The Biopause Project: Balloon Experiments to Observe the Upper Boundary of the Terrestrial Biosphere

By Sohsuke OHNO,[1)] Norimune MIYAKE,[1)] Osamu OKUDAIRA,[1)] Ko ISHIBASHI,[1)] Yuko KAWAGUCHI,[1),2)] Keisuke MAEDA,[1),3)] Manabu YAMADA,[1)] Issei IIJIMA,[4)] Yusuke TAKAHASHI,[5)] Satoshi NONAKA,[4)] Takahiro SEGAWA,[6)] and Hideyuki FUKE[4)]

[1)] *Planetary Exploration Research Center, Chiba Institute of Technology, Narashino, Japan*
[2)] *Saitama University Research Administration Office, Saitama University, Saitama, Japan*
[3)] *Department of Space Systems Engineering, Kyushu Institute of Technology, Kitakyushu, Japan*
[4)] *Institute of Space and Astronautical Science, JAXA, Sagamihara, Japan*
[5)] *Faculty of Engineering, Hokkaido University, Sapporo, Japan*
[6)] *Graduate Faculty of Interdisciplinary Research Faculty of Medicine Basic Science for Clinical Medicine, University of Yamanashi, Chuo, Japan*

(Received March 10th, 2023)

The “biopause” is the upper boundary of the biosphere. Understanding the biopause and the biological flux across the biopause is key to understanding the universality, distribution, origin, and evolution of life in the universe. However, the biopause has not been extensively investigated before and remains poorly understood. The most direct information available for analyzing the biopause is the distribution and dynamics of life in the stratosphere. The Biopause project is a stratospheric bioaerosol sampling project aimed at determining the altitude of the biopause observationally. In this paper, we summarize the results of the balloon experiments conducted as part of the Biopause project, report the results of the most recent balloon experiment from last year, and discuss future experimental plans to determine the biopause observationally.

Key Words: Biopause, Astrobiology, Scientific Balloon Experiments, Stratosphere, Microorganism

1. Introduction: What is the Biopause?

The “biopause” is the upper boundary of the terrestrial biosphere. Understanding the biopause and the biological flux across it is key to comprehending the habitability, universality, distribution, origin, and evolution of life in the universe. Information about the biopause is crucial, especially for testing the panspermia hypothesis, which explores whether life can travel between planets and celestial bodies. By determining the location of the biopause in Earth's atmosphere, we can constrain the limits of biological survival and the potential for life to spread into space beyond Earth. However, the biopause has not been extensively investigated before and remains poorly understood.

Where, then, is biopause located? While biological presence is well established in the lowest layer of Earth's atmosphere (the troposphere), the distribution of microorganisms in the middle atmosphere, i.e., stratosphere and mesosphere, remains insufficiently understood. Therefore, the most direct information available for investigating the biopause is the distribution and dynamics of life in the terrestrial middle atmosphere.

It is widely accepted that the tropospheric atmosphere contains a diverse array of living microbes (e.g., Ref. 1). Organisms are universally present in the troposphere because the temperature and pressure conditions are conducive to their survival. Additionally, since the tropospheric atmosphere is well mixed, microorganisms from the ground and water can easily spread throughout the troposphere.

On the other hand, the stratosphere's biological mass is considered small due to both biological and dynamical reasons. The stratospheric environment is biologically severe for living things, as high UV radiation, low temperatures, low pressures, low humidities, and scarce nutrients make it difficult for microorganisms to survive or proliferate. Additionally, it is dynamically challenging for microorganisms from the surface and troposphere to reach the stratosphere, as the stratosphere has low atmospheric pressure, causing microorganisms with cells to settle rapidly. Moreover, due to the stratified nature of the stratospheric atmosphere, the vertical mixing rate is slow, making it difficult for microorganisms to be lifted by air currents. The dynamical lifetime of microbes in the stratosphere would be short.

However, the possible presence of microbes in the stratosphere and mesosphere has been reported in previous experiments using balloons, aircraft, and rockets, as shown in Table 1.[2-10)] These reports are not negligible. Nevertheless, the problems of earlier studies, such as the possibility of contaminating tropospheric microbes during the sampling procedure and limited analytical methods, hinder the determination and understanding of the biopause. Cleaner experiments that minimize tropospheric contaminations and more diverse sample analysis methods are required to gain a deeper understanding of the biopause.[11)]

For the past several years, we have been conducting a stratospheric microorganism collection experiment called the 'Biopause Project' to make an observational determination of the biopause.[11-13)] In this paper, we summarize the method of the balloon experiments in the Biopause Project, report the results of the latest balloon experiment, which tested a new sampler, and discuss plans to determine the biopause observationally.

2. The Biopause Project

The scientific objectives of the Biopause project are to observe the microbial number density distribution in the stratosphere and lower troposphere to determine the altitude of the biopause. The key is establishing experimental methods that ensure no detectable contamination from the ground or the troposphere. This chapter provides an overview of the procedures for balloon experiments in the Biopause Project and summarizes past balloon experiments conducted over the past several years.

2.1. Overview of a balloon experiment

We used the scientific balloons of the Japan Aerospace Exploration Agency (JAXA) for the Biopause project. The balloon experiment is conducted at JAXA's Taiki Aerospace Experiment Site as part of JAXA's collaborative research. In the Biopause Project's balloon experiment, the balloon is separated from the experimental equipment after ascending to the stratosphere. During its descent with a parachute, microparticles and microorganisms floating in the upper troposphere and stratosphere are collected. Figure 1 shows the overview of the balloon experiments of the Biopause project. By collecting microorganisms during descent, contamination from those that may have adhered to the exterior of the experimental equipment, including the balloon itself (picked up while on the ground and during the ascent in the troposphere), was significantly prevented (see Section 3.1).

The experimental system is equipped with remotely operable valves, allowing the collection of airborne microorganisms at specific altitudes. Multiple sampling units are mounted in parallel and operate at different altitudes, enabling the observation of altitude distribution within a single balloon flight. After the sampling, the equipment lands in the Pacific Ocean, where it is retrieved by a fishing vessel. The collection devices are then disassembled in a laboratory, and the collected samples are analyzed.

2.2. Past balloon experiments of the Biopause project

The Biopause Project has conducted five balloon experiments to date.

The first experiment was carried out in 2016 to validate the experimental methodology, as shown in Fig. 2. It included one set of sampling units for sample collection and another set for negative control. In this first experiment, the collected samples were successfully observed using a fluorescence microscope and a scanning electron microscope (SEM), confirming the viability of the experimental methodology. Although the negative control samples were lost, the experiment succeeded in estimating the upper limit of microorganism density in the stratosphere. The results of the 2016 experiment can be summarized as follows: 1) Successfully observed, for the first time in the world, the upper limit of microorganism density in the stratosphere, including those that cannot be cultured. 2) Successfully collected and analyzed stratospheric particles using a newly developed descending impactor-type sampler.[12)]

The second experiment was conducted in 2017. This balloon experiment aimed to achieve four objectives: 1) Observing stratospheric microorganisms' morphology and numerical density at different altitudes in the exact location, including uncultivable microorganisms. 2) Comparing microorganism detection using fluorescence microscopy with conventional culturing methods. 3) Collecting and analyzing control samples, which had not been successfully retrieved in the first experiment. 4) Establishing an experimental method for measuring airflow within the sampling unit. Multiple sampling units were mounted in parallel for the balloon experiment to achieve these objectives. However, due to water intrusion into the sampling units, the retrieval of stratospheric samples failed, and the experiment could not accomplish its intended goals.

Measures were implemented to prevent water intrusion into the sample collection section based on the water intrusion in the sampling unit during the second experiment. Specifically, the control mechanism of the sampling unit's valve was completely redesigned, and water drop tests were conducted under conditions simulating water landing for the entire sampling unit. Although preparations for the balloon experiment were completed in the summer of 2018, the launch was ultimately postponed due to unfavorable weather conditions; the third balloon experiment was scheduled to be conducted in the summer of 2019.[13)]

Following the second experiment, multiple sampling units were mounted in parallel to observe the vertical distribution in the third balloon experiment of 2019, as shown in Fig. 3. As a result of the third balloon experiment, no stratospheric microorganisms were detected at any sampling altitudes (13 km–26 km). However, we successfully recovered the sample, and non-biological aerosol particles were detected. This indicates that the number density of stratospheric microorganisms has an upper limit even lower than the results obtained from the first experiment. Here is the summary of the 2019 experiment results: 1) Although some equipment malfunctions occurred, the collection of microparticles in the stratosphere and the analysis of recovered samples were successful. 2) An experimental procedure was established that eliminates the impact of contamination. 3) Data acquisition was successfully conducted using an internal flow measurement device for samples. 4) On the day of the experiment, observations over Hokkaido indicated that the number density of microorganisms in the stratosphere, between altitudes of 12.8 km and 26.5 km, was extremely low, including unculturable species, and was below the detection limit of this study.

The fourth balloon experiment of the project, conducted in the 2021 fiscal year, aimed to reliably observe the biopause. The goal of this experiment was to comprehensively observe the morphology and number density of microorganisms, including unculturable species, at different altitudes at the exact location and time, covering not only the stratosphere but also the upper troposphere. The experimental setup was constructed with the same configuration as the third experiment, and the balloon experiment was carried out in the summer of 2021. The experimental equipment successfully ascended to an altitude of 33 km as planned, and after the balloon was detached, it operated as expected during parachute descent. After landing in the Pacific Ocean, a fishing vessel successfully retrieved the experimental apparatus. However, upon disassembling the sealed sample collection unit at Chiba Institute of Technology, it was discovered that water had infiltrated the interior. Unfortunately, the recovery of samples from the stratosphere and upper troposphere was unsuccessful.

The latest experiment in the Biopause project, the fifth experiment, was conducted in 2024. Its primary objective was to validate the new ball valve developed for the sampling unit in a balloon-based environment, specifically addressing issues encountered in the fourth balloon experiment, particularly water ingress into the sampling unit. Further details on this experiment are provided in Chapter 4.

3. Descending Inertial Impactor Sampler.

3.1. Principle and characteristics

In the balloon experiment of the Biopause project, the sampler is released from the balloon after ascending to the stratosphere. During its descent with a parachute, microorganisms floating in the stratosphere and/or upper troposphere are collected. By collecting bioaerosol samples during descent, contamination from microorganisms attached to the outer walls of the experimental equipment, including those attached on the ground before launch and those acquired in the troposphere during ascent, is significantly reduced. The descent velocity of the sampler, controlled by the size of the parachute and the sampler's weight, is much greater than the falling velocity of microorganisms that detached from the equipment’s exterior. This minimizes the likelihood of ground microorganisms re-entering the sampling unit.

The descending inertial impactor sampler of the Biopause project passively introduces atmospheric air into the sampler during descent, capturing microorganisms onto an impaction plate in the sampler. The inlet and outlet of the sampler open and close according to predetermined timing using valves, allowing samples to be collected at specific altitudes. The conceptual diagram of the impactor-type sampler for the balloon experiment illustrates its vertical cylindrical airflow path, with the impaction plate placed inside. Figure 4 shows the mechanism of the descending inertial impactor. The impaction plate is made of glass (for fluorescence microscopy observation) and precision-cleaned metal plates (for SEM observation). Air entering from the bottom of the device passes through a nozzle, flowing in a manner that avoids the impaction plate; however, airborne particles collide with and adhere to the impaction plate.

Using the descending inertial impactor sampler developed in the Biopause project, multiple sampling units can be mounted in parallel, allowing for a comprehensive observation of the altitude distribution of microorganisms at a specific location in a single flight. Previous studies on the altitude distribution of microorganisms in the middle atmosphere have compiled data obtained using different methods—rockets, balloons, aircraft—at different locations and times. However, there has been no systematic study using the same method to examine microbial distribution at various altitudes in the exact location. Due to variations in methodologies, biases, horizontal density differences, seasonal fluctuations, and errors inherent in each approach, past studies have been unable to quantitatively evaluate vertical transport mechanisms or the residence time of microorganisms in the middle atmosphere. The experimental method in the Biopause project effectively resolves these issues. Additionally, in high-altitude balloon experiments, an advantage of using the Descending Inertial Impactor Sampler is that the only moving component above is the valve, simplifying operation.

3.2. Development of the valve

The air inlet and outlet of the sampling unit must be sealed before the device descends into the lower troposphere during sampling. To achieve this, a remotely operable valve is required at high altitudes.

This valve must function reliably in stratospheric conditions, particularly at low temperatures (below -60°C). Additionally, the valve must provide sufficient conductance while maintaining a straight airflow path for effective sample collection. It must also withstand the cleaning and sterilization procedures necessary for microbial analysis. Furthermore, the sampling unit must be lightweight enough to be mounted on the balloon experiment, yet durable enough to endure impacts upon water landing or other shocks. Finally, the valve must operate rapidly (within several seconds) during descent with the parachute.

During the first to fourth balloon experiments in the Biopause project, vacuum gate valves were installed at the air inlet and outlet of the sampling unit. A compressed air-driven valve was used instead of an electric power source, as gate valves were chosen because they provide high conductance, can be disassembled for dry heat sterilization, and ensure reliable function. However, since modifying the O-rings inside the valve for low-temperature tolerance was difficult, the entire valve must be kept warm at high altitudes to maintain functionality. Additionally, operating air pressure helped prevent malfunctions and improved impact resistance, especially during water landings. To further verify durability, water intrusion tests were conducted at speeds equal to or exceeding those of balloon splashdowns.

However, after four balloon experiments, it became evident that water ingress into the sampling unit through the gate valve was possible. To address this issue, a new ball valve was developed. The impactor was placed inside a single ball valve unit, as shown in Fig. 5. By constructing the housing and ball entirely from aluminum alloy, the device remained within the allowable weight limit for balloon experiments (Fig. 6). The valve was electric motor-driven rather than air-driven. Comprehensive sealing tests, dry heat sterilization tests, low-temperature and low-pressure operation tests, and simulated balloon experiment environmental tests were conducted, confirming no performance issues. Importantly, only the motor required heating, eliminating the need for overall temperature control and significantly decreasing the mass of the batteries required.

3.3. Development of the impactor

The sampling system's particle size and collection efficiency depend on several factors, including the shape of the airflow path, the size and positioning of the collection plate, and other design elements. Maintaining smooth airflow through the device is a critical requirement for collecting microorganisms with high efficiency.

To understand the overall flow field within the device, we conducted wind tunnel experiments and fluid simulations, comparing the results using a model. The wind tunnel tests were performed at both atmospheric and low pressures using JAXA's planetary environment wind tunnel (Fig. 7). To analyze the airflow, we measured the static pressure at the inlet and outlet of the device, as well as at the throat section of the nozzle. The fluid simulation results closely matched the wind tunnel experiment data, confirming that the simulations accurately reproduced the airflow field observed in the experiments.

Additionally, using the fluid simulation data, we estimated the probability of particle deposition on the collection plate when airborne particles entered the sampling unit. Since microorganisms are known to be larger than approximately 1 μm, the system was designed to capture microorganisms of 1 μm or larger effectively.

3.4. Cleaning, sterilization, and biological analysis

To ensure reliable analysis of microorganisms collected from the atmosphere, the sampling unit must be sterilized in advance. Each component of the sampling unit was sterilized, then assembled and sealed inside a clean bench, as shown in Fig. 8.

The procedures for biological analysis, including cleaning and sterilization, have already been established through previous experiments. In the fourth experiment conducted in 2019, our biological methodology successfully demonstrated that no contamination from the ground or troposphere was detected. Regarding analysis techniques, previous experiments involved fluorescence microscopy observations and microbial culturing.

4. Balloon Experiment in 2024

The fifth experiment of the Biopause project, conducted in 2024, aimed to validate the newly developed ball valve for the sampling unit in an actual balloon experiment. This experiment specifically addressed issues encountered in the fourth balloon experiment, particularly the problem of water ingress into the sampling unit.

4.1. Objectives of the fifth balloon experiment

Based on the water instruction issue encountered in previous balloon experiments, we developed a new sampling unit that prevents negative pressure inside the collection unit upon water landing. By designing the ball valve for the sampling unit from scratch, we achieved a compact and lightweight structure while minimizing the risk of water infiltration upon landing.

In the fifth balloon experiment of the Biopause project, a piggyback high-altitude balloon test was conducted to verify the newly developed ball valve unit through operational testing in the stratosphere and water ingress testing upon landing. This experiment successfully eliminated the major challenge of water ingress into the sampling unit, ensuring the viability of future full-scale stratospheric microbial sampling experiments. Ultimately, it aims to contribute to the observational determination of the biosphere-stratosphere interface, known as the biopause.

We put some success criteria for the fifth balloon experiment before doing it. The minimum success criteria of the fifth balloon experiment of the Biopause project were: Obtain the data and insights necessary for utilizing the newly developed ball valve in the stratospheric microbial sampling unit during balloon experiments. And the nominal success criteria were: 1) Operate the newly developed ball valve for the microbial sampling unit in the stratosphere or upper troposphere. 2) Recover the experimental system after a water landing and verify that no water intrusion has occurred inside the sampling unit. The extra success criteria were: 1) Conduct verification tests on the protective lid used to shield against water droplets during launch and ascent. 2) Observe the impactor surface under a microscope to confirm whether aerosols have been successfully collected.

4.2. Experimental equipment

In the fifth balloon experiment, the newly developed ball valve for the stratospheric microbial sampling unit was mounted on the same gondola as another experiment (Fig. 9). The system also included a valve drive motor, control electronics, battery, sensors (such as an atmospheric pressure gauge and rotation sensor), and a camera. The sealing valve used was the newly developed ball valve.

For valve control, three approaches were prepared: 1) ground command activation, 2) autonomous control based on onboard pressure readings, and 3) timer-based activation after balloon separation. These control methods were divided into primary and redundant control systems for reliability.

Before launch, the ball valve was sealed in the laboratory at Chiba Institute of Technology and then transported to Taiki Experiment Field, where it was integrated into the gondola for the balloon experiment. The valve system underwent extensive pre-experiment testing, including sealing tests, cleaning tests, simulated sterilization tests, and low-temperature tests to ensure functionality. Additionally, a real-time flight simulation was conducted in a large, low-temperature, and low-pressure chamber, replicating the stratospheric environment, which confirmed the absence of operational issues. Furthermore, a two-stage filtration system—a biological contamination prevention filter and a seawater intrusion prevention filter—

was developed to prevent negative pressure inside the valve. These filters were subjected to post-low-temperature immersion tests, ensuring their readiness for the actual experiment.

4.3. Execution of B24-01 experiment

JAXA's scientific balloon, carrying our equipment, was launched from the Taiki Aerospace Research Field on Saturday, August 3, 2024, at 4:50 AM JST, as part of the JAXA scientific balloon experiments (Fig. 10). During its descent by parachute after balloon separation from the sampler system, the newly developed ball valve for the sampling units, along with its upper and lower covers, functioned exactly as expected. The photos of the ball valve in open and closed positions are shown in Fig. 11.

After a successful water landing, the entire system was safely recovered, and the sampling unit remained sealed as planned. It was then transported back to the Taiki Experiment Field via a fishing vessel. Figure 12 shows the recovered experimental equipment from the Pacific Ocean.

4.4. Post-experiment analysis

After cleaning the outer surface, we immediately inspected the interior of the valve inside a clean bench at the Taiki Experiment Field. The water ingress detection seal showed no abnormalities, and no signs of water infiltration were observed inside the sampling unit.

Following this, the device was transported to Chiba Institute of Technology, where the internal collection plate was examined using a microscope in the laboratory. Optical microscopy and SEM analysis were performed on the impaction plate within the sampling unit to verify whether aerosols were successfully collected in the stratosphere. No traces of salt crystals or any evidence of water ingress were detected. Figure 13 shows the interior of the sampling unit after the experiment.

4.5. Summary of the balloon experiment in 2024

In the fifth experiment, a high-altitude balloon test was conducted to validate the newly developed ball valve for the Biopause sampling unit. The experiment included operational testing in the stratosphere and water ingress testing upon landing.

The results confirmed that the ball valve functioned properly in the stratosphere without any issues. Additionally, after the experiment, the collection plate inside the sampling unit and the water ingress detection seal were examined, confirming that no water infiltration occurred during landing.

Moreover, the prototype cover, designed to prevent rainwater or other moisture from entering during launch and ascent, was tested and successfully operated as expected. Furthermore, optical microscopy and SEM analysis were performed on the impaction plate within the sampling unit to verify whether aerosols were successfully collected in the stratosphere.

All predefined success criteria—minimum, nominal, and extra success benchmarks—were fully achieved in this experiment.

5. Conclusion

This paper introduces the Biopause project, a stratospheric microorganism sampling experiment conducted using a high-altitude balloon. The scientific goal is to observationally determine the upper boundary of Earth's biosphere, known as the biopause, through balloon experiments. Several high-altitude balloon experiments have been conducted so far, yielding specific scientific results, but there have also been failures. The biggest issue in past experiments was water intrusion into the microorganism sampling unit. To address this, a new ball-valve-type sampling unit was developed, designed to be small, lightweight, and resistant to water intrusion.

This paper demonstrates that high-altitude balloon experiments successfully conducted operational tests of the sampling unit in the stratosphere, including its water resistance. The unit operated as expected at high altitudes, and no water intrusion was observed, confirming its effectiveness.

In the future, high-altitude balloon experiments will be conducted with multiple new sampling units in parallel, aiming to observe the altitude distribution of microorganisms in the stratosphere and upper troposphere.

Table 1. Summary of Previous Observations of Microorganisms in the Middle Atmosphere.

Methods and Papers	Altitudes
Balloon Experiments	
Rogers and Meier, 1936	11-21 km
Soffen, 1965	38-41 km
Wainwright et al., 2003	41 km
Yang et al., 2009	12-35 km
Bryan et al., 2019	1.3-38 km
Rocket experiment	
Imshenetsky et al.,1976	48-77 km
Aircraft experiments	
Griffin, 2004	20 km
Yang et al., 2008	0-12.3 km
Smith et al., 2018	~12 km

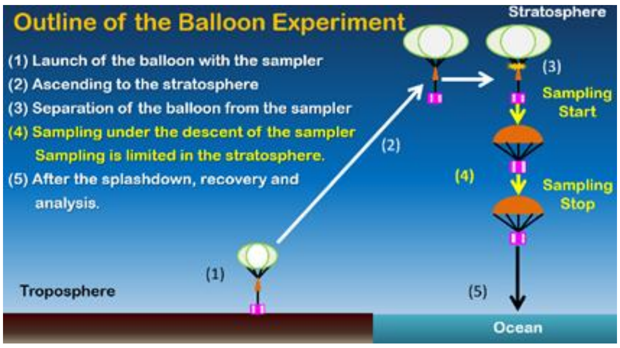

Fig. 1. A schematic diagram of the balloon experiments for the Biopause project overview.

Fig. 2. Launch of the first balloon experiment of the Biopause project, using a scientific balloon of JAXA on June 8, 2016, from Taiki Aerospace Research Field, JAXA, in Hokkaido, Japan.

Fig. 3. The experimental system for the third balloon experiment of the Biopause project in 2019.

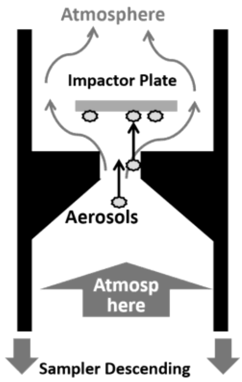

Fig. 4. Schematic diagram of the descending inertial impactor sampler.

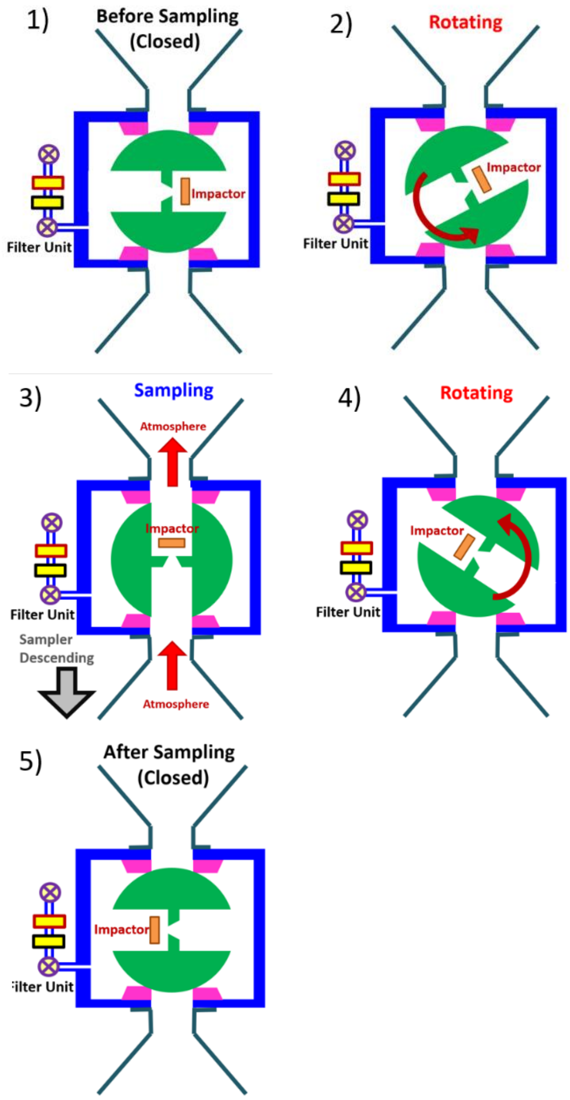

Fig. 5. A schematic diagram of the newly designed ball valve and sampling unit.

Fig. 6. The newly designed sampling unit of the Biopause project.

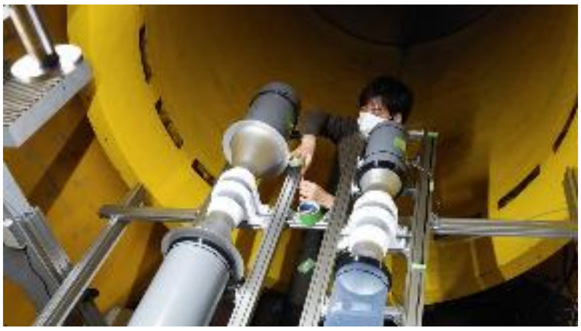

Fig. 7. Wind tunnel experiment to test the sampling unit using JAXA's Planetary Environment Wind Tunnel.

Fig. 8. Each component of the sampling unit was sterilized, then assembled and sealed inside a clean bench.

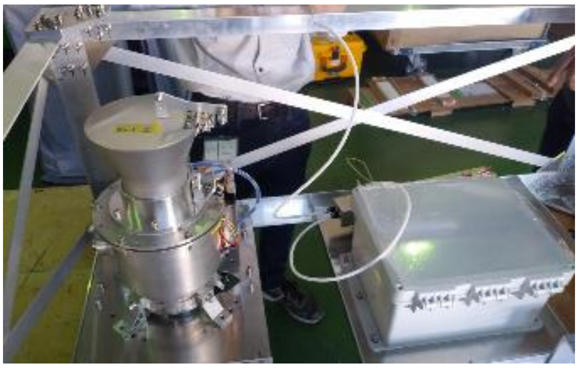

Fig. 9. In the fifth balloon experiment, the newly developed ball valve for the stratospheric microbial sampling unit was mounted on the same gondola as the one used in another experiment.

Fig. 10. Balloon launch of the fifth balloon experiment of the Biopause project in 2024.

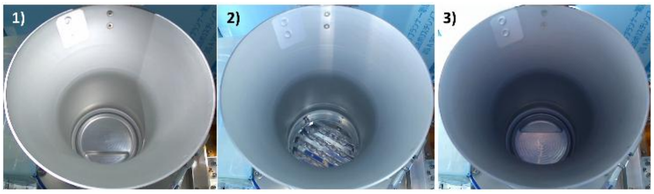

Fig. 11. The photos of the ball valve in open and closed positions during the fifth balloon experiments of the Biopause project. 1): Before the valve operation, 2): The valve is in the open position during the sampling, and 3): After the sampling, the valve is closed.

Fig. 12. Recovered experimental equipment from the Pacific Ocean.

Fig. 13. The interior of the sampling unit after the experiment.

Acknowledgments

The scientific balloon (DAIKIKYU) flight opportunity was provided by ISAS, JAXA. We extend our profound gratitude to Mr. Kenich Kato, Stella Precision, Fujitechnology Inc., and the Manufacturing Center of the Chiba Institute of Technology for their invaluable contributions and unwavering support in the execution of this experiment.

References

1) Maki, T., Aoki, K., Kobayashi, F., Kakikawa, M., Tobo, Y., Matsuki, A., Hasegawa, H., and Iwasaka, Y.: Characterization of halotolerant and oligotrophic bacterial communities in Asian desert dust (KOSA) bioaerosol accumulated in layers of snow on Mount Tateyama, Central Japan, *Aerobiologia,* **27** (2011), pp. 277–290.
2) Rogers, L.A. and Meier, F.C.: The collection of microorganisms above 36,000 feet, *Nat. Geo. Soc. Stratosphere Se.,* **2** (1936), pp. 146–151.
3) Soffen, G.A.: Atmospheric collection at 130,000 feet, Jet Propulsion Laboratory, Document number 65N24000., 1965.
4) Wainwright, M., Wickramasinghe, N.C., Narlikar, J.V., and Rajaratnam, P.: Microorganisms cultured from stratospheric air samples obtained at 41 km, *FEMS Microbiol. Lett.,* **218** (2003), pp. 161–165.
5) Yang, Y., Itahashi, S., Yokobori, S., and Yamagishi, A.: UV-resistant bacteria isolated from upper troposphere and lower stratosphere, *Biol. Sci. Space.,* **22** (2008), pp. 18–25.
6) Bryan, N.C., Christner, B.C., Guzik, T.G., Granger, D.J., and Stewart, M.F.: Abundance and survival of microbial aerosols in the troposphere and stratosphere, *The ISME Journal,* **13, 11** (2019), pp. 2789–2799.
7) Imshenetsky, A.A., Lysenko, S.V., Kazakov, G.A. and Ramkova, N.V.: On microorganisms of the stratosphere, *Life Sci Space Res,* **14** (1976), pp. 359–362.
8) Griffin, D.W.: Terrestrial microorganisms at an altitude of 20,000 m in Earth's atmosphere, *Aerobiologia,* **20** (2004), pp. 135–140.
9) Yang, Y., Itahashi, S., Yokobori, S., and Yamagishi, A.: UV-resistant bacteria isolated from upper troposphere and lower stratosphere, *Biol. Sci. Space,* **22** (2008), pp. 18–25.
10) Smith, D.J., Griffin, D.W., and Schuerger, A.C.: Stratospheric microbiology at 20km over the Pacific Ocean, *Aerobiologia,* **26** (2010), pp. 35–46.
11) Ohno, S., The Biopause project: Microbe sampling in the stratosphere to determine the upper boundary of the biosphere, *Viva Origino*, 46 (2018), pp. 1–3.
12) Ohno, S., Ishibashi, K., Miyake, N., Kawaguchi, Y., Kakehashi, Y., Okudaira, O., Yamada, M., Yamada, K., Takahashi, Y., Harada, D., Yamagishi, A., Segawa, T., Nonaka, S., Ishikawa, Y., Tokoro, G., Yamanouchi, K., Kobayashi, M., Fuke, H., Yoshida, T., Matsui, T.: A report on the B16-02 balloon experiment: Biopause- bioaerosol sampling at the stratosphere, JAXA-RR-17-007, 2018, pp. 15-23 (in Japanese).
13) Miyake, N., Ohno, S., Ishibashi, K., Kawaguchi, Y., Okudaira, O., Maeda, K., Iijima, I., Kakehashi, Y., Yamada, M., Kato, K., Yamada, K., Takahashi, Y., Yamagishi, A., Segawa, T., Nonaka, S., Fuke, H., Yoshida, T., Matsui, T.: A report on the 2017-2019 balloon experiments of the stratospheric bioaerosol sampling (Biopause project), JAXA-RR-20-009, 2020, pp. 1-17 (in Japanese).