resulting pressure difference was estimated as <0.5% and was expected to lead to an insignificant error in [O₂] in the gas mixture. The viscosities of N₂, air, and O₂ at 20 °C and atmospheric pressure are 1.747×10^{-5} , 1.837×10^{-5} , and 2.023×10^{-5} Pa·s, respectively. That is, the viscosities of air and O₂ are higher than the viscosity of N₂ by factors of $k_a = 1.05$ and $k_o = 1.16$, respectively. Therefore, one could expect to obtain a desired value of [O₂] (in % of pure O₂) by setting the duty cycle at

$$t_{ON}/T = \frac{k_o[\text{O}_2]}{100\% + (k_o - 1)[\text{O}_2]} \quad (1),$$

when mixing O₂ and N₂, and at

$$t_{ON}/T = \frac{k_a[\text{O}_2]}{20.9\% + (k_a - 1)[\text{O}_2]} \quad (2),$$

when mixing air and N₂.

One could also expect, however, that the presence of the finite dead volume of gas between the plunger of the valve and the inlet of the fluidic resistor (Fig. S-2) would necessitate corrections to the calculated duty cycles. Indeed, when the valve is switched from N₂ to O₂, the viscosity of the gas flowing through the capillary is lower than the viscosity of O₂, until the entire dead volume is purged of N₂. Vice-versa, immediately after the valve is switched from O₂ to N₂, the viscosity of the gas flowing through the capillary is higher than the viscosity of N₂. Therefore, the direct application of the equations 1 and 2 to calculate the duty cycle was likely to result in V_o/V_i somewhat

higher than t_{ON}/T (and somewhat higher than desired $[O_2]$ in the gas mixture). The magnitude of this effect was expected to be proportional to the ratio between the dead volume and V_t . Therefore, we used face-mounted solenoid valves with a small dead volume and placed the capillaries very close to the valve outlets. (A large dead volume would also cause another undesirable effect, disproportionately high sensitivity of $[O_2]$ in the mixtures to small mismatches between the pressures at the two valve inlets.)

The values of $[O_2]$ in the gas mixtures produced by the mixer were evaluated with a commercial oxygen analyzer (MAXO₂ by Maxtec) with a nominal accuracy of 1%.¹ In addition, $[O_2]$ was also evaluated by measurements of fluorescence of RTDP in a microchannel (as described in the main text and in Ref. 1) in a microfluidic device similar to one used in this study, with an expected absolute accuracy of better than 1% in $[O_2]$ (and down to 0.1% in $[O_2]$ at low $[O_2]$). Typical results of measurements of $[O_2]$ (in %) for mixtures of air and N₂ with t_{ON}/T from eq. 2 are shown in Table 1.

Table 1. Measurements $[O_2]$ (in %) for mixtures of air and N₂ with t_{ON}/T from eq. 2

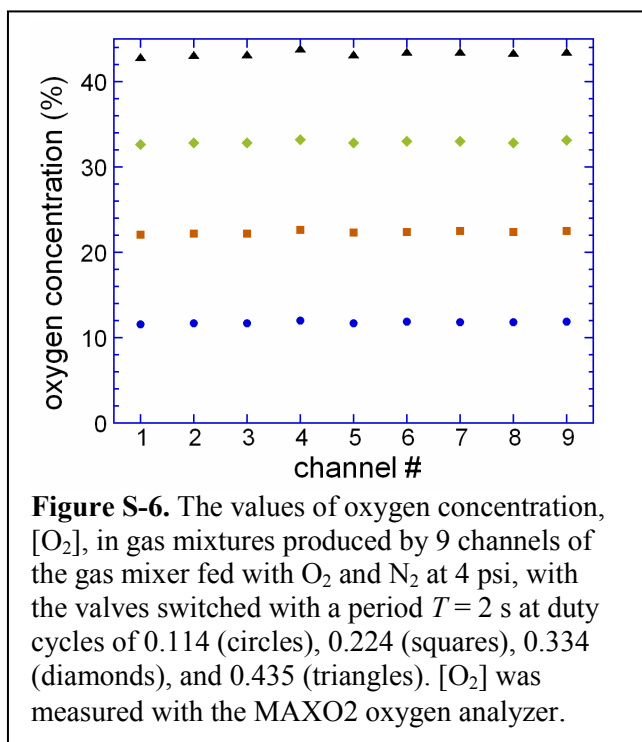
expected from eq. 2	0	2	4	6	8	10	12	14	16	18
measured with analyzer	0.2	2.3	4.2	6.1	8.1	10.4	12.0	14.1	16.0	18.0
measured by fluorescence	0.00	2.16	4.25	6.18	8.26	10.22	12.25	14.07	16.15	17.94

As expected, the direct application of eq. 2 resulted in somewhat higher than desired values of $[O_2]$. Therefore, to improve the accuracy of $[O_2]$ in the mixtures, we corrected the values of $[O_2]$ plugged into eq. 2, subtracting from them 0 to 0.2%, depending on the range. The results obtained with the corrections are shown in Table 2.

Table 2. Measurements $[O_2]$ (in %) for mixtures of air and O₂ with corrected t_{ON}/T

expected after corrections	0	2	4	6	8	10	12	14	16	18
measured with analyzer	0.1	2.0	4.0	6.0	7.9	9.9	11.9	14.0	16.0	17.9
measured by fluorescence	0.00	1.93	4.03	6.01	7.96	9.95	11.96	13.94	15.96	17.86

The values of $[O_2]$ measured with both the oxygen analyzer and fluorescence microscopy were very close to the desired values, with an absolute standard deviation 0.05% for both measurement techniques, which was as low as one could expect given the instrumental errors.



We also tested variations of $[O_2]$ between the 9 channels of the gas mixer, when they were all operated at the same period $T = 2$ s with identical duty cycles. In this test, the mixer was fed with O_2 and N_2 at 4 psi (resulting in $Q = 0.2$ mL/s), the duty cycles were set at 0.114, 0.224, 0.334 and 0.435 (values producing $[O_2] = 10\%$, 20%, 30%, and 40%, according to eq. 1), and $[O_2]$ was measured with the oxygen analyzer. The results of the test (Fig. S-6) indicated a good consistency of $[O_2]$ in different

channels. Its absolute standard deviations at $t_{ON} / T = 0.114$, 0.224, 0.334 and 0.435 were 0.12%, 0.17%, 0.19%, and 0.29%, respectively, which were well within the instrumental error of the analyzer. The variations of $[O_2]$ between measurements taken on different days at the same Q , T , and duty cycle (not shown) were somewhat higher, but still within the error of the analyzer. Therefore, once calibrated and corrected, the gas mixer could be reliably used without the necessity of monitoring $[O_2]$ in the generated gas mixtures.

The hydrodynamic resistance of the fluidic resistors in the gas mixer was chosen to obtain volumetric flow rates of air in the range of 0.1 – 0.3 mL/s at air pressures of 2 – 6 psi at the mixer inlets. Pressures in this range do not require special reinforced tubing and are simple to adjust and to measure accurately. The flow rates in the range 0.1 – 0.3 mL/s result in small gas consumption (with each of the 10 channels of the mixer operating at

0.2 mL/s, a standard 200 ft³ gas cylinder is expected to last for more than a month) and from our previous work,¹ we expected a flow rate of 0.2 mL/s to be sufficiently high to prevent contamination of the gas mixtures fed to the device by the atmospheric air. (The sources of contamination include the diffusion through the porous PDMS chip and uncontrolled leakages at the tubing connection points and its effect on [O₂] in the gas mixtures is expected to be inversely proportional to the gas flow rate.¹) Importantly, when the gas channels have a depth of 150 μm (as in the presented device), which is easy to produce using standard microfabrication procedures, $Q = 0.2$ mL/s corresponds to a relatively low pressure at the gas inlets. Indeed, for a fully developed laminar flow of air with viscosity $\eta_a = 1.8 \cdot 10^{-5}$ Pa·s at a volumetric flow rate $Q = 0.2$ mL/s in a square channel with a width $w = 150$ μm and length $L = 12$ mm (typical dimensions for the gas channels of the device), the pressure drop across the channel is $\Delta P = 28 \frac{L \eta_a Q}{w^4} \approx 2.4$ kPa.

Because the gas outlet is vented to atmosphere, ΔP also represents the gauge pressure at the gas inlets and the maximal gauge pressure inside the gas channels, and at its estimated value of 2.4 kPa, this pressure is expected to cause only minor stresses in the device and minor deformations of gas channels. Moreover, the pressure drop of 2.4 kPa at $Q = 0.2$

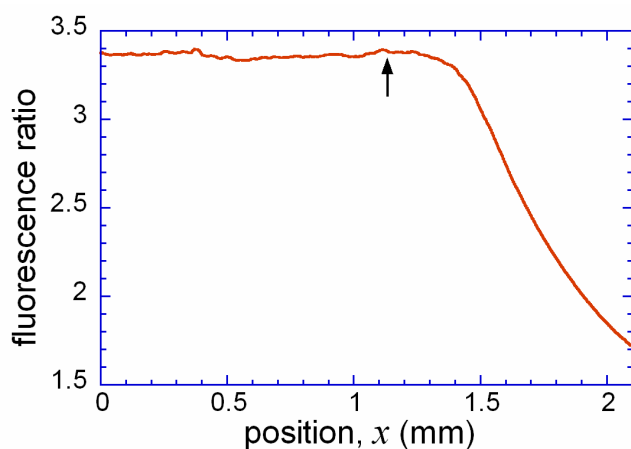


Figure S-7. The ratio of intensities of fluorescence in the 90 μm wide test channel (filled with a 250 ppm solution of RTDP) between the cases when N₂ and O₂ is fed to all 9 gas inlets. The fluorescence ratio is plotted as a function of the position along the test channel, x , with $x = 0$ corresponding to the middle of gas channel 5 (the center of the gas channel array; Fig. 1 in the main text). $x = 1.125$ mm (indicated by an arrow; cf. Fig. 1b in the main text) corresponds to the center of gas channel 9 (cf. Fig. 3 in Ref. 1).

In the internal region, between $x = 0$ and 1.125 mm, the mean value of the measured fluorescence ratio is 3.35 and the standard deviation is 0.0135, corresponding to ~0.5% in [O₂], which is within the measurement error. Therefore, the results of the measurements are consistent with the result of numerical simulations (cf. Fig. 1b in the main text) that when the gas mixtures with the same [O₂] are fed to all gas channels, [O₂] in the internal region of the test channel is uniform and identical to [O₂] in the gas channels.

mL/s is ~ 10 times smaller than the pressure drop across the fluidic resistors at the same gas flow rate (4 psi \approx 28 kPa). Therefore, connecting the gas mixer to the microfluidic device is expected to have little influence on the operation of the mixer (other than $\sim 8\%$ reduction in Q), and $[O_2]$ in the gas channels is not expected to change due to variations in the dimensions of the channels or layout of the device, provided that the channel resistances remain sufficiently small.

References.

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2. K. Campbell, Y. Fainman and A. Groisman, *Applied Physics Letters*, 2007, **91**.