Supplementary Information (SI) for Lab on a Chip.
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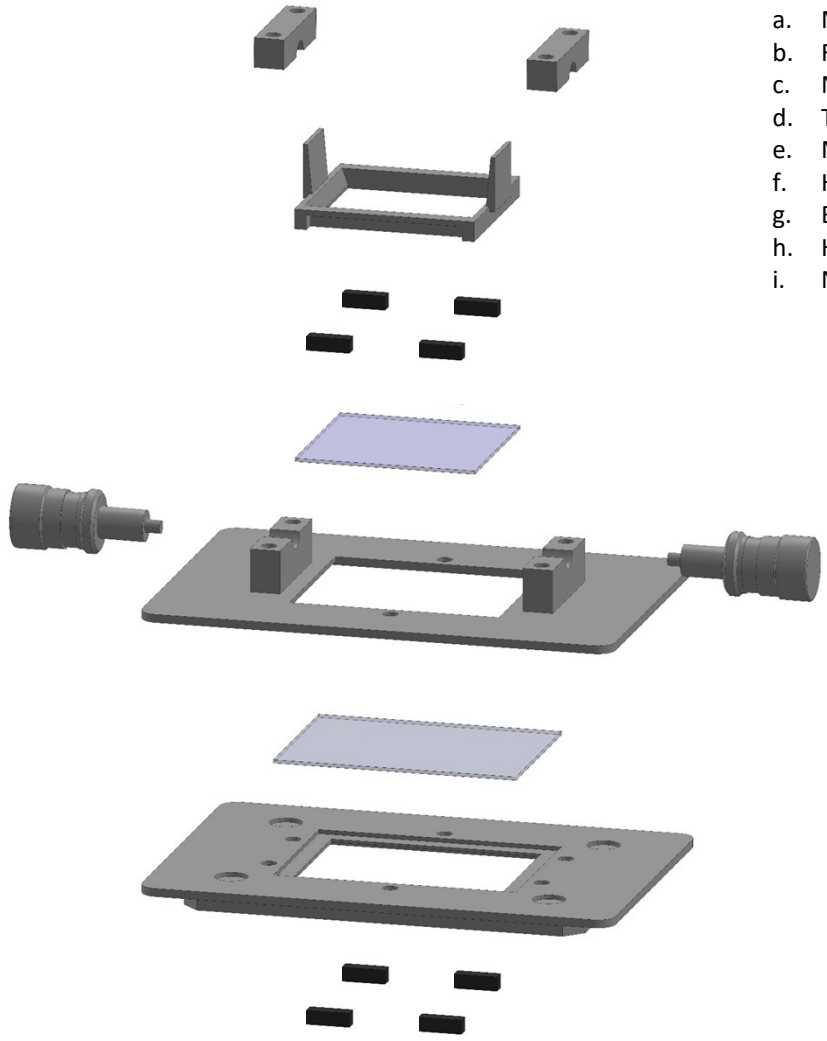
Supplementary Information

Table S1 Vitamin components in L1 media (values sourced from the L1 media kit, NCMA at Bigelow Laboratory, US).

Component	**Concentration in final media [M]**
Thiamine · HCl (vit. B1)	2.96×10^{-7}
Biotin (vit. H)	2.05×10^{-9}
Cyanocobalamin (vit. B12)	3.69×10^{-10}

Table S2 Trace elements components in L1 media (values sourced from the L1 media kit, NCMA at Bigelow Laboratory, US).

Component	**Concentration in final media [M]**
$Na_2EDTA \cdot 2H_2O$	1.17×10^{-5}
$FeCl_3 \cdot 6H_2O$	1.17×10^{-5}
$MnCl_2 \cdot 4H_2O$	9.00×10^{-7}
$ZnSO_4 \cdot 7H_2O$	8.00×10^{-8}
$CoCl_2 \cdot 6\ H_2O$	5.00×10^{-8}
$CuSO_4 \cdot 5\ H_2O$	1.00×10^{-8}
$Na_2MoO_4 \cdot 2\ H_2O$	8.22×10^{-8}
H_2SeO_3	1.00×10^{-8}
$NiSO_4 \cdot 6\ H_2O$	1.00×10^{-8}
Na_3VO_4	1.00×10^{-8}
K_2CrO_4	1.00×10^{-8}



a. Micrometer screw fixer
b. Frame of the top channel plate
c. Neodymium magnets (fixed)
d. Top channel plate
e. Micrometer screw
f. Holder top
g. Bottom microwell plate
h. Holder bottom
i. Neodymium magnets (movable)

Fig. S1 Explosion diagram of the 3D printed holder of SlipO_2Chip. This holder affixes the top channel plate and the bottom microwell plate of SlipO_2Chip and enables for the accurate slipping motion of the top channel plate via a pair of micrometer screws and the frame part which drives the top channel plate. Both glass plates are maintained in close proximity to each other through the careful placement of neodynium magnets. Four magnets are glued onto four corners of the top channel plate and perfectly fit in the duct of the frame part. The other four magnets are placed underneath the bottom microwell plate and are movable along slipping manipulations.

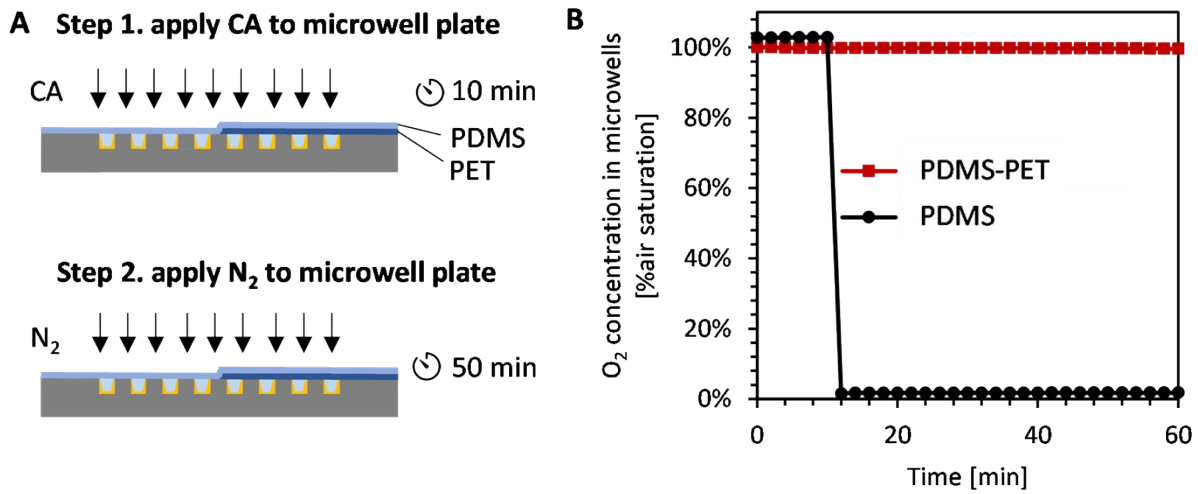


Fig. S2 Verification of the gas-tightness of SlipO_2Chip. **(A)** Cross-sectional visualization of the experiment conducted to ensure gas tightness of SlipO2Chip. The microwell plate saturated with L1 media was placed into a customized gas control chamber. Half of the microwell plate was covered by PDMS film and the rest part was covered by a PDMS-PET film with the PET side facing the microwells. The microwell plate was first exposed to compressed air (CA, 100% air saturation) and, after a 10-min interval, 100% N_2 gas was introduced into the chamber. During this stepwise exposure to different O_2 concentrations, the O_2 concentration within microwells was monitored every 2 min using automated microscopy and an RGB camera. **(B)** Change of O_2 concentrations in microwells during the 60-min gas-tightness experiment. Microwells sealed with a PET film successfully maintained 100% air saturation even after a 50-min exposure to N_2. In contrast, microwells covered solely with a PDMS layer displayed a rapid decline in O_2 after the surrounding was saturated with N_2, reaching 0% O_2 concentration. This difference underscores the role of the PET layer in ensuring the gas-tightness of the microwells.

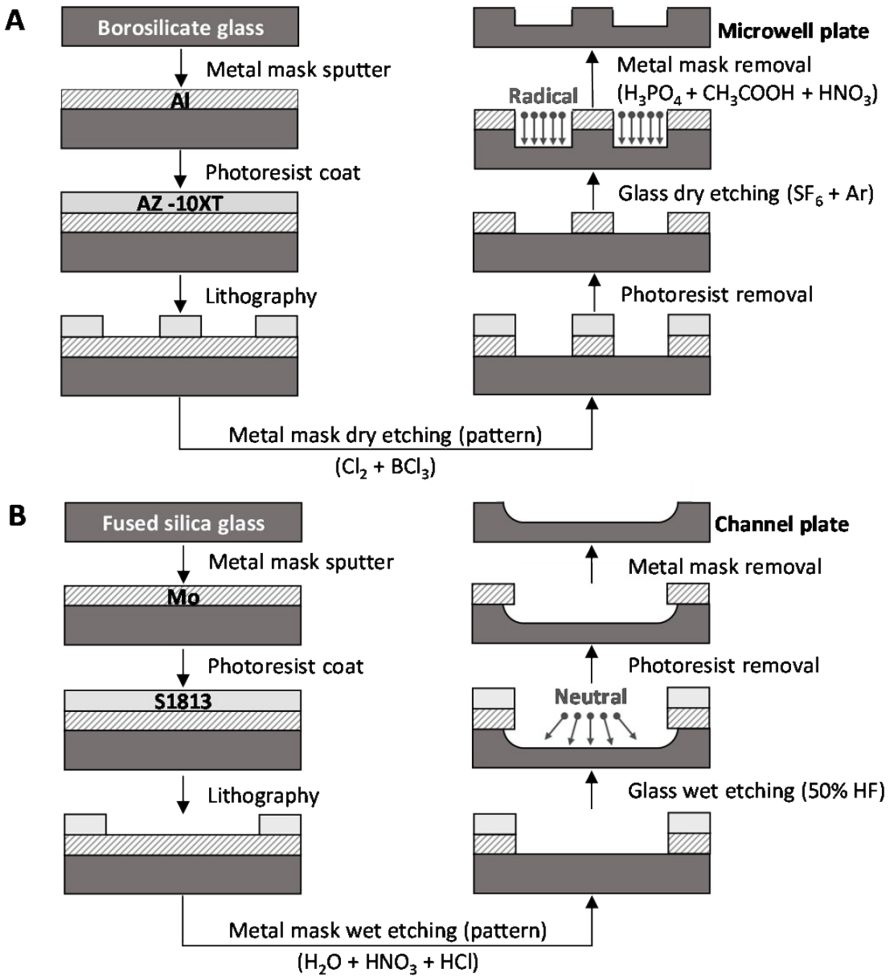


Fig. S3 Schematic protocol of dry- and wet etching protocols used in the production of SlipO_2Chip. **(A)** Dry etching protocol used to generate the microwell plate. Reactive ion etching (RIE) of borosilicate glass was carried out using SF_6 and SF_6/Ar plasma. Sputtered Al on the glass structure using patterned AZ-10XT photoresist mask was used as a hard-mask for plasma etching. **(B)** Wet etching protocol used to generate the channel structure. The wet etching of fused silica glass was carried out using 50% HF acid. Sputtered Mo on the glass structure using patterned S1813 photoresist mask was used as a hard-mask for the wet etching.

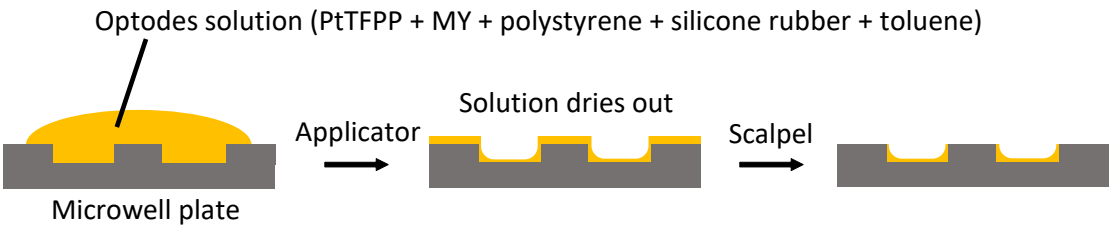


Fig. S4 Protocol to decorate microwells with O_2 sensitive optode materials. A cocktail of PtTFPP, reference dye (MY), polystyrene and silicone rubber were dissolved in toluene and this optode chemistry was evenly spread on the microwell plate surface via a surface applicator tool. After solvent evaporation, a thin layer of optode chemistry was formed across the entire microwell surface and subsequently removed using a scalpel. This resulted in a thin layer deposition of optode chemistry at the sides and the bottom of the microwell.

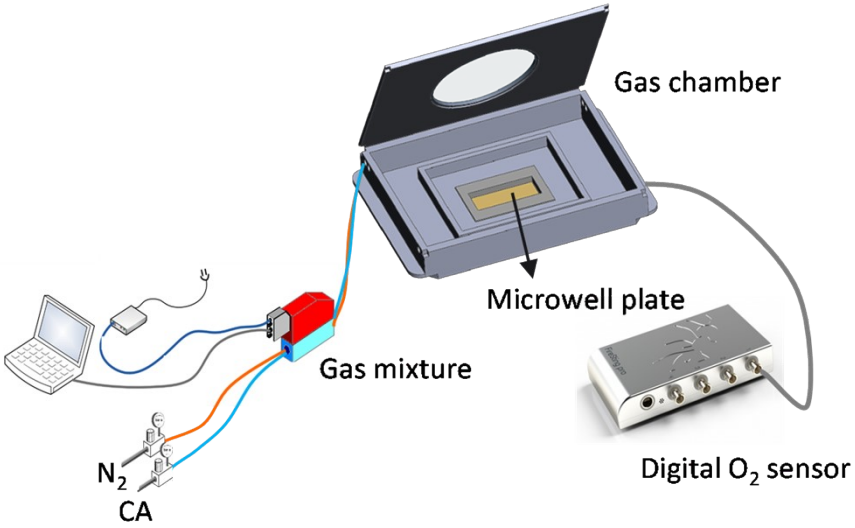


Fig. S5 Experimental setup for calibrating deposited optode chemistry within microwells. Here shown is a custom gas chamber composed of the gas-impermeable material Polyoxymethylene (POM). The excitation light used to stimulate optode chemistries was applied through the objective of an inverted microscope connected to an RGB camera for imaging. By connecting the chamber to a digital gas mixer consisting of 2 gas flow meters (High-precision thermal mass flow meter, Vögtlin Instruments GmbH, CH), the flow rates of nitrogen gas (N_2) and compressed air (CA) were tuned to saturate the chamber with different O_2 concentrations. A multi-point O_2 calibration was realized for each microwell plate. The actual O_2 concentrations in the chamber were confirmed by inserting a commercial O_2 sensor (FireSting, Pyroscience GmbH, DE).