Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Widefield Frequency Domain Fluorescence Lifetime Imaging Microscopy (FD-FLIM) for Accurate Measurement of Oxygen Gradients within Microfluidic Devices

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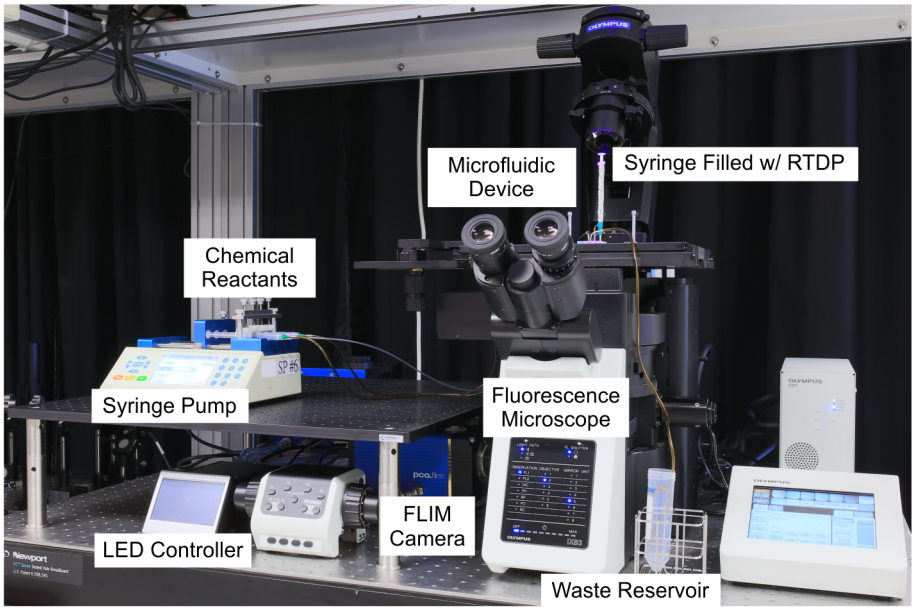

Fig. S1. Photo of the experimental setup for the FD-FLIM measurements. One four-syringe syringe pumps (Fusion 400, Chemyx Inc., Staford, TX) are utilized for flow rate control of the oxygen scavenging chemical reactants. The FLIM sCMOS camera (PCO.FLIM, PCO AG, Germany) is attached to an inverted fluorescence microscope (Olympus IX 83, Olympus, Japan). The light source is provided by a collimated LED with a center wavelength of 405 nm (M405LP1-C1, Thorlabs Inc., Newton, NJ) which is controlled by a LED controller (DC2200, Thorlabs Inc., Newton, NJ). The microfluidic device is connected with syringes by Tygon tubings (Saint-Gobain Performance Plastics, Akron, OH), and gauge 14 blunt needles (Jensen Global, Santa Barbara, CA).

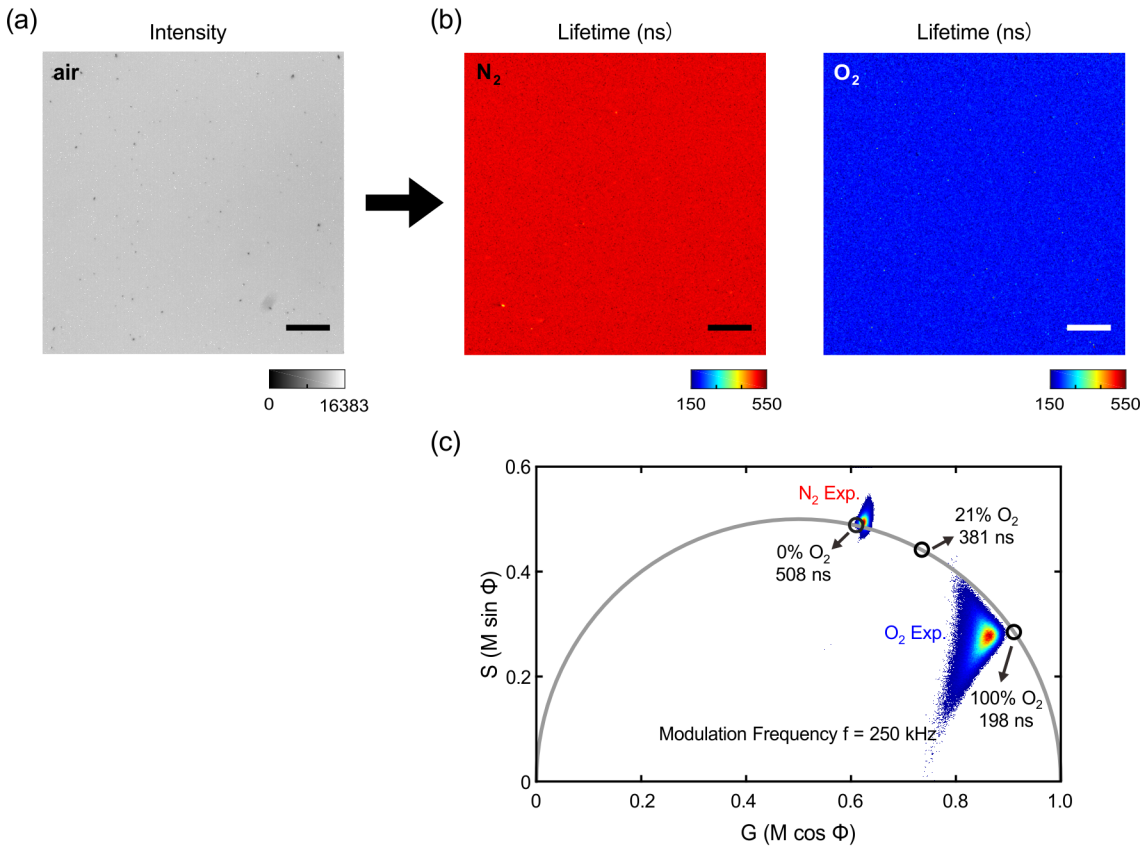

Fig. S2. Evaluation of the quenching constant of the oxygen sensitive dye, RTDP. (a) A typical 14-bit raw image of RTDP solution obtained from FLIM sCMOS camera at ambient environment as reference. (b) The calculated lifetime distributions of RTDP solutions with saturated nitrogen (0% oxygen level; left figure) and oxygen (100% oxygen level; right figure) by directly bubbling gases into the solution for 8 minutes. Scale bars are 200 μm. (c) The phasor plot analysis of the RTDP solutions with saturated nitrogen and oxygen.

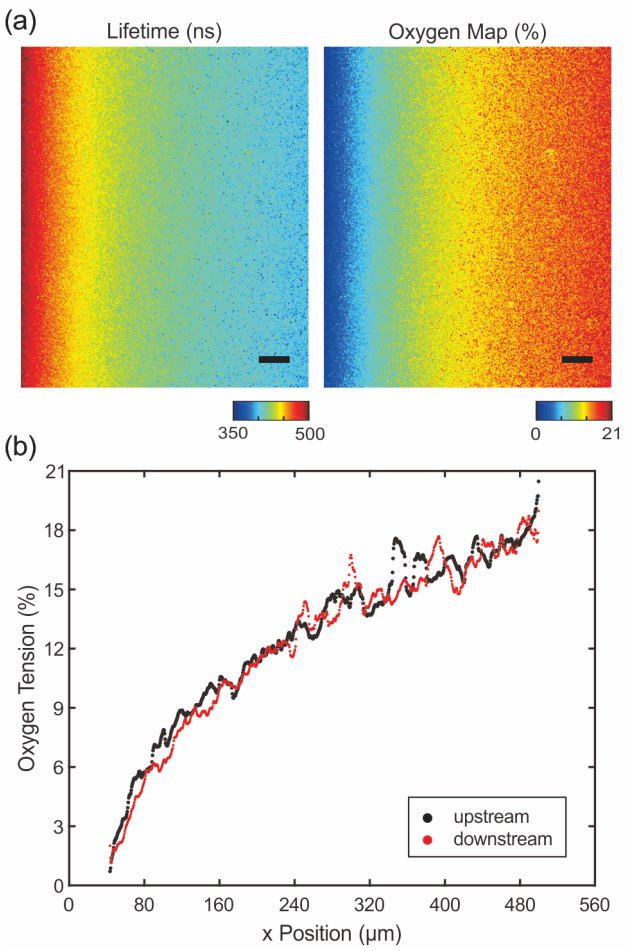

Fig. S3. (a) Fluorescence phase lifetime (left) and oxygen tension (right) distributions in a device with 30 μm wall width after introducing oxygen-scavenging chemicals, 50 mg/ml pyrogallol and 1M NaOH, into the microfluidic channel. Scale bars are 50 μm. (b) The oxygen gradient profiles across the width of the side channel in the upstream and downstream.

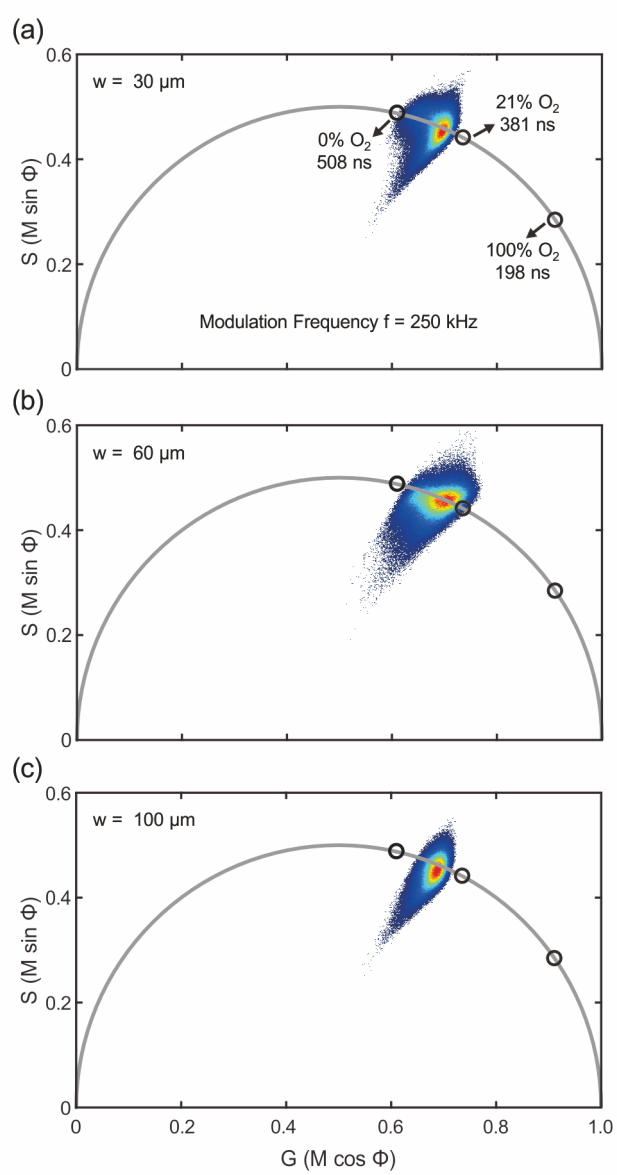

Fig. S4. The phasor plot analysis of RTDP in three devices with (a) 30, (b) 60, and (c) 100 µm wall width after introducing oxygen scavenging chemicals (1M NaOH and 50 mg/ml pyrogallol) into the microfluidic channels. The phasor distributions for RTDP are between the lifetimes of 508 ns to 381 ns, indicating the range of oxygen gradients (0% to 21% oxygen levels). The bandwidth of the distribution is narrower and the distribution shifted to the right along the semicircle in 100 µm wall width device, representing the smaller range of the oxygen gradient (8% to 19%).