Supplementary Information

Continuous Surveillance of Bioaerosols On-site by an Automated Bioaerosol Monitoring System

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Figure S1

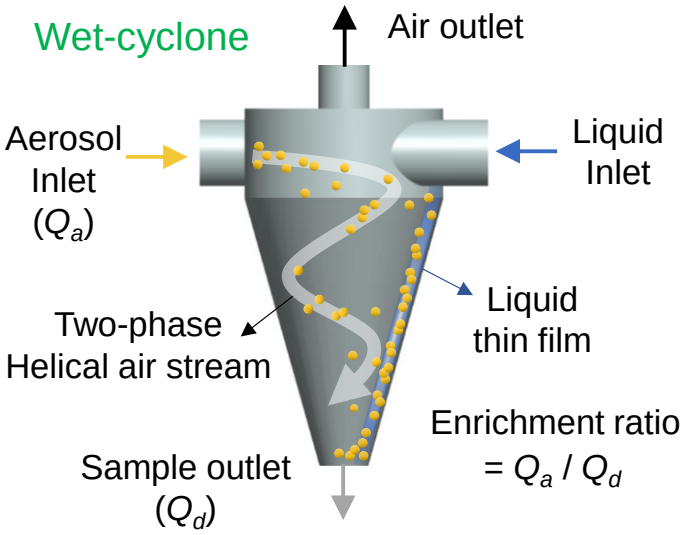


Fig. S1. The principle of the continuous aerosol sampling in the wet-cyclone unit of the ABMS. A wet-cyclone collects aerosols in a liquid film on the inner wall of the cyclone using particle centrifugal force and liquid surface tension. These systems achieve high sampling performance and enable sample concentration due to the high flow rate ratio between the incoming air and drainage liquid. The aerosols are gradually influenced by the centrifugal and shear forces generated by the internal helical air stream and are continuously collected in a stable liquid thin film that forms on the inner wall of the wet-cyclone. The optimal air-to-liquid enrichment ratio of ~9.6×10^5 is achieved by setting the sampling air flow rate to 16 L/min, liquid supply to 67 μL/min, and liquid drainage to 17 μ L/min.

Figure S2

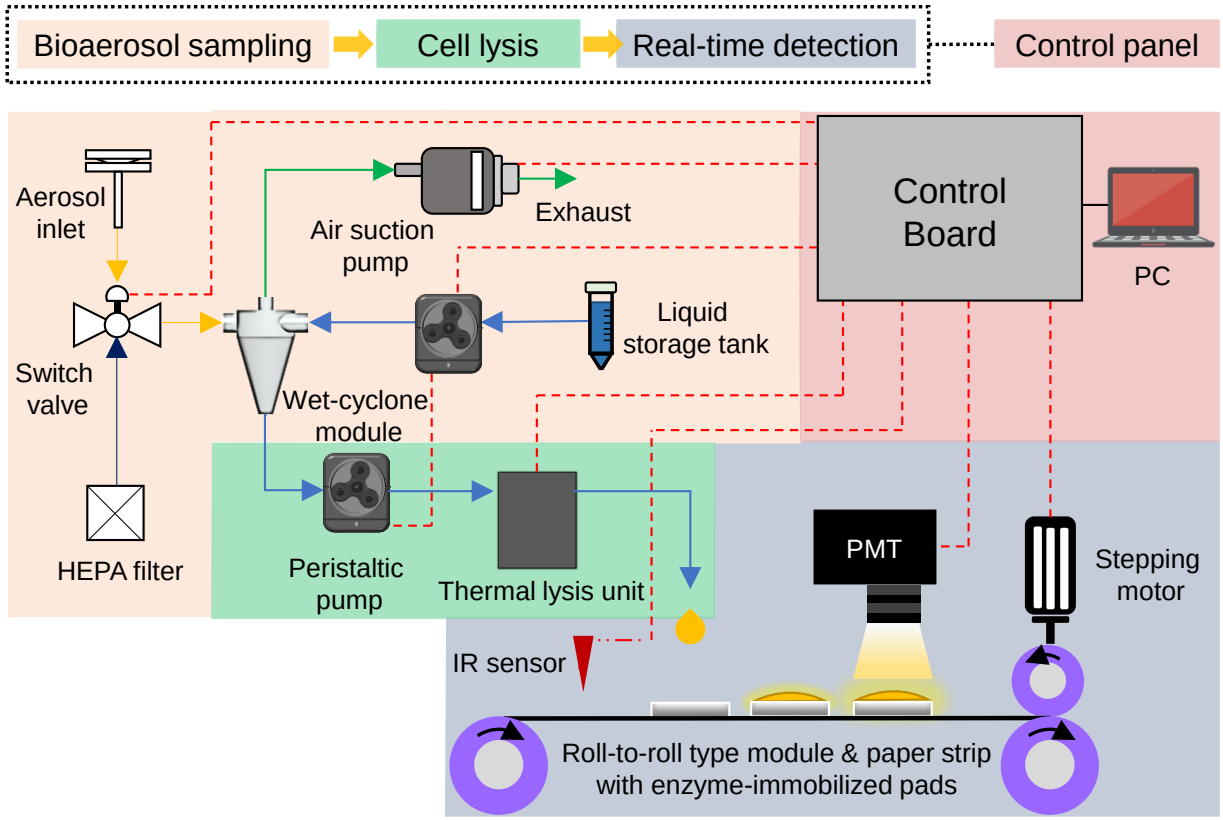


Fig. S2. ABMS system configuration and operation procedure. Bioaerosols initially pass through the sampler inlet with a flow rate of 16 L/min$_{air}$ and enter the wet-cyclone unit through the switching valve via an air suction pump. Simultaneously, sterilized deionized water (SDW) is injected by the peristaltic pump at a rate of at 67 μL/min and bioaerosols are continuously collected in a stable liquid thin film. A drainage liquid flow (~ 17 μL/min) continuously delivers the sampled specimens to the cell-lysis unit. The HEPA filter is used to supply clean air during the cleaning mode of the system. The cell-lysis unit has an aluminum chamber filled with mineral oil maintained at 95°C. The suspension— which includes the specimens—passes through a tube immersed in the mineral oil, with a residence time of 3 min. After the thermal cell-lysis process, the lysed specimens move to the detection unit. A total of 30 μL of lysed specimens is delivered

to the immobilized-enzyme pad, which is attached to a roll-to-roll module, through a capillary needle. An infrared sensor and stepping motor are used to accurately control the pad position. The lysed specimens are added to each pad every 2 min and the bioluminescence intensity of each pad is measured in relative luminescence units (RLUs) using a photomultiplier tube (PMT). All parts are operated automatically using the control board with the custom LabVIEW program.

Figure S3

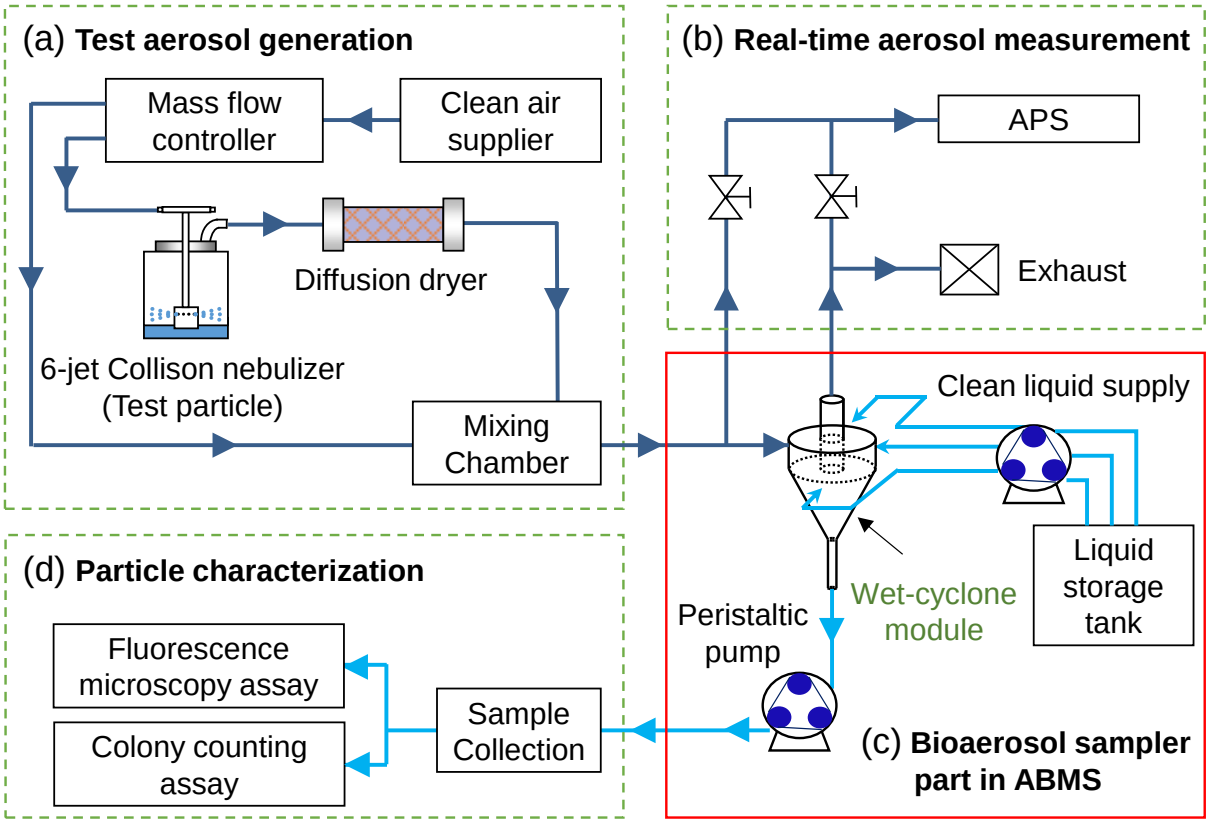


Fig. S3. Schematic diagram of the experimental setup for evaluating the performance of the wet-cyclone unit of the ABMS. (a) Test aerosol generation. Standard sizes of FPSL particles (0.48–2.1 μm) and *E. coli* bacteria were used as test particles. (b) Real-time aerosol measurement. Particle characteristics and collection efficiency are measured in real-time using an APS. (c) The bioaerosol sampler component of the ABMS. There is one aerosol inlet and three sampling liquid inlets on the side of the wet-cyclone unit, and the outlets for exhausted air and hydrosol liquid (sampled specimens) are on the upper and bottom sides, respectively. (d) Particle characterization. The sampled particles in liquid are tested using fluorescence microscopy and colony-counting assays.

Figure S4

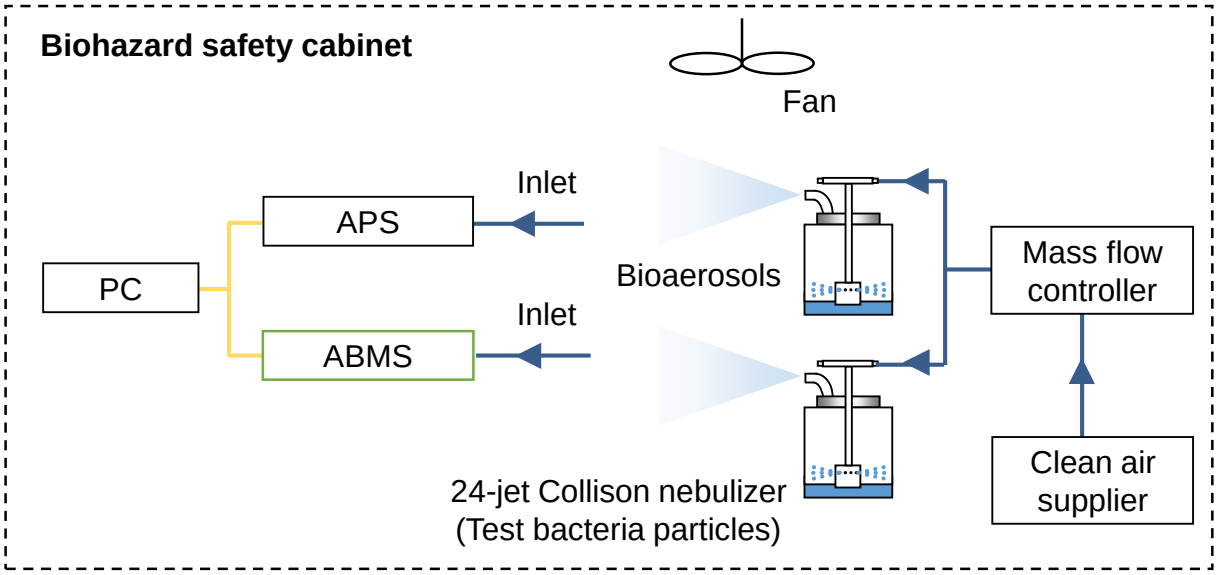


Fig. S4. Schematic diagram of the test-bed experimental setup for evaluating ABMS performance. To evaluate the bioaerosol detection performance in terms of real-time sensitivity and responsivity, the *E. coli* aerosols were generated using two 24-jet Collison nebulizers in a biosafety cabinet. To evaluate the sensitivity of the ABMS, serially diluted bacterial suspensions were nebulized stably and constantly. To evaluate responsivity, the bioaerosol concentration was adjusted by operating the two nebulizers at 30-min intervals. While the ABMS measures relative luminescence every 5 min, the APS (3321; TSI Inc., St. Paul, MN, USA) monitors aerosol concentration with a time-resolution of 1 min. A time delay of a few minutes is required for the sampled specimens to travel through the ABMS units.

Figure S5

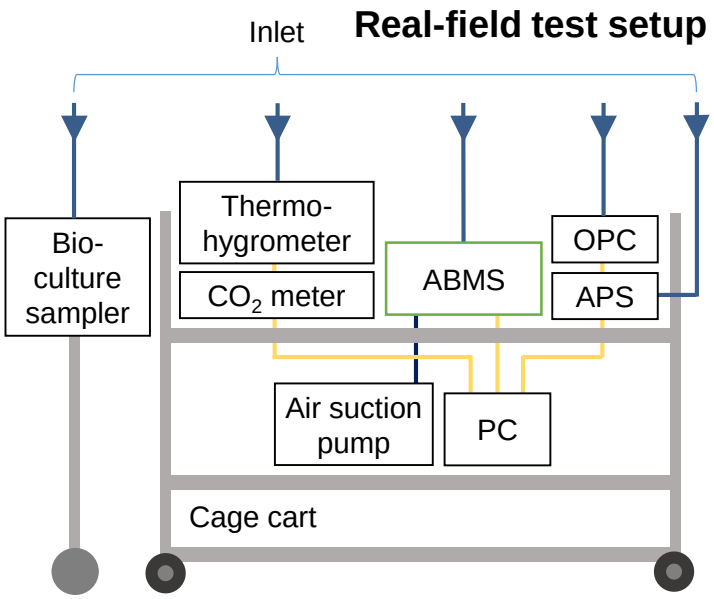


Fig. S5. Schematic diagram of the field test setup. To validate the applicability of the ABMS to real-world situations for the real-time monitoring of atmospheric bioaerosols, a field test of the ABMS was conducted at a subway station (Dongdaemun History & Culture Park station on Line 2) in Seoul, Republic of Korea, on August 5, 2019 (13:00–17:00). The temperature, relative humidity, and carbon dioxide (CO_2) concentration were monitored using a NDIR CO_2 meter (TES□1370; TES Electrical Electronic Corp., Taipei, Taiwan). The atmospheric particulate matter (PM) concentration was monitored using an optical particle counter (OPC) (model 1.109; GRIMM Aerosol Technik Ainring GmbH & Co. KG, Ainring, Germany) and APS (3321; TSI Inc., St. Paul, MN, USA). The Buck Bio-Culture sampler (model 708320; A.P. Buck Inc., Orlando, FL, USA) was used to measure total culturable bioaerosol concentration (TCBC; CFU) for comparisons with ABMS readings.

Figure S6

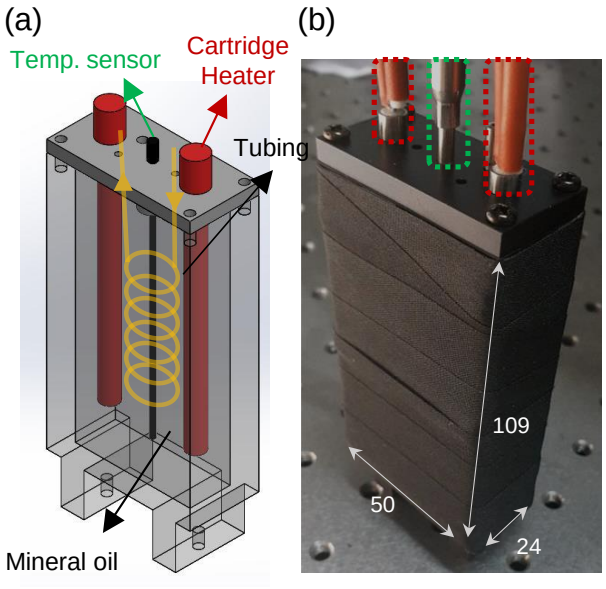


Fig. S6. Design and fabrication of the thermal-lysis unit. (A) The thermal-lysis unit consists of a cartridge heater (20 W; Taeyoung, Bucheon, Republic of Korea) and a temperature sensor (pt100; Taeyoung) in an aluminum chamber (50 × 24 × 109 mm) filled with mineral oil (M5904-500ML; Sigma Aldrich, St. Louis, MO, USA). The product of bioaerosol cell lysis flows through tubing (Tygon microbore tubing, 0.02-inch inner diameter, 0.06-inch outer diameter; Cole-Parmer, Chicago, IL, USA) placed in the mineral oil heated to 95°C by the cartridge heater; the sample moves through the unit in ~3 min at a flow rate of 1 mL/h. (B) Photograph of the thermal-lysis unit.

Figure S7

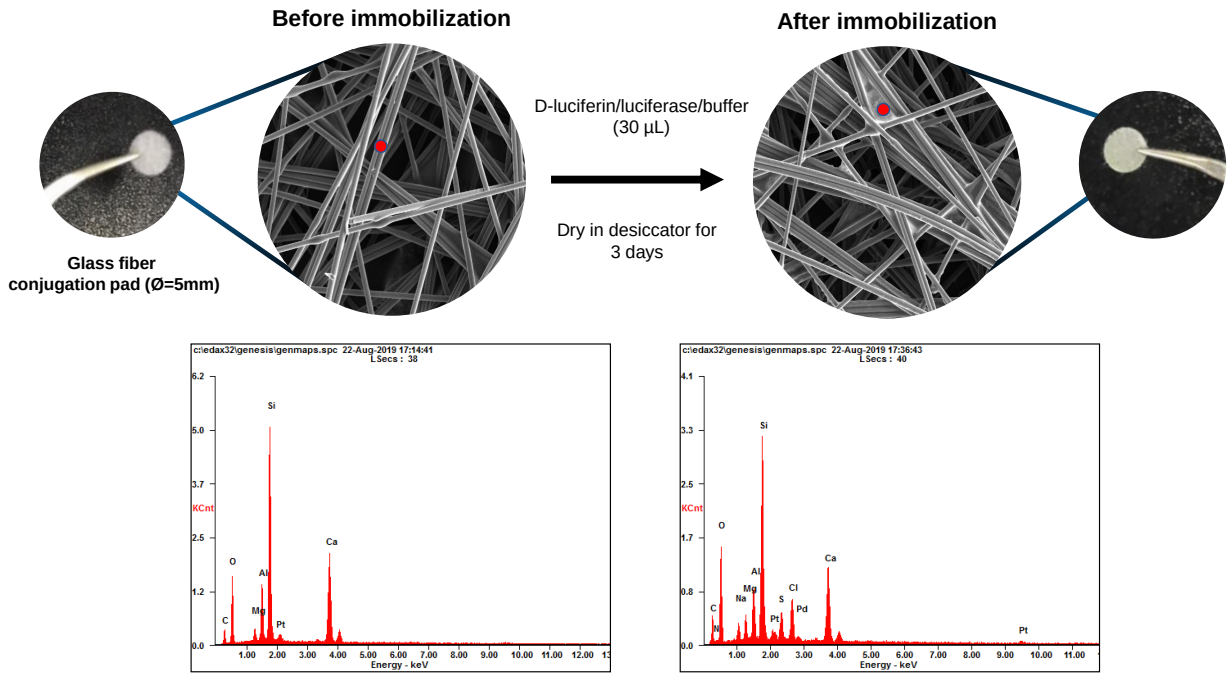


Fig. S7. SEM images of the glass fiber conjugation pad before and after immobilization of luciferase/luciferin. To immobilize all ingredients on the glass-fiber-conjugation pad, an immobilization mixture was prepared by mixing luciferase (5 mg/mL), luciferin (10 mM), and reaction buffer in a 1:1:8 (v/v/v) ratio, respectively. Sodium and sulfur were observed after immobilization in the pores of pads. These elements originated from the enzyme ingredients.

Figure S8

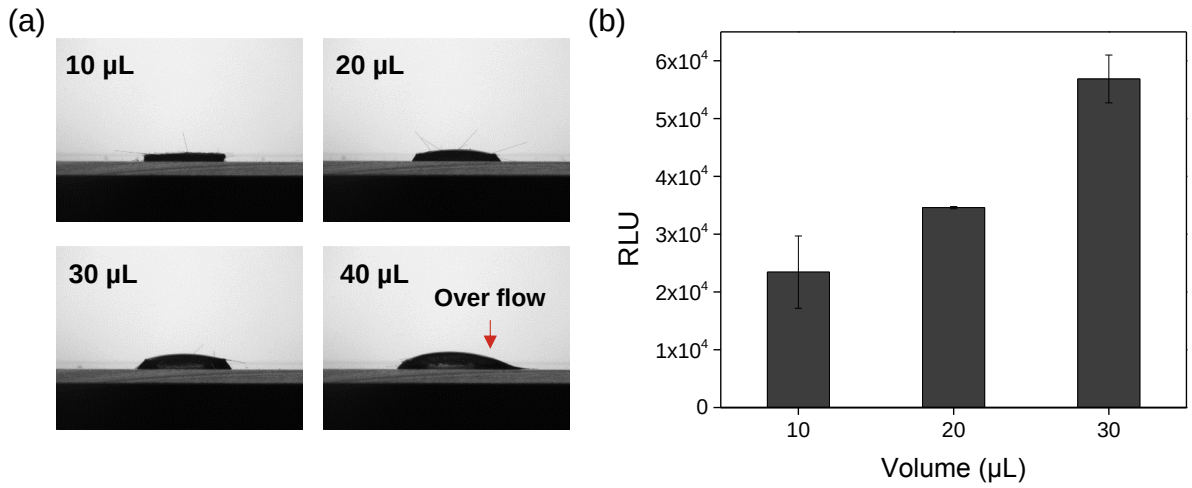


Fig. S8. Determining the appropriate volume to use on the PMT-based detector slit of the ABMS. (a) Various volumes of distilled water were dropped onto the glass-fiber-conjugation pad with luciferase/D-luciferin immobilized and directly evaluated using the contact angle test. When 40 µL of distilled water was added to the immobilized-enzyme glass-fiber-conjugation pad, the distilled water overflowed from the pad. Therefore, 40 µL is not an appropriate volume for the inlet slit of the PMT-based detector. (b) A total of 5 mol of ATP standard solution was dropped onto the glass-fiber-conjugation pad with immobilized luciferase/D-luciferin, and bioluminescence intensity was directly monitored using a bioluminometer. The 30-µL volume produced the highest bioluminescence intensity. Therefore, a volume of 30 µL was appropriate for the PMT slit. Error bars indicate SDs (n = 3).

Figure S9

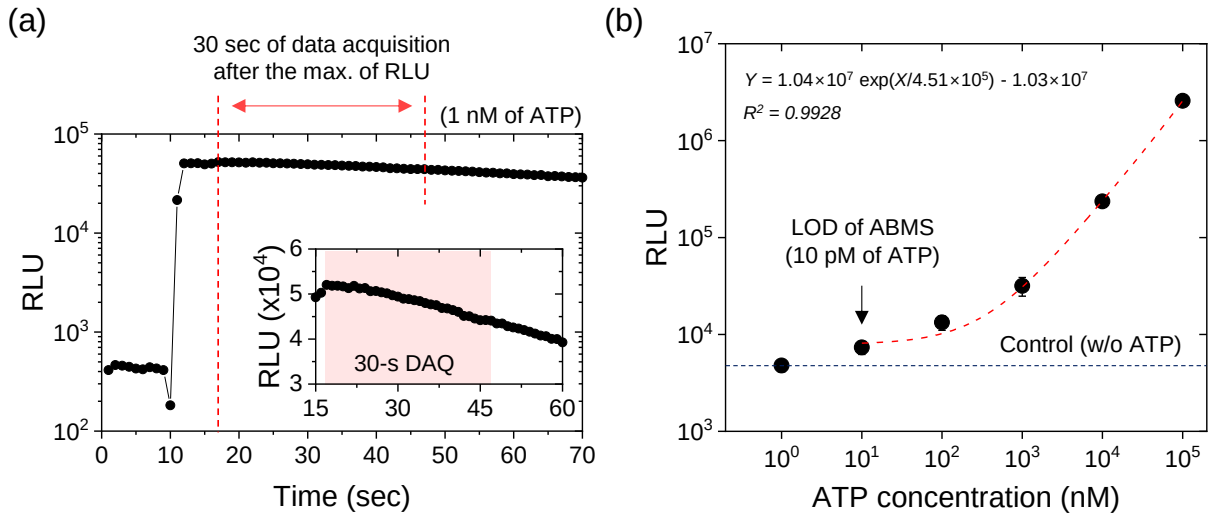


Fig. S9. Evaluation of the performance of the ATP-bioluminescence-detection unit of the ABMS. (a) The response duration time. Once 30 μL ATP solution (1 nM) was dropped onto the immobilized-enzyme glass-fiber pad, the relative luminescence increased to its maximum value (~5.78×10^4 RLU) immediately during the reaction, and then decreased gradually thereafter. The average variation over 30 s was compared for the quantification of the ATP-bioluminescence reaction. (b) ATP-bioluminescence reactions using sequentially diluted ATP standard solutions. The experimental sensitivity (i.e., LOD) of the ABMS was 10 pM. Error bars indicate SDs (n = 3).

Figure S10

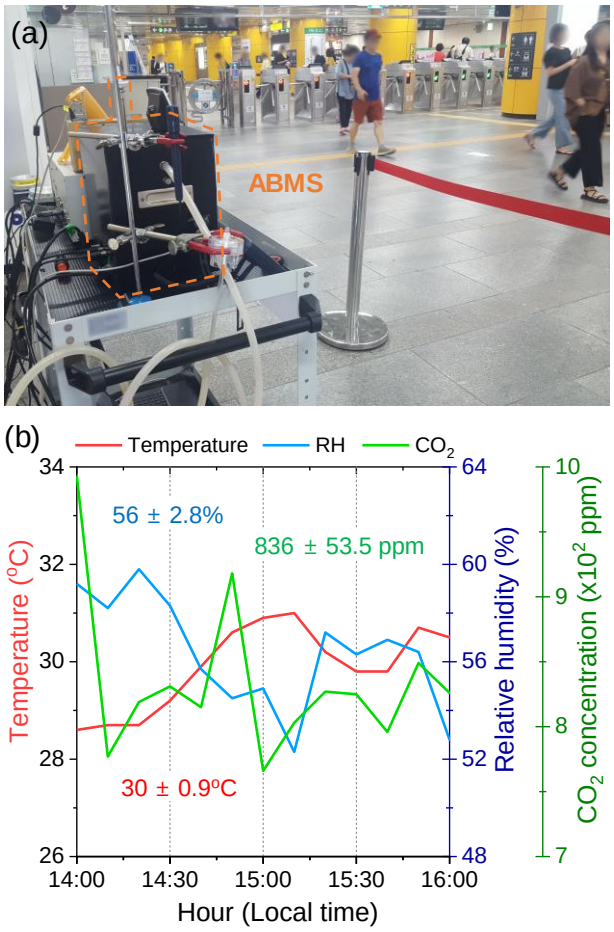
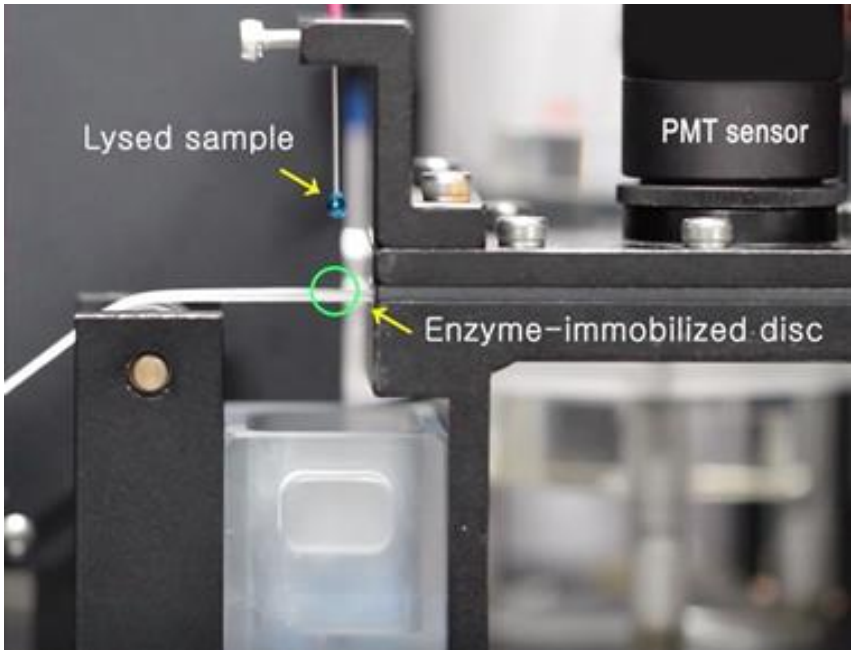


Fig. S10. ABMS field test. (a) Photograph of the real-time monitoring of bioaerosols at a subway station (Dongdaemun History & Culture Park station on Line 2) in Seoul, Republic of Korea, on August 5, 2019 (13:00–17:00). Various environmental sensors as well as the ABMS were used together (Fig. S5). (B) Variations in temperature, relative humidity, and CO_2 concentration during the field test. Environmental data were continuously recorded using a NDIR CO_2 meter (TES□1370; TES Electrical Electronic Corp., Taipei, Taiwan). The variation in CO_2 concentration (coefficient of variation: 0.06) was relatively large compared to those for temperature (0.05) and relative humidity (0.03). During the field test, the floating population in the subway station was tallied at 4,118. (14:00–16:00).

Movie S1



Mov. S1. Continuous and real-time detection of bioaerosols using the ABMS. The ATP-bioluminescence reaction starts from the moment the thermally lysed specimens are delivered to the immobilized-enzyme pad (or disc), which is attached to a roll-to-roll module. The bioluminescence intensity of each pad is measured in RLUs using a PMT (H11890; Hamamatsu Photonics, Hamamatsu, Japan).