

## Supplementary Materials for

### Light-induced assembly of living bacteria with honeycomb substrate

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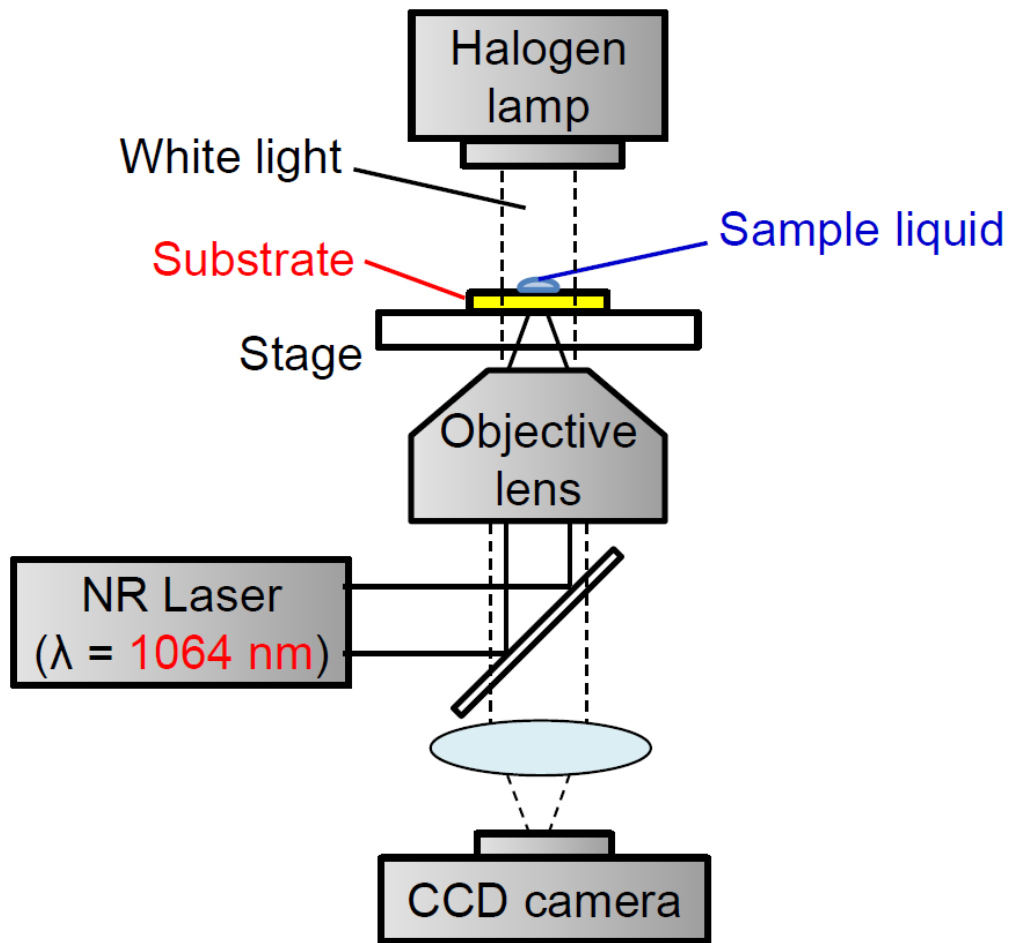
#### The PDF file includes:

- Fig. S1. Schematic of experimental setup of LIA of bacteria.
  - Fig. S2. Preparation and structural evaluation of honeycomb substrate.
  - Fig. S3. Elemental analysis of honeycomb substrate.
  - Fig. S4. Behavior of bacteria (*P. aeruginosa*) trapped in pores of honeycomb substrate during laser irradiation under bright-field condition (transparent optical image).
  - Fig. S5. Survival confirmation of bacteria on honeycomb substrate.
  - Fig. S6. Fluorescence image of trapped *P. aeruginosa* by changing laser power (20-s irradiation for each).
  - Fig. S7. Fluorescence image of trapped *S. aureus* by changing laser power (20-s irradiation for each).
  - Fig. S8. Comparative experiment of LIA of bacteria with flat gold film [thickness: 10 nm in (27)].
  - Fig. S9. Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 100 mW through 10× dry objective lens (initial power was 200 mW).
  - Fig. S10. Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 35 mW through 10× dry objective lens (initial power was 90 mW).
  - Fig. S11. Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 20 mW through 10× dry objective lens (initial power was 65 mW).
  - Fig. S12. Model for the calculation of light-induced convection in honeycomb substrate.
  - Fig. S13. Simulation of optical response of a gold thin film on the substrate.
- Legends for movies S1 and S2

**Other Supplementary Material for this manuscript includes the following:**

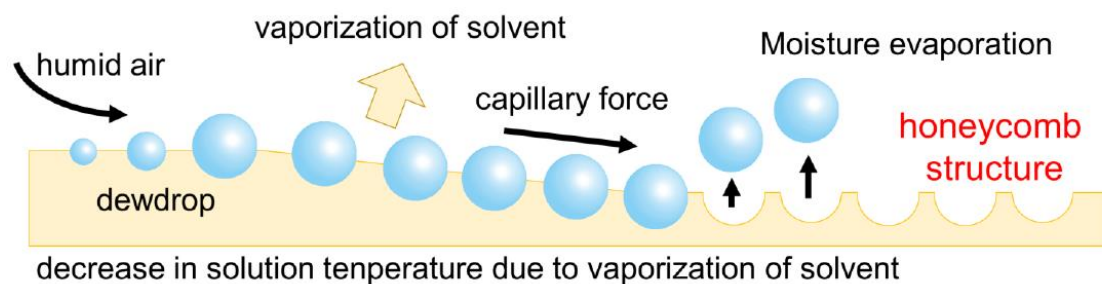
(available at [advances.sciencemag.org/cgi/content/full/6/9/eaaz5757/DC1](https://advances.sciencemag.org/cgi/content/full/6/9/eaaz5757/DC1))

Movie S1 (.mp4 format). An example of fluorescence image of LIA process of *S. aeruginosa* into Au-coated honeycomb substrate (SYTO 9) with a laser power of 40 mW similar to Fig. 2B.  
Movie S2 (.mp4 format). Transparent image of *S. aeruginosa* in Au-coated honeycomb substrate after LIA, which corresponds to fig. S4.

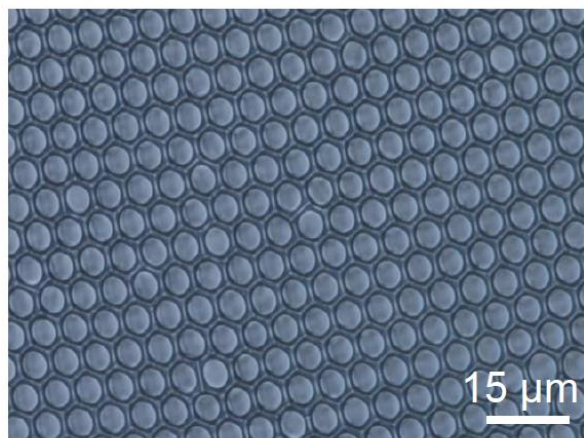


**Fig. S1. Schematic of experimental setup of LIA of bacteria.**

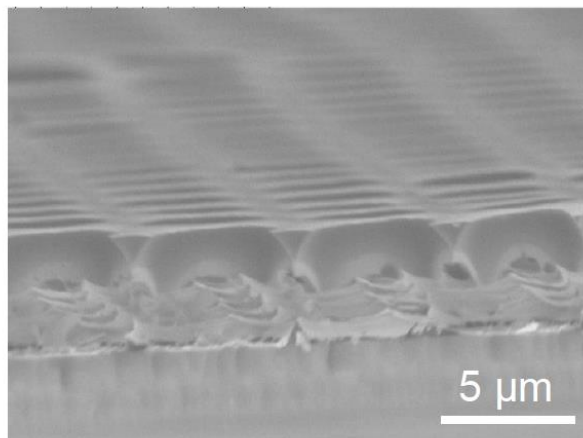
## A Preparation of the honeycomb polymer film



B

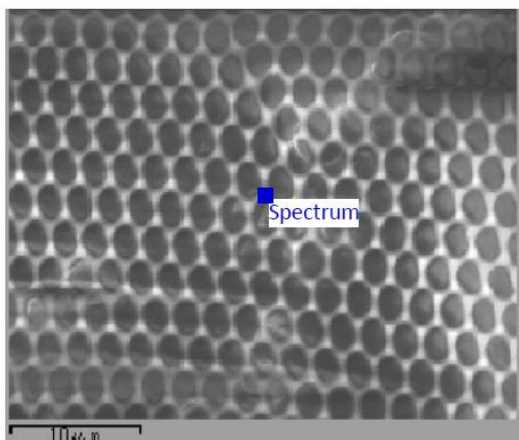


C



**Fig. S2. Preparation and structural evaluation of honeycomb substrate.** (A) Schematic image of production process of honeycomb substrate. (B) Wide area stereomicroscope image of honeycomb substrate (top view). (C) SEM image of the cross section of a honeycomb substrate (side view).

**A** Before gold sputtering

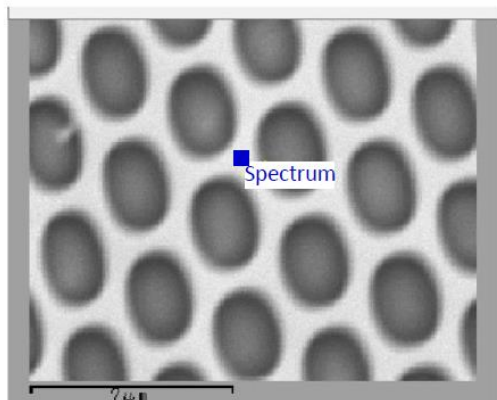


Element	MC (%)	ANC (%)
C	72.7	81.4
O	16.5	13.9
Na	1.10	0.643
Al	0.472	0.235
Si	6.63	3.17
K	0.862	0.296
Ti	0.397	0.112
Zn	1.30	0.267

MC: Mass concentration

ANC: Atomic number concentration

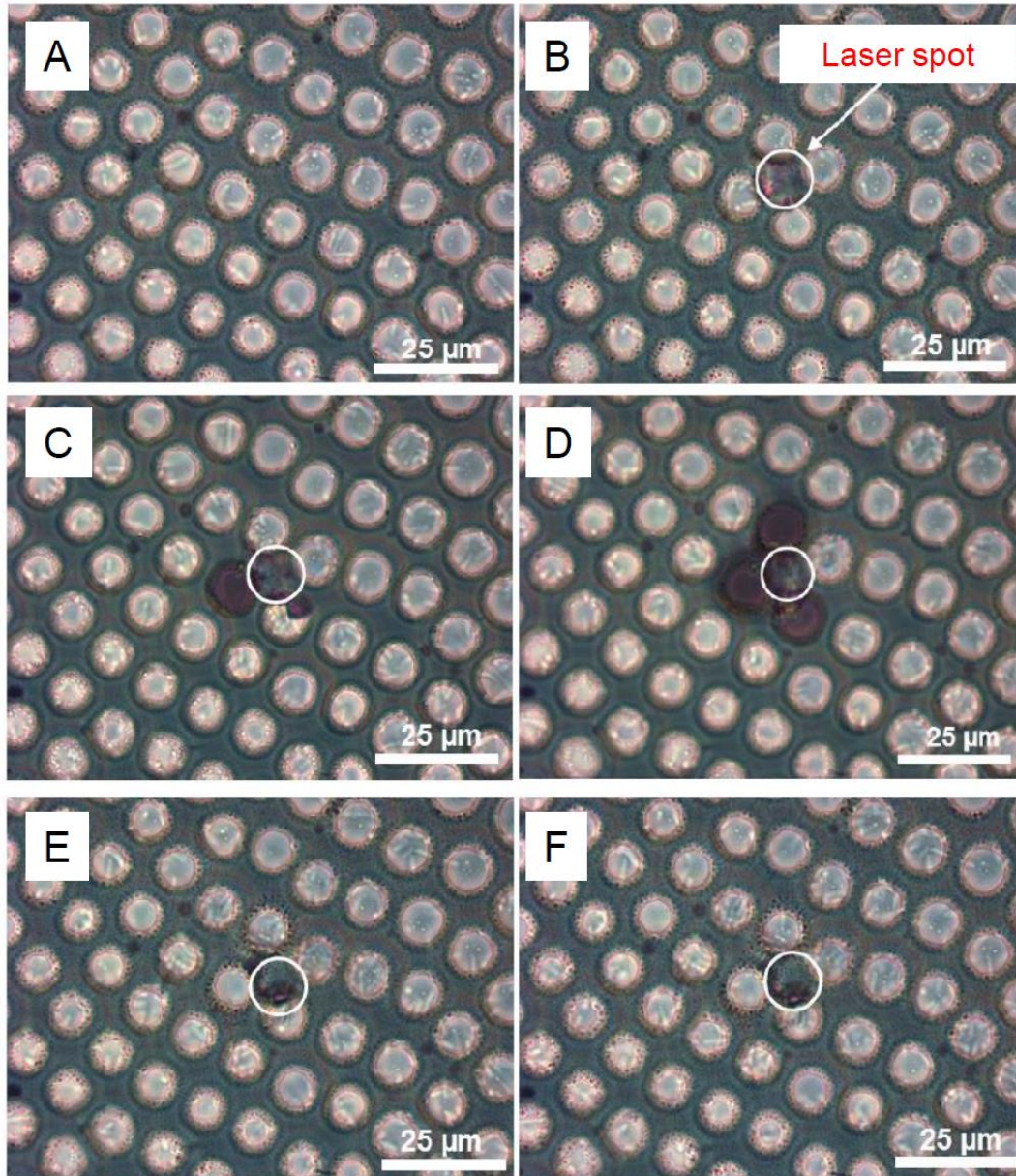
**B** After gold sputtering



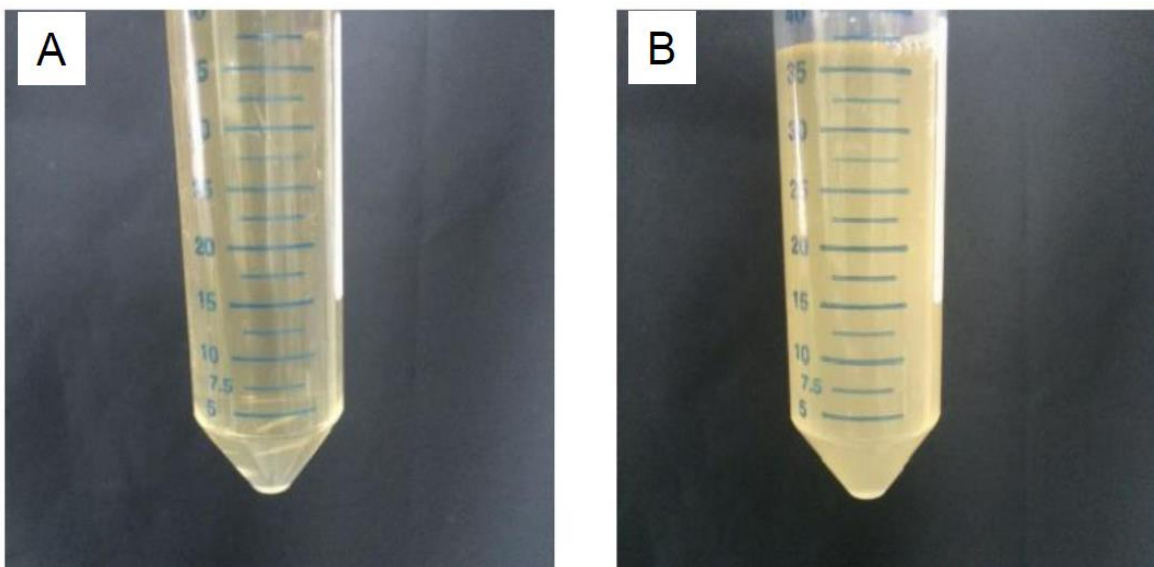
Element	MC (%)	ANC (%)
C	54.1	87.4
O	3.10	3.76
Na	0.634	0.535
Al	0.435	0.313
Si	5.53	3.82
K	0.936	0.465
Ti	0.443	0.180
Zn	0.595	0.177
Au	34.2	3.37

**Fig. S3. Elemental analysis of honeycomb substrate.** (A) SEM image and result of elemental analysis of honeycomb polymer film before gold sputtering. (B) SEM image and EDX spectrum of honeycomb polymer film after gold sputtering.



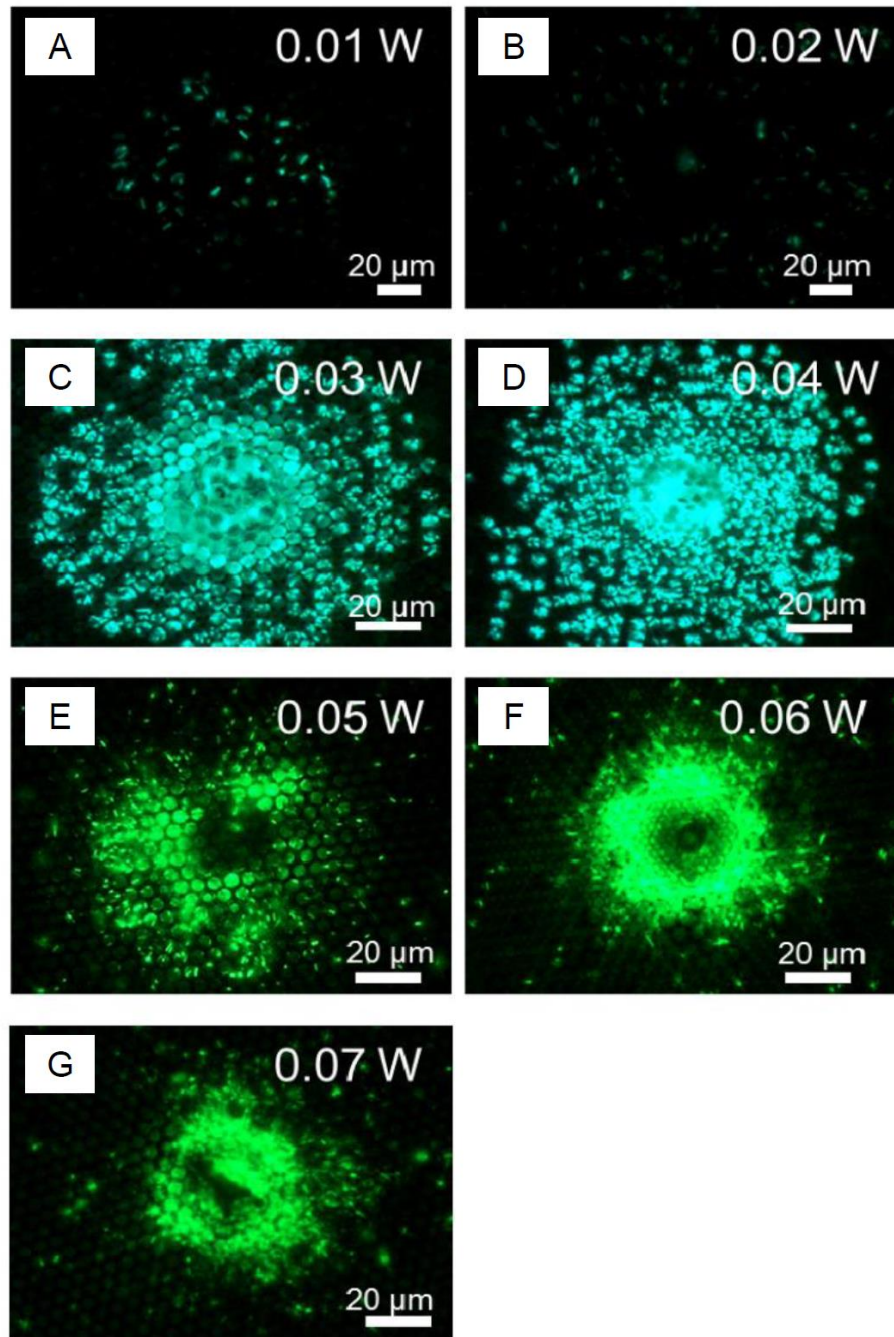


**Fig. S4. Behavior of bacteria (*P. aeruginosa*) trapped in pores of honeycomb substrate during laser irradiation under bright-field condition (transparent optical image). (A) Start of laser irradiation (0 s). (B) 5 s, (C) 50 s, (D) 53 s, and (E) 56 s after the start of laser irradiation. (F) 30 s after the stopping laser (86 s after (A)).**



**Fig. S5. Survival confirmation of bacteria on honeycomb substrate.** (A) Liquid medium before cell culture. (B) Liquid medium after culturing by immersing the honeycomb substrate containing *P. aeruginosa* trapped under light-induced assembly. [Photo Credit: Shinya Kurita, Osaka Prefecture University].

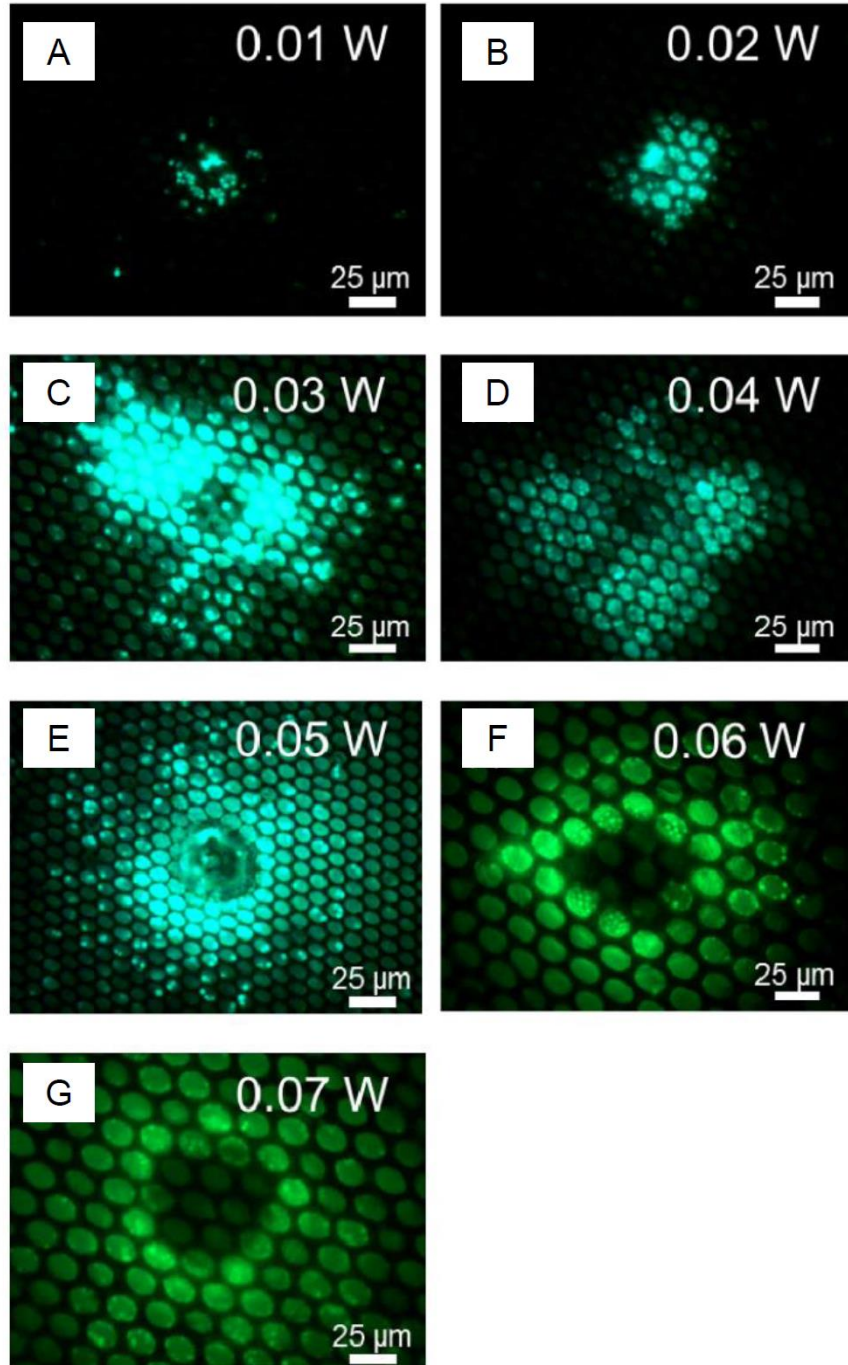
*P. aeruginosa*



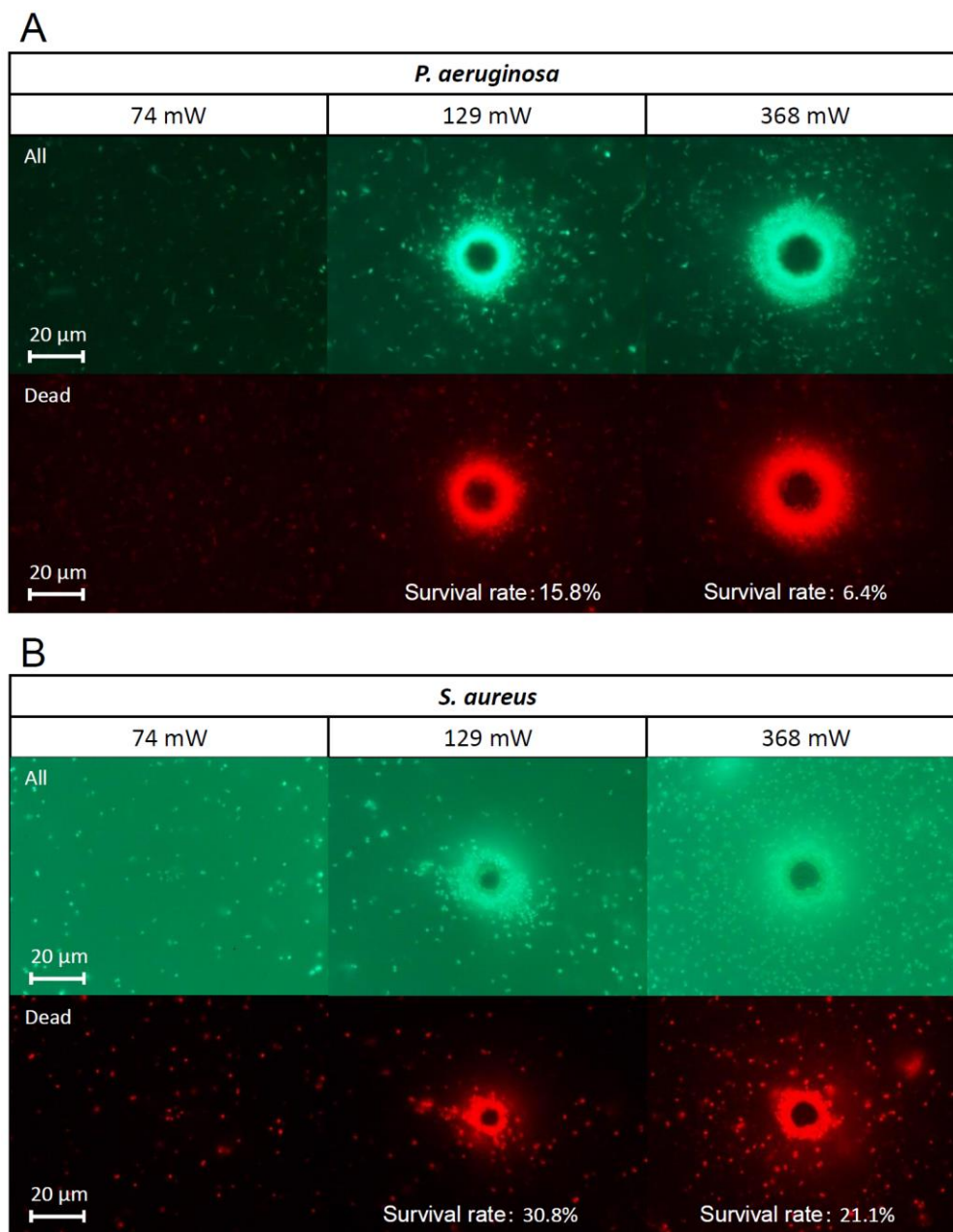
**Fig. S6.** Fluorescence image of trapped *P. aeruginosa* by changing laser power (20-s irradiation for each).



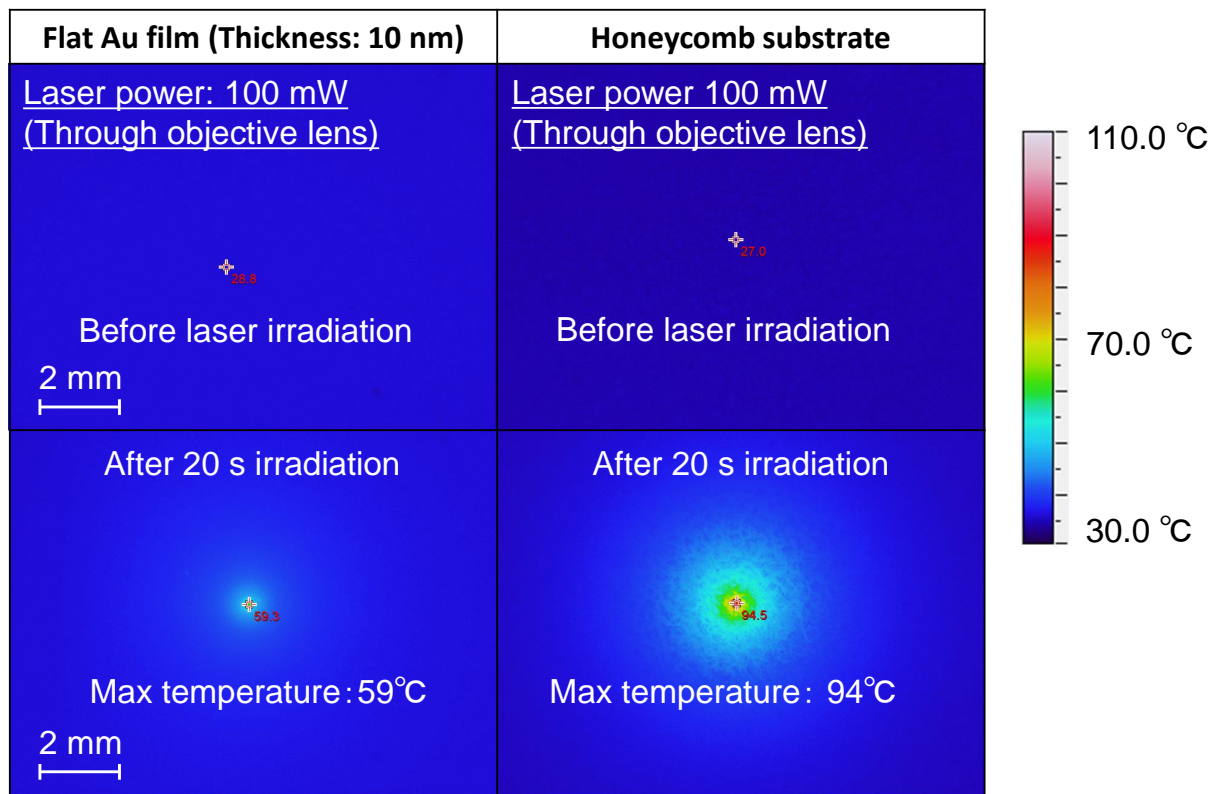
*S. aureus*



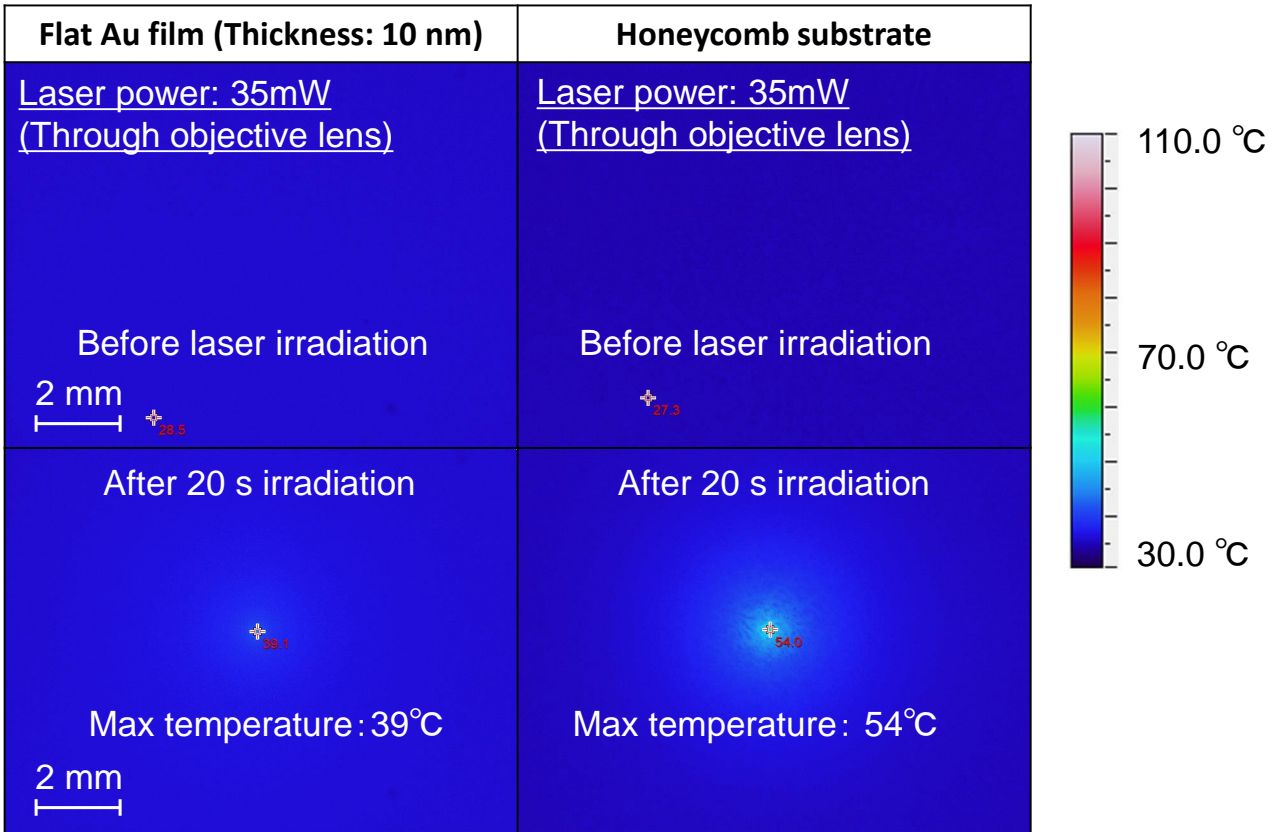
**Fig. S7.** Fluorescence image of trapped *S. aureus* by changing laser power (20-s irradiation for each).



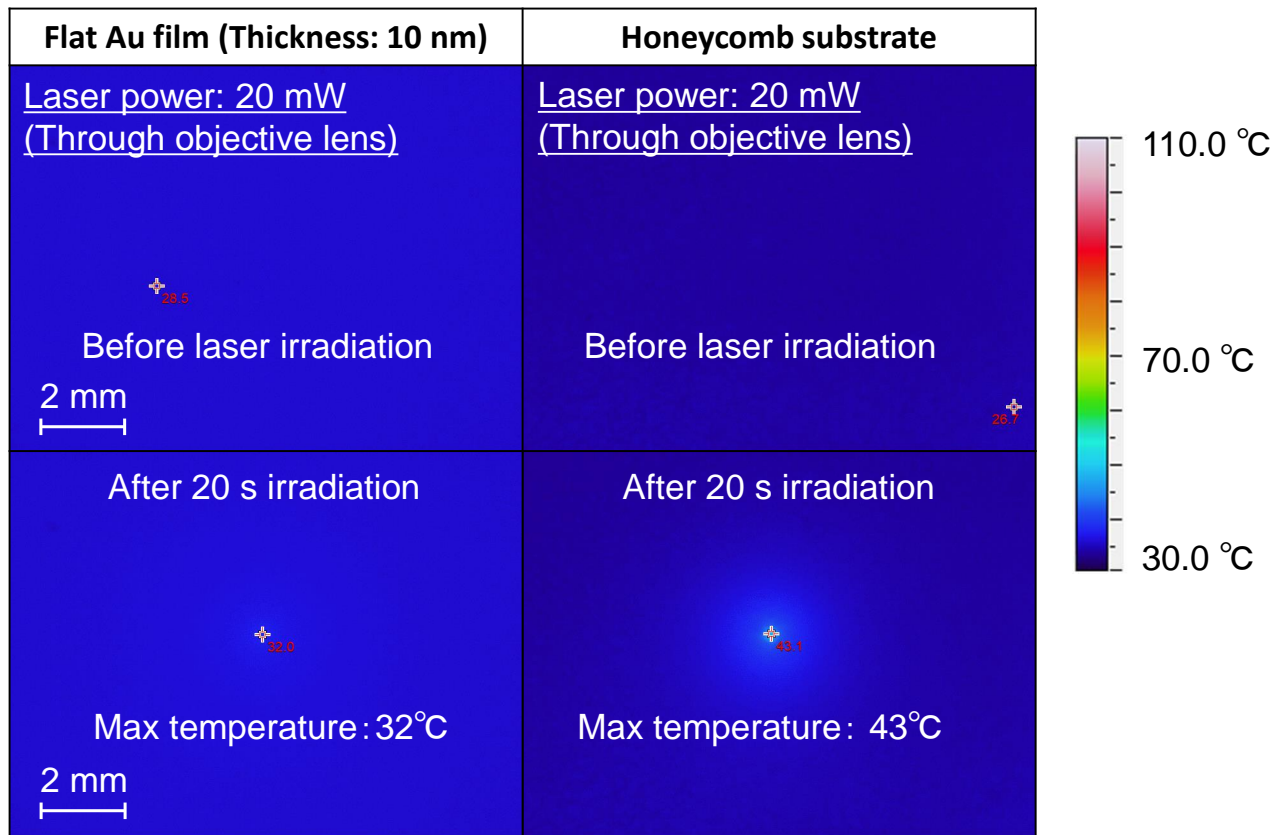
**Fig. S8. Comparative experiment of LIA of bacteria with flat gold film [thickness: 10 nm in (27)].** (A) Fluorescent images of *P. aeruginosa* under laser irradiation with different powers, and (B) fluorescent images of *S. aureus* under laser irradiation with different powers. In the both cases of (A) and (B), the upper panels show the green fluorescence from SYTO9 and the lower panels show red fluorescence from PI. The laser powers are reduced to be 20 mW, 35 mW, and 100 mW through 100 $\times$  oil immersion objective lens used in main experiments in Fig. 2 and so on.



**Fig. S9.** Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 100 mW through 10× dry objective lens (initial power was 200 mW).



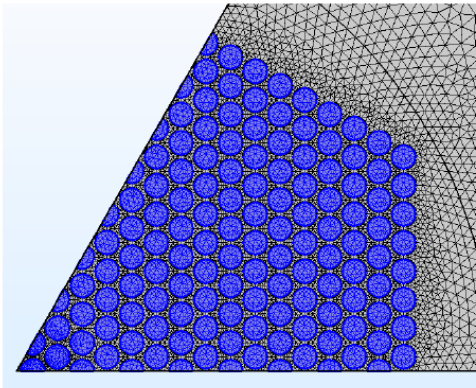
**Fig. S10.** Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 35 mW through 10× dry objective lens (initial power was 90 mW).



**Fig. S11. Thermographic images of flat Au film and Au-coated honeycomb substrate before and after infrared laser irradiation of 20 mW through 10× dry objective lens (initial power was 65 mW).**



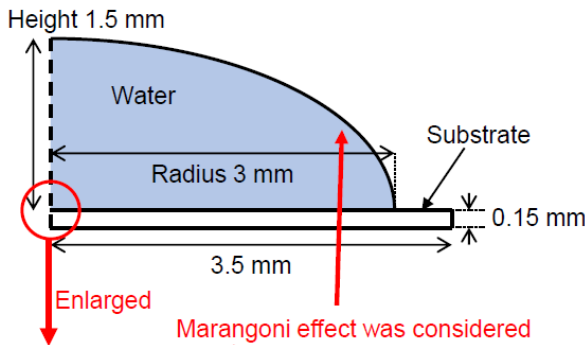
**A** Interval between pores was set to  $1\ \mu\text{m}$



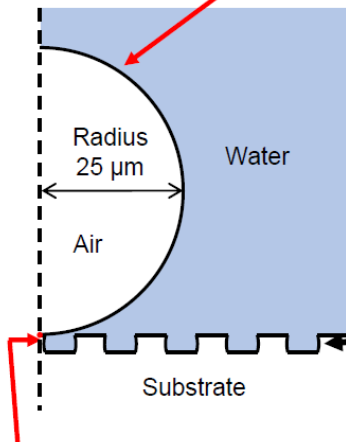
**Boundary condition:**

Symmetry conditions were imposed on the xz section and the  $60^\circ$  section. For other interfaces, following conditions were assumed.  
 #Gas-liquid interface: with slip.  
 #Solid-liquid interface: without slip.  
 #Marangoni effect at gas-liquid interface.  
 #With regard to heat transfer, the condition of losing energy at  $5\ \text{W}/\text{m}^2\text{K}^2$  was imposed on all interfaces except symmetrical interfaces.

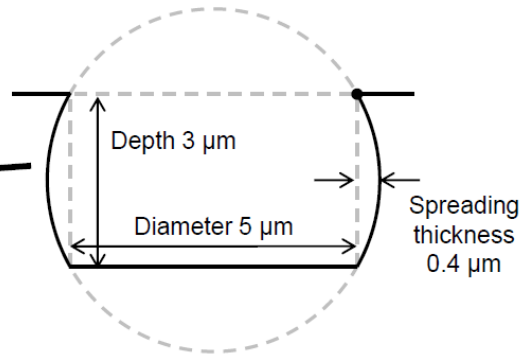
**B**



#Assuming that the water is an incompressible fluid, the Boussinesq approximation was adopted.

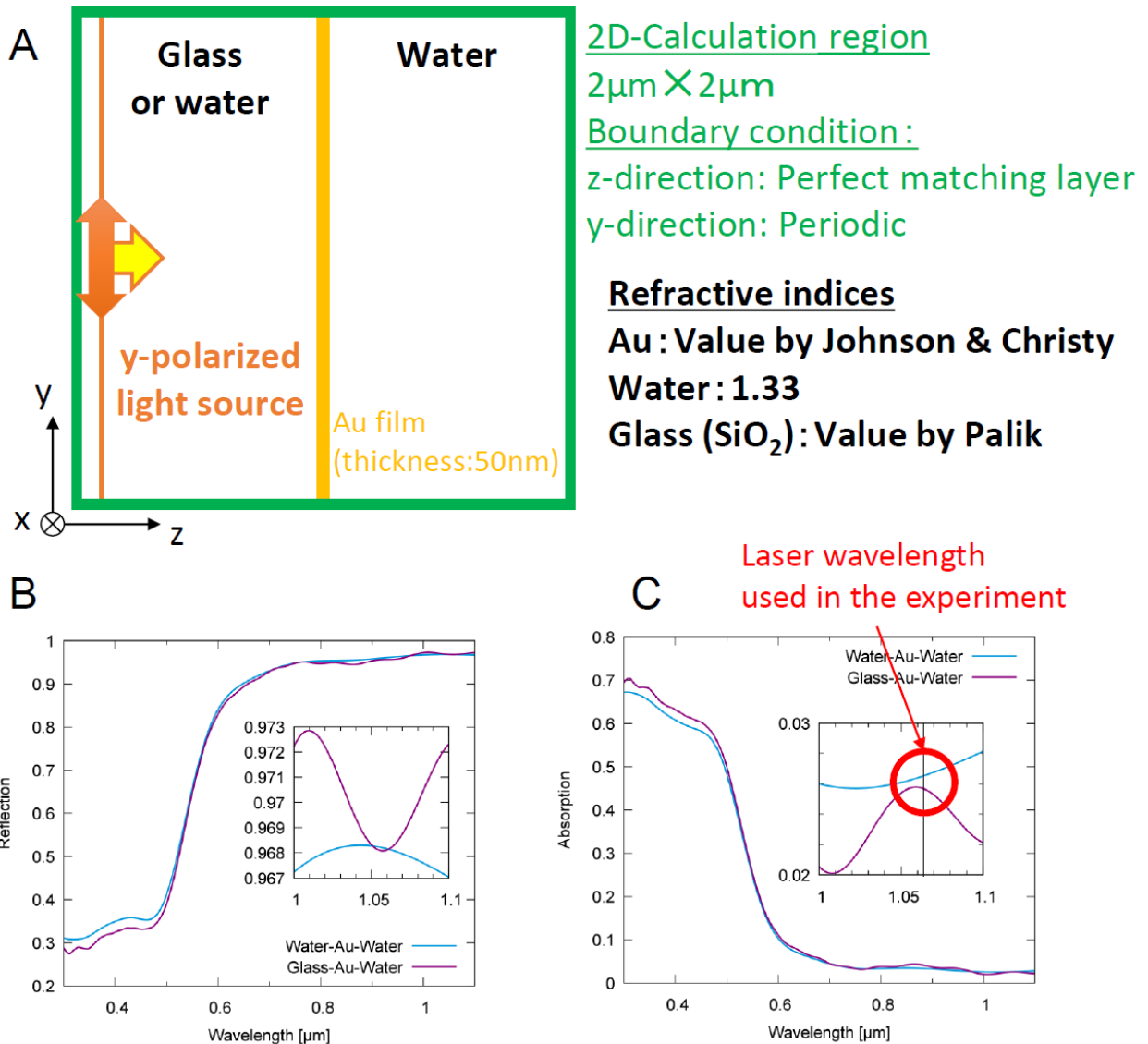


Pore is the same size as a dewdrop in Fig. S2



Point heat source:  $Q=PA$   
 $P=20\ \text{mW}$ ,  $A=0.03$

**Fig. S12. Model for the calculation of light-induced convection in honeycomb substrate. (A)** Top view and boundary conditions. **(B)** Side view and detailed information on a light-induced bubble and pores.



**Fig. S13. Simulation of optical response of a gold thin film on the substrate.** (A) Model for the calculation of optical response of Au thin film on the honeycomb substrate. (B) Reflection spectra and (C) Absorption spectra for two types of media with different refractive indices.

**Movie S1. An example of fluorescence image of LIA process of *S. aeruginosa* into Au-coated honeycomb substrate (SYTO 9) with a laser power of 40 mW similar to Fig. 2B.**

**Movie S2. Transparent image of *S. aeruginosa* in Au-coated honeycomb substrate after LIA, which corresponds to fig. S4.**