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Supporting Information

Imaging the oxygen wave with single bioluminescent bacteria

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Experimental section

1. *Bacterial strain and reagents*

The freeze-dried luminescent bacteria (*P. phosphoreum T3*) was obtained from Institute of Soil Science, Chinese Academy of Sciences. Yeast extract was purchased from Adamas Reagent, Ltd. Pepton was purchased from Qingdao Hope Bio-Technology Co., Ltd. Sodium chloride, dibasic sodium phosphate, potassium dihydrogen phosphate, and glycerol were purchased from Nanjing Chemical Reagent CO., Ltd. Poly-D-Lysine was purchased from Sigma-Aldrich.

2. *Bacteria culture and immobilization*

The bacterial sterile growth medium contained 30.0 g/L NaCl, 5.0 g/L NaH_2PO_4, 1.0 g/L K_2HPO_4, 5.0 g/L yeast extract, 5.0 g/L pepton, and 3.0 mL glycerin with a pH of 7.0 ± 0.2. The bacteria were cultured in a test tube at 20 °C and shaken overnight at the speed of 200 rpm, in which the $OD_{600\ nm}$ (OD is short for optical density) of liquid culture medium was up to 1.5.

A chamber made with PDMS (polydimethylsiloxane) was located on the glass coverslip. 100 μL poly-D-Lysine (0.02 mg/mL) solution was added to the chamber incubating for 30 minutes. Then the poly-D-Lysine solution was rinsed with sterile water for three times. The chamber was placed in the air for drying thoroughly. Then 100 μL bacterial solution was added to the chamber incubating for 30 minutes to make bacteria attach on the coverslip. Next, bacterial solution was removed and rinsed with liquid culture medium for several times.

3. *Optical setup*

The microscopy setup was based on an Olympus IX83 inverted microscope with a 100x oil immersion objective (N.A. =1.49) for imaging single bacteria (Fig. 2a&b, Fig. 3, Fig. 6), or a 1.25x objective (N.A. = 0.04) for imaging the bacteria monolayer (Fig. 2c-f, Fig. 5). Halogen lamp was used as an external illumination for bright field imaging. The Olympus IX83-ZDC module can maintain continuous focus by a near-infrared light. An electron multiplying charge-coupled device (EMCCD, Andor IXon Ultra 897, 512 × 512 pixels, 16 μm per pixel) camera was employed to collect photons emitted by bacteria. Bioluminescence imaging was taken with different exposure times, such as 0.63 s, 1 s, and 10 s. The color pictures in Movies S1 were captured with Canon EOS 70D camera. All bioluminescent imaging process was carried out in the darkroom. The averaged signal intensity in a rectangle region of interest (ROI) was utilized to quantify the bioluminescence intensity of bulk and single bacteria. The data quantitative analysis was processed by Matlab and ImageJ software.

4. *Electrochemical experiments on the microscopy*

A PDMS chamber was adhered to the coverslip and a monolayer of bacteria was immobilized on it. Half of the coverslip was coated with 30 nm-thick platinum (Pt) layer for the water oxidation (routine Pt-sputtering procedures with the evaporator), and another half was original glass. 200 μL culture medium as electrolyte was dropped in the chamber. The working electrode (WE): Pt-coated coverslip; counter electrode (CE): a 0.3 mm Pt wire; reference electrode (RE): a 0.5 mm Ag wire. The potential was applied by a commercial electrochemical workstation (CHI 760E, Shanghai Chenhua Instrumental CO., Ltd., China).

5. *Perfusion system*

A gravity-based perfusion system (SF-77B, Warner, Instruments, Connecticut) was used to regulate local oxygen concentrations around single bacteria. The solutions can be quickly switched within 1~2 seconds.

Supporting Figures

6. *The diffusion of oxygen produced by different potentials*

We have applied three different potentials (0.5 V, 1.0 V, 1.5 V) to produce oxygen at Pt layer surface. BL images snapshots (Fig. S1a) showed that bioluminescent intensity and diffusion rate had gradually increased with applying higher potentials. As shown in Fig. S1b, when applying a potential of 0.5 V, the BL intensity at the left side gradually increased to reach a plateau, indicating a stable production of oxygen and good viability of bacteria. At the same time, the BL intensity at the right side only increased by ~10%, likely due to the insufficient supply of oxygen from the left side. It is understandable because of three reasons. First, the production rate of oxygen was limited under such a mild potential. Second, the bacteria on the electrode surface were alive and actively consumed oxygen. Third, the vertical diffusion towards the bulk solution further reduced the amount of oxygen that diffused to the right side.

When applying a potential of 1.0 V, the BL intensity in the left side rapidly increased to a maximum and then gradually decreased, suggesting the higher production rate of oxygen and the partial damage to the bacterial viability. As a result, there was a larger increase in the BL intensity of the right side.

The application of 1.5 V immediately increased the BL intensity at the left side, which then rapidly decreased, indicating the effective production of oxygen and the significant inhibition of the bacteria viability. Consequently, the large number of oxygen molecules diffused towards the right side, resulting in an obvious diffusion wave on the right side in the time-lapsed BL images. In order to better demonstrate the diffusion feature, we chose to apply a potential of 1.5 V in the study.

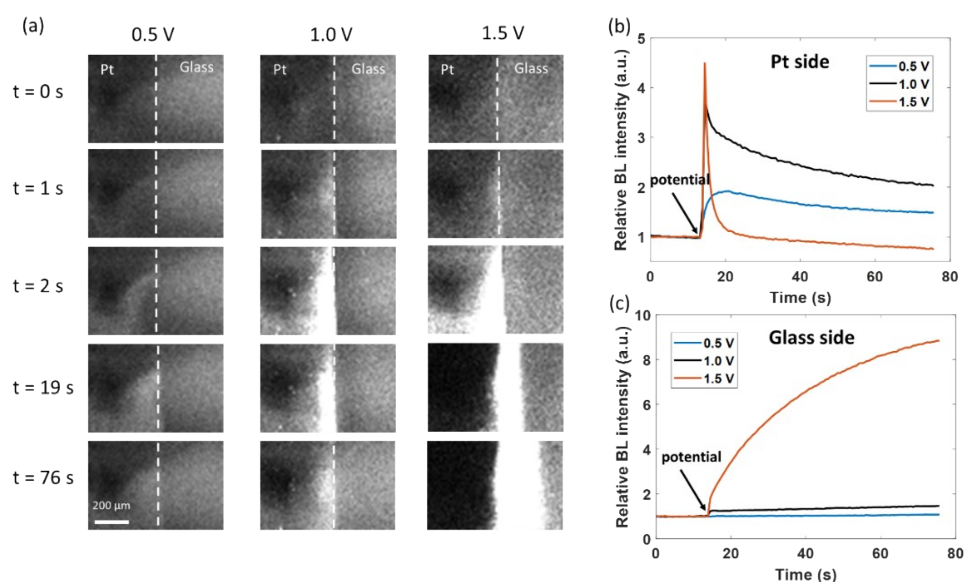

Fig. S1. (a) BL images snapshots (0.5 V, 1.0 V, 1.5 V potentials) revealed the generation and diffusion of

oxygen waves in an artificially triggered water electrolysis process. (b)-(c) Time-lapsed BL intensity curves at Pt side and glass side when applying 0.5 V, 1.0 V, 1.5 V potentials, respectively. (Black arrows refer to the moment of applying potential.)

7. *The dynamics of bioluminescence with adding autoinducers*

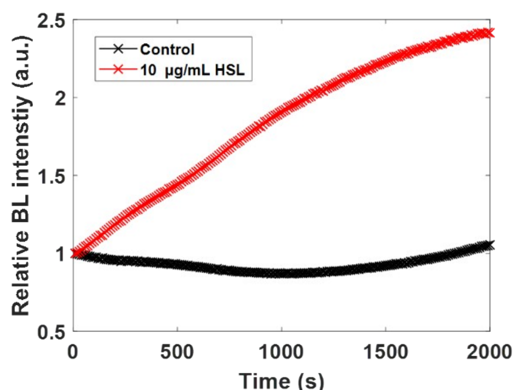

Fig. S2. The addition of 10 μg/mL HSL enhances bioluminescence emission in 30 minutes.

8. *Quantitative analysis of diffused oxygen molecules*

The amount of oxygen was also estimated by integrating the chromoamperimetric curve (*i*-*t* curve), which gave a total amount of oxygen molecules of 4.9×10^{-8} mol in an electrolysis time of 40 seconds (20th to 60th second in Fig. 2). Local oxygen concentration could be calculated based on the Michaelis-Mention equation, since the $I(r, t)$, I_{max}, and K_m were known. The calculated result of local oxygen concentration has been provided in Fig. S3b.

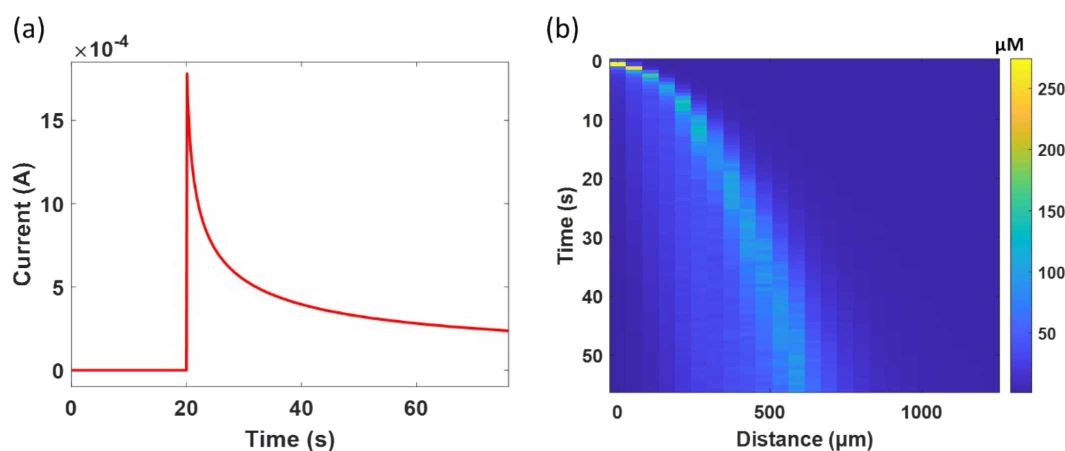

Fig. S3. (a) *i-t* curve when 1.5 V potential was applied at the Pt layer. (b) Time-dependent local oxygen concentration profiles during the electrolysis process.

9. *The trajectories of bacteria and BL wave*

In some experiments, we found that a tiny amount of bacteria suspending in the solution moved with the BL wave. Their movement trajectories are shown in Fig. S4. The movement directions of bacteria and BL wave were coincident. As a result, it demonstrates that micro-convection exists in the culture medium.

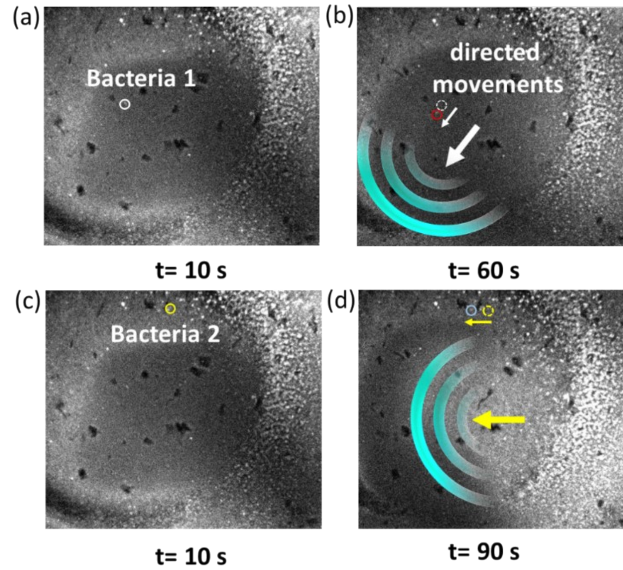

Fig. S4. The original position of bacteria 1 (a) and bacteria 2 (c) are marked with white and yellow circles, respectively. Micro-convection drives the directed movement towards new positions after (b, d).

10. The influence of evaporation on the oxygen wave

An EMCCD camera was used to record the spontaneous and stochastic BL wave, when a coverslip was placed on top of the chamber and was in contact with the culture medium to get rid of the gas-liquid interface (top interface) to minimize evaporations. Then the coverslip was removed from the top of PDMS chamber and BL wave was recorded. Comparative results showed that the introduction of glass coverslip largely inhibited the BL wave, in terms of not only the fluctuation ratio but also the time scale. These results validated that the formation of the BL wave resulted from micro-convection of liquid induced by evaporation.

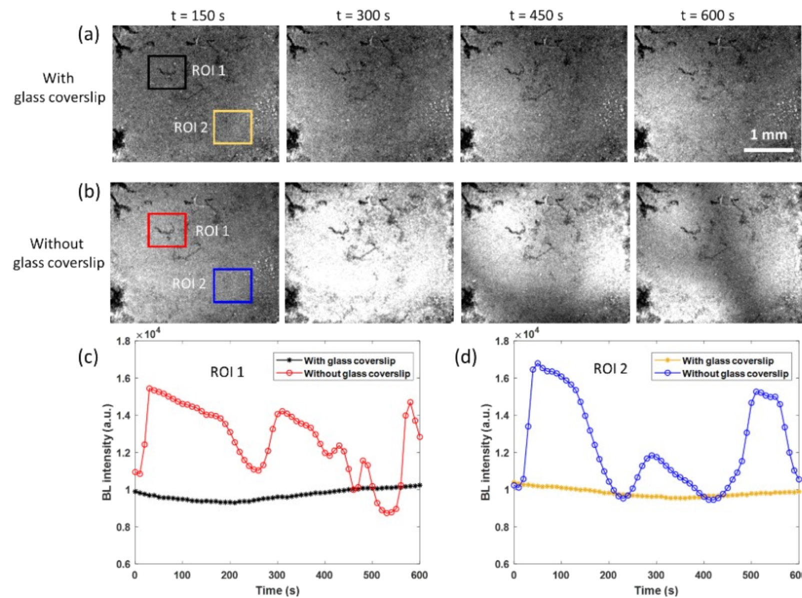

Fig. S5. (a, b) BL intensity change on the interface at a different time with/without glass coverslip. (c, d) Time-dependent intensity profiles in different ROIs.

11. The influence of thermal fluctuation on the oxygen wave

A local heating system by taking the conductive indium tin oxide (ITO, 15-30 Ω) film as a resistor was used

to heat the coverslip. When increasing the temperature of the coverslip underneath the solution, significant bioluminescent waves were observed as a result of the enhanced thermal fluctuations

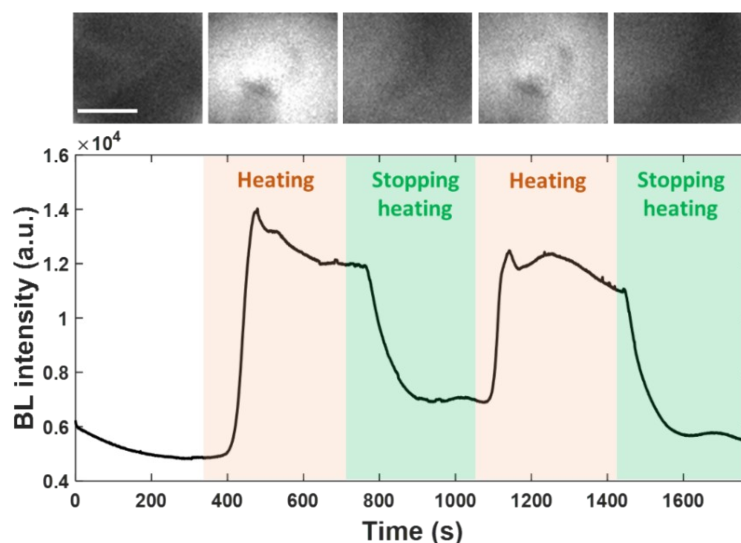

Fig. S6. BL intensity was enhanced obviously in the heating process (marked with orange rectangles). On the top of BL intensity curve, BL images were shown in corresponding processes. (Scale bar: 500 μm.)

12. Supporting Movies

MovieS1. The bacterial suspension was shaken and stood in a test tube three times.

MovieS2. The bacteria colonies on the agar plate were blown with nitrogen gas and then re-supplied air.

MovieS3. Artificial oxygen wave was produced by water electrolysis process, diffusing to the right side.

MovieS4. BL intensity of single bacteria was changed during water electrolysis process.

MovieS5. Spontaneous and stochastic BL wave was recorded in the chamber.

MovieS6. The BL wave was further validated by single bacteria BL imaging.