Life-signature Detection Microscope for Cloud Layer Particles

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Keywords:

Life-signature Detection Microscope, cell, organic compounds

Please check correct the affiliation

https://www.youtube.com/watch?v=2WtjORvN-_Y


https://popcornshow.net/tenkinoko-water

Summary

- Inertial Impactor Sampler

...can capture particles on targets without the needs of power/movable parts

...being designed to fit the microscope

- Fluorescence Microscope

...can get morfology, density, and biochemical information of particles

...tiny version is now being developed

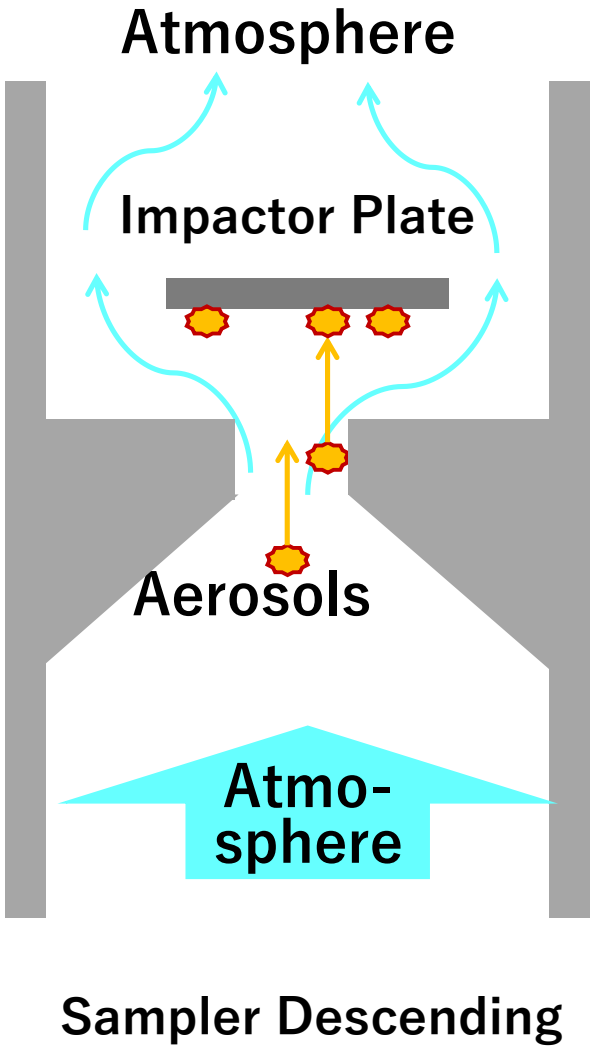
- Dyes

...candidates were found to stain DNA?, membrane and metabolism product? in 75% H_2SO_4 condition

How to collect

Descending Inertial Impactor Sampler

- We developed a new descending inertial impactor sampler for the Biopause project.
- The stratospheric atmosphere is introduced into the sampler using the descending velocity of the sampler and atmospheric aerosol particles are collected on the impactor plates.
- This method reduces biological contamination dramatically as the particles that adhere to the balloon and the wall of the sampler cannot enter the sampler during the descent because the descent velocity of the particles (as determined by Stokes' law) is much less than the descent velocity of the sampler.



Inertial impactor sampler

Balloon Experiment Procedure

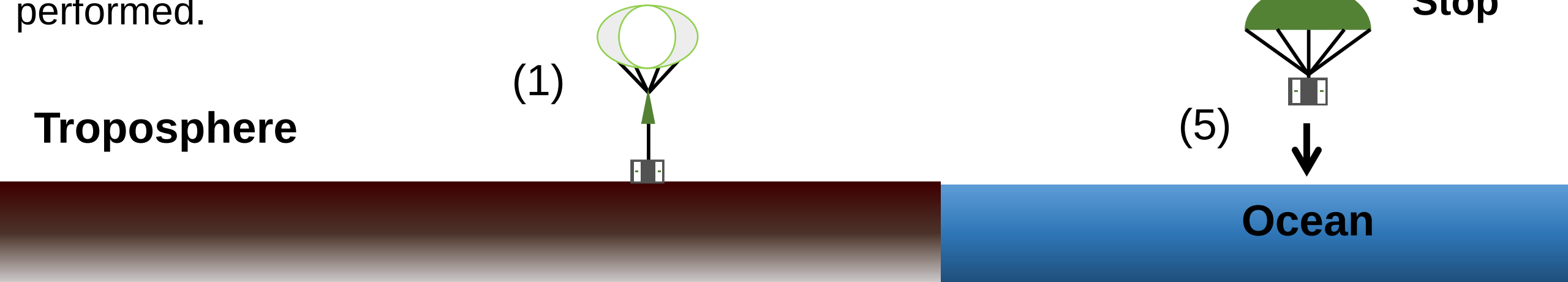
(1) Balloon carrying the sampler was launched.

(2) Balloon ascended to the stratosphere (28.5 km). The balloon traveled at this height for 30 min.

(3) Sampler was separated from the balloon.

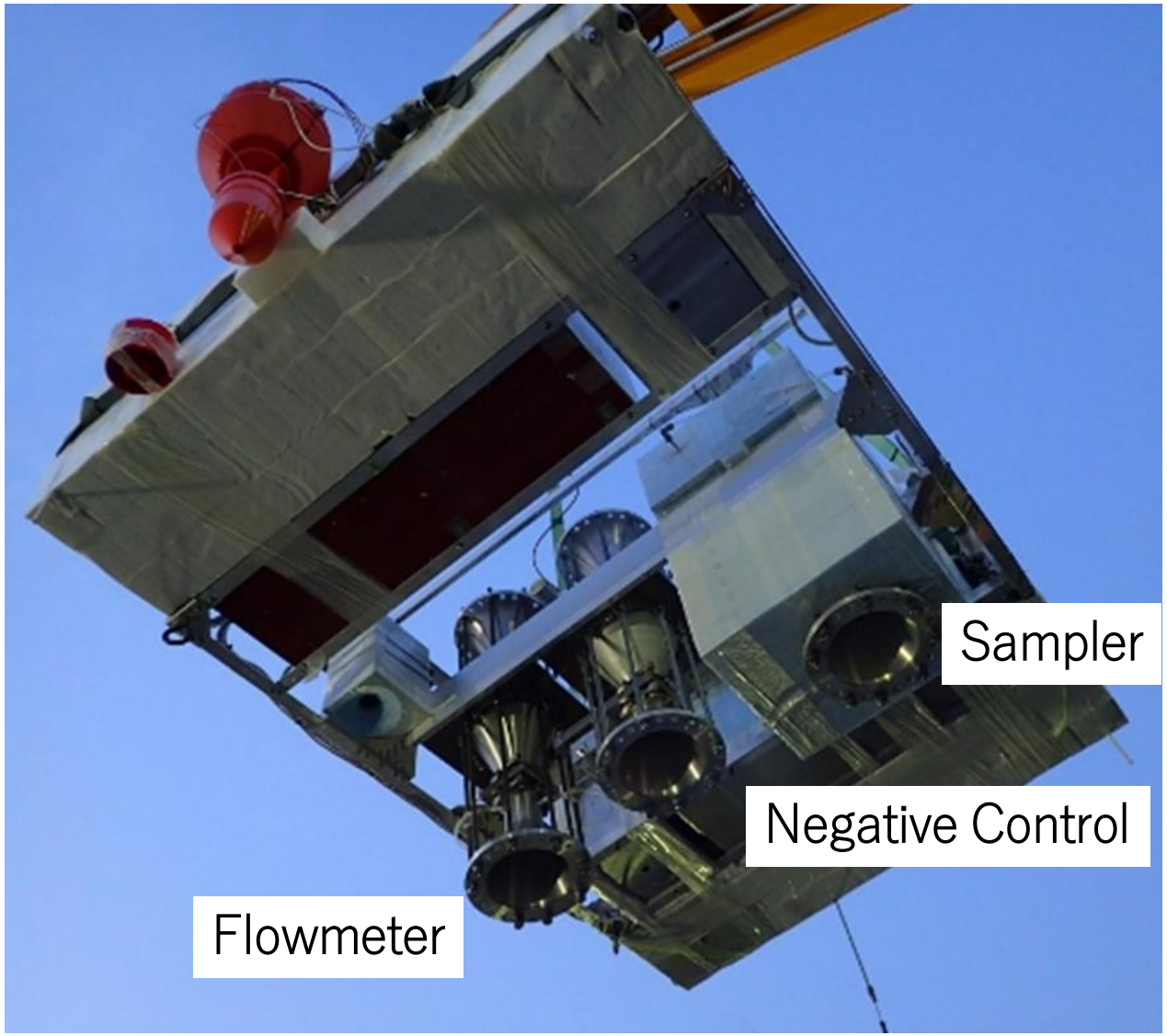
(4) During the descending (27 -> 13 km) at ca. 30 m s^{-1} in stratosphere, air inlet/exhaust valves were opened. Sampling were performed only in the stratosphere.

(5) Sampler splashdown. Recovery and analysis were performed.



Inertial impactor sampler

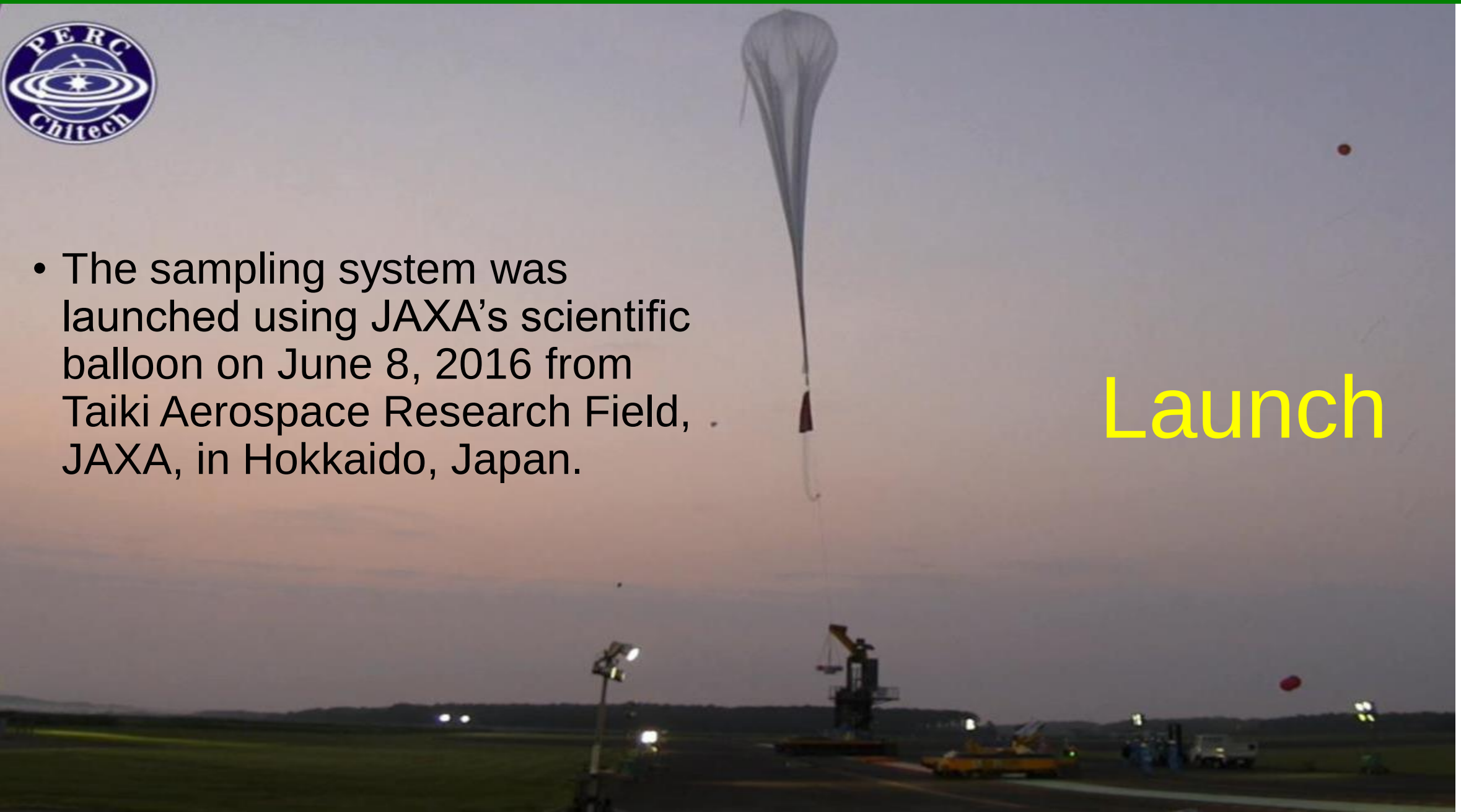
The Descending Inertial Impactor



The Experimental System

Inertial impactor sampler

- The sampling system was launched using JAXA's scientific balloon on June 8, 2016 from Taiki Aerospace Research Field, JAXA, in Hokkaido, Japan.

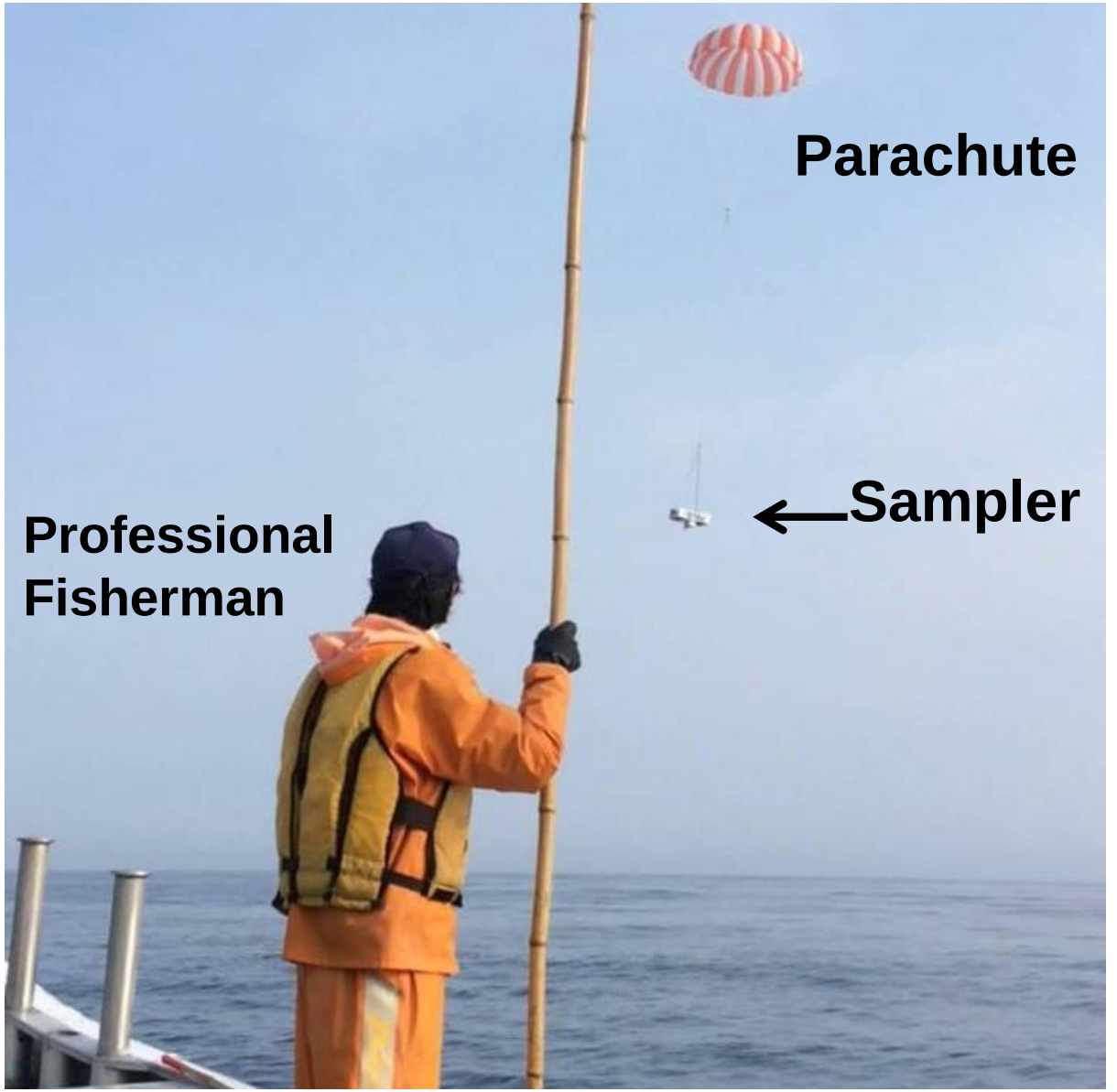


Inertial impactor sampler

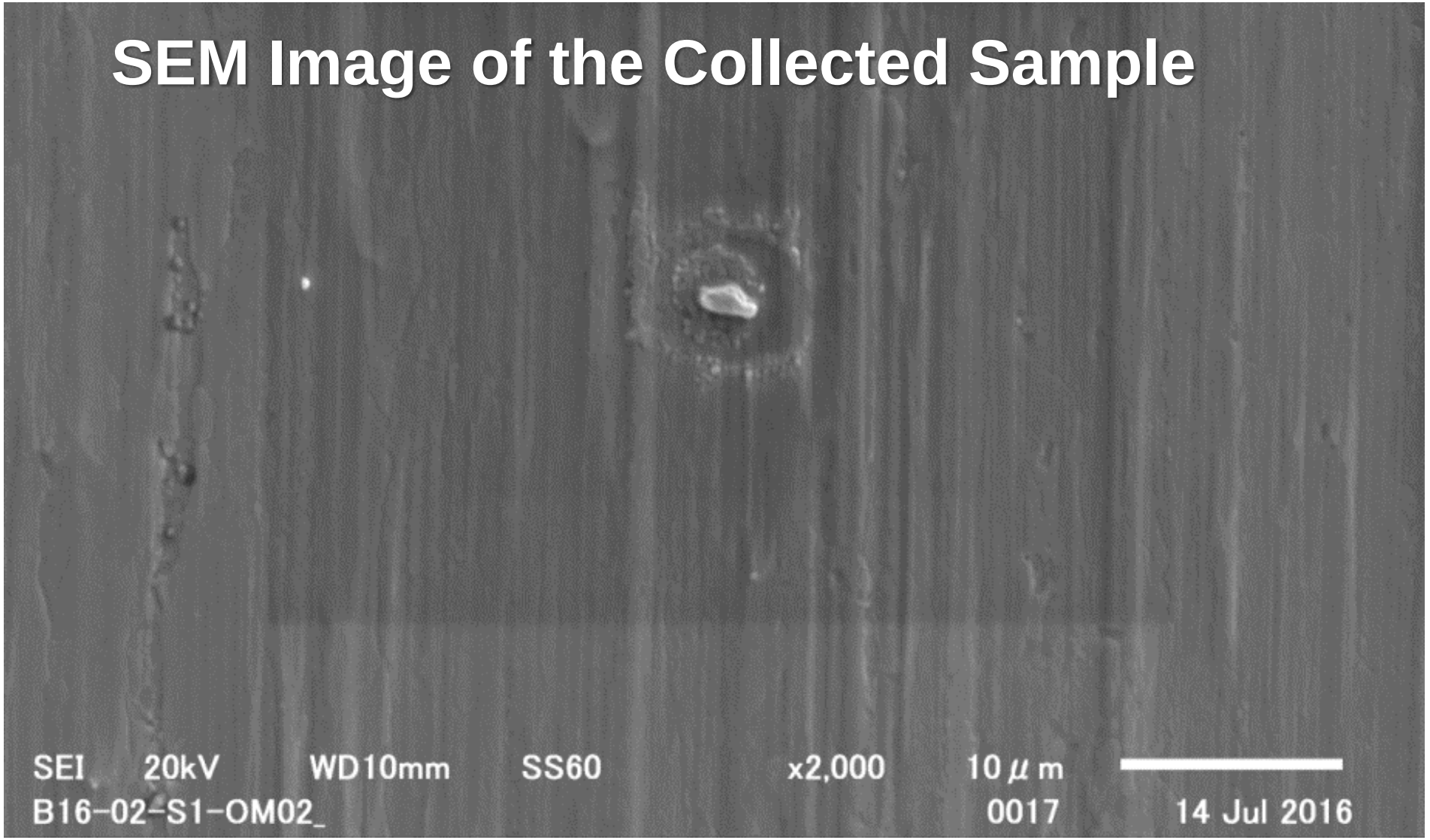
Balloon Experiment 2016

- After splashdown in the Pacific Ocean, the sampler was recovered using a fishing boat.

http://www.gisresources.com/russia-reduces-reliance-on-foreign-remote-sensing-satellite-data/russia-map/



Inertial impactor sampler



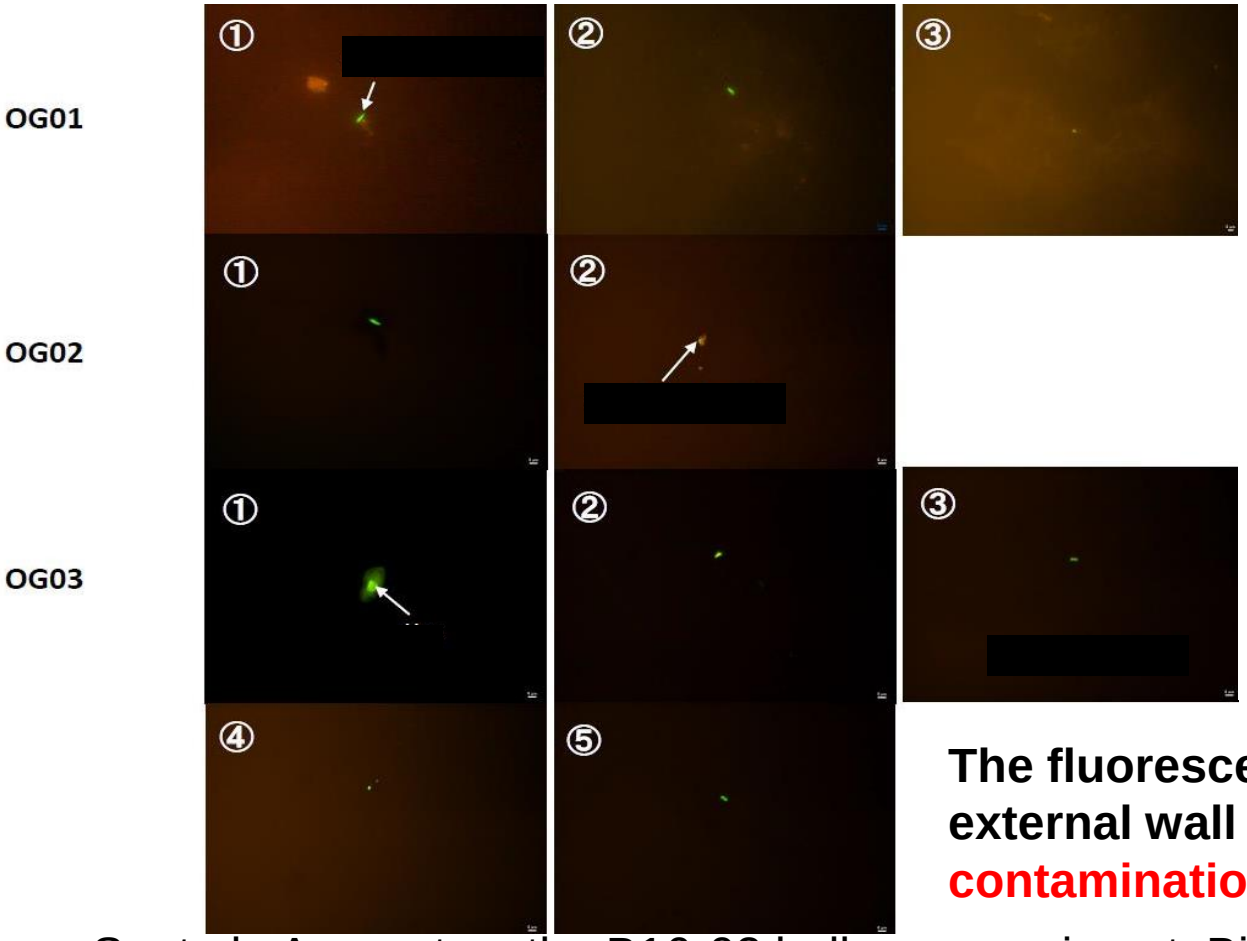
We found 46 particles with concentric rings called “satellite structure”, which are specific to the aerosols captured using an impactor. These are the evidence that we surely collected aerosols in the stratosphere, because contaminated particles does not have such a structure.

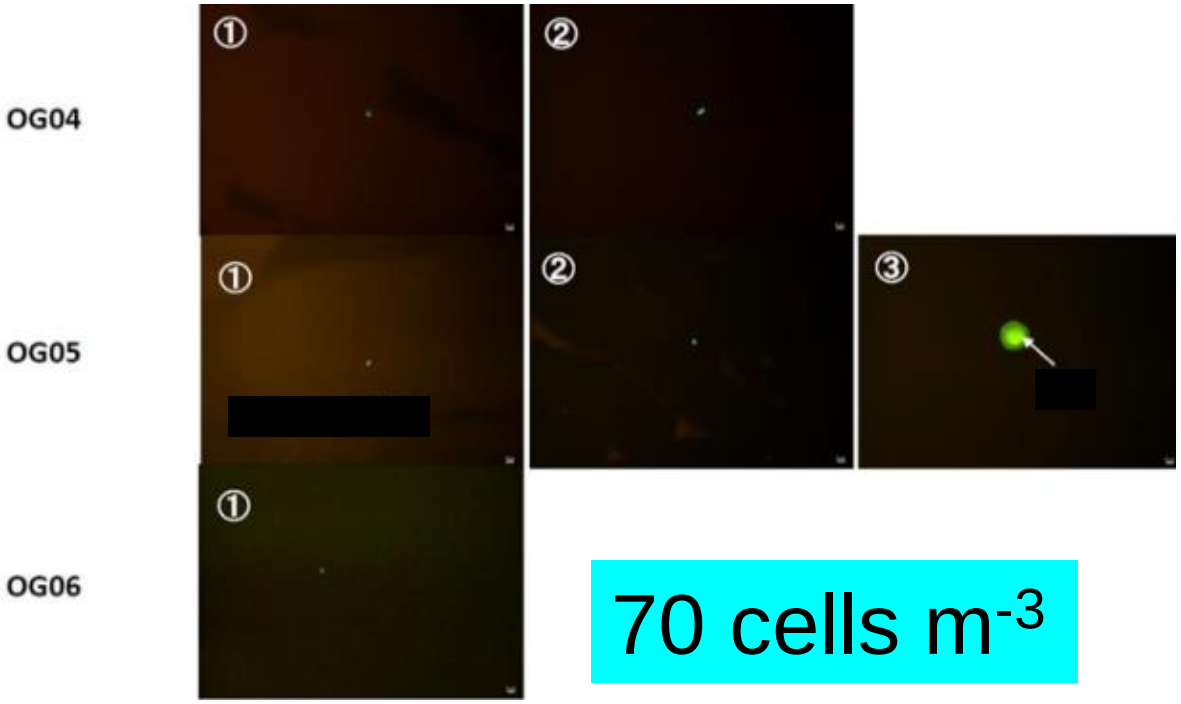
Ohno, S. et al., A report on the B16-02 balloon experiment: Biopause-bioaerosol sampling at the stratosphere, JAXA Research and Development Report, JAXA-RR-17-007 (2018) 15-23.

Inertial impactor sampler

Fluorescence Microscope Images of the Collected Samples

Using fluorescence pigment (SYBR green) and fluorescence microscope, only the particles containing DNA show green light. We distinguished microbes from other types of particles. Using a fluorescence microscope, we identified 21 microbes on the impactor plate in the sampler.





70 cells m^{-3}

The fluorescent beads for positive control, painted on the external wall of the sampler before flight, were not detected. The contamination of the sampling process was not significant.

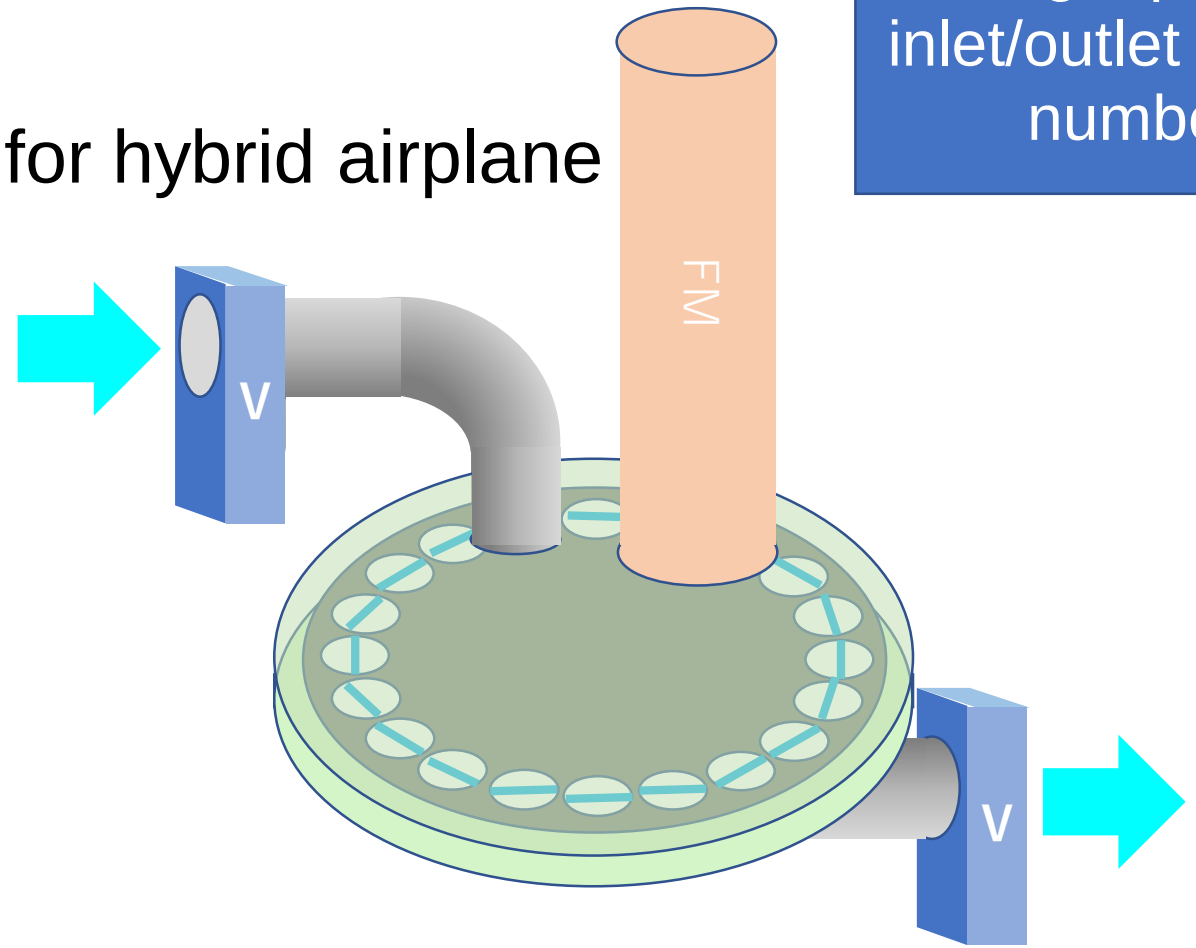
Ohno, S. et al., A report on the B16-02 balloon experiment: Biopause-bioaerosol sampling at the stratosphere, JAXA Research and Development Report, JAXA-RR-17-007 (2018) 15-23.

Inertial impactor sampler

Our idea

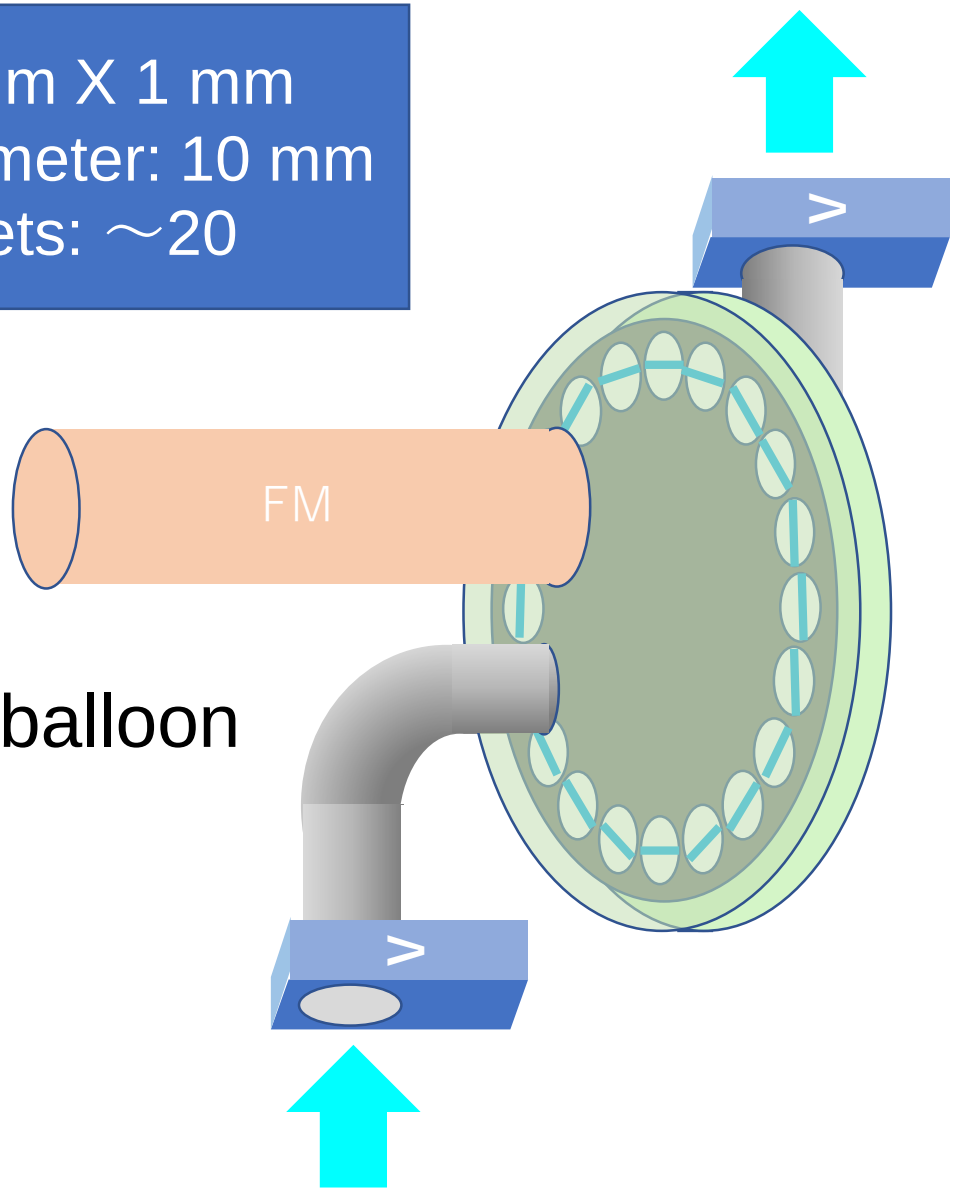
target plate: 10 mm X 1 mm
inlet/outlet inner diameter: 10 mm
number of targets: ～20

for hybrid airplane



electric power for 2 valves, turret,
and microscope is required

for balloon

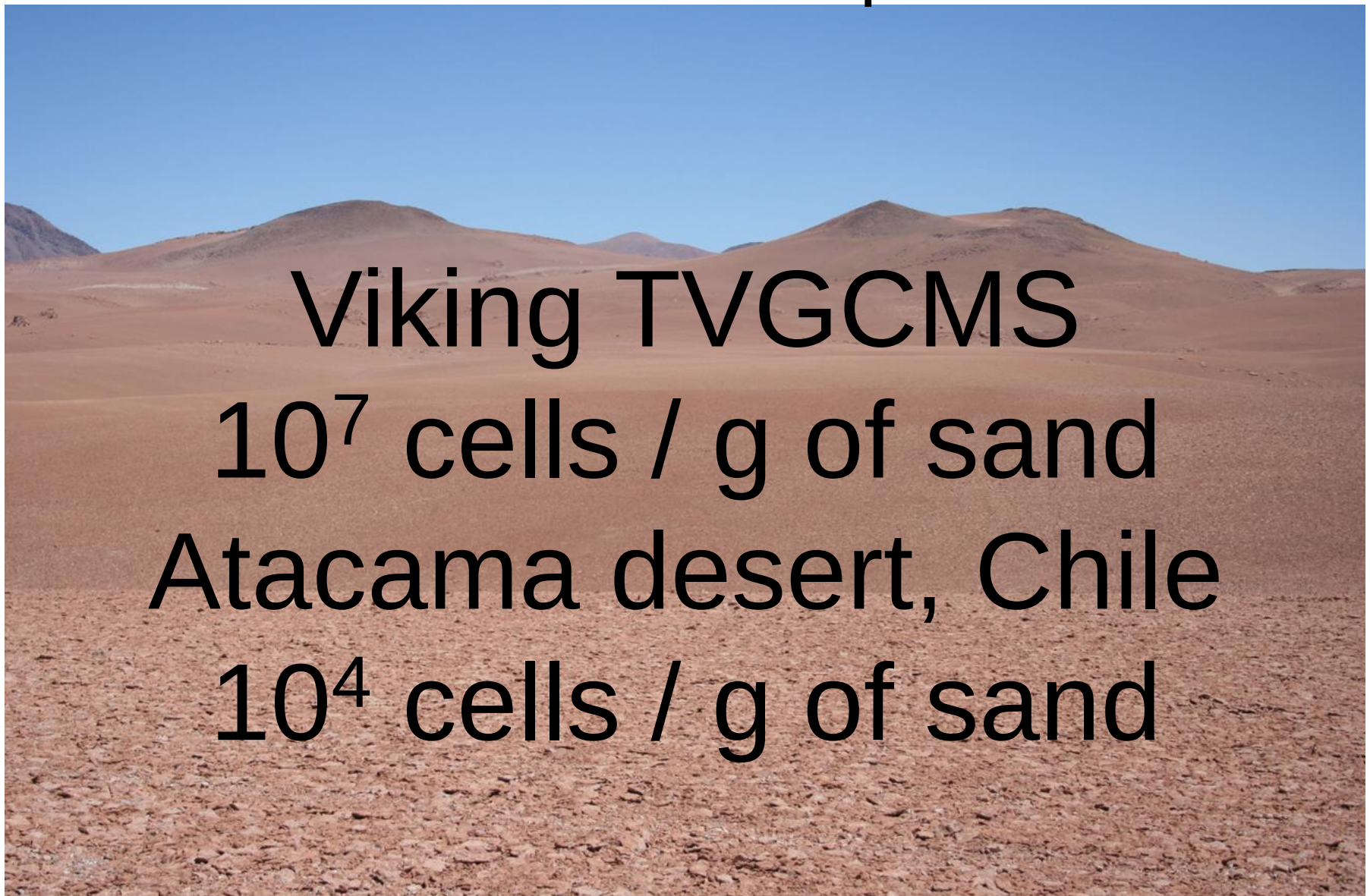


All equipped with air tight rotor cover / A. Yamagishi

What to see

Fluorescence microscope

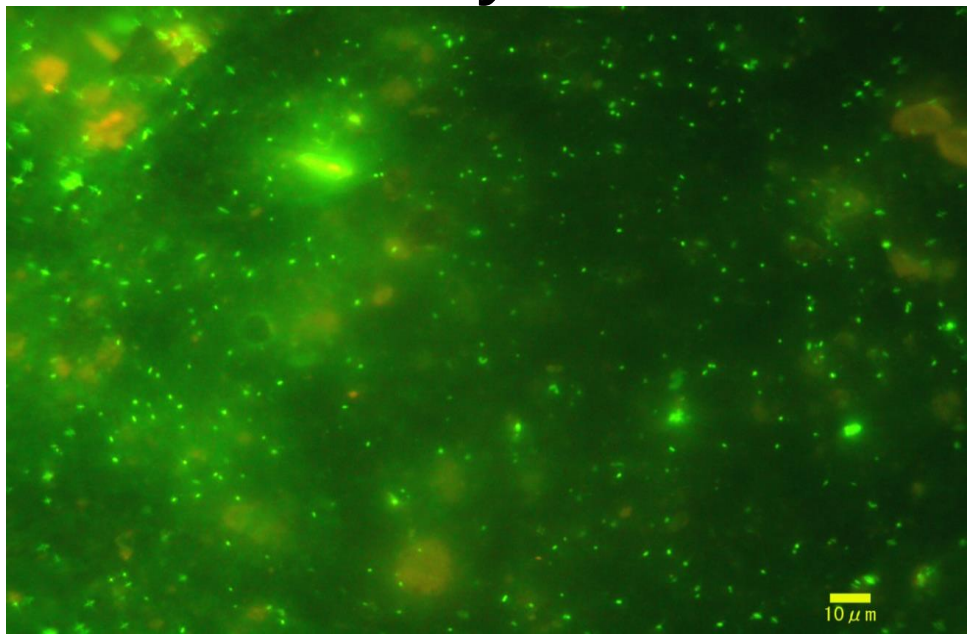
Detection limit of microscope



Fluorescence microscope

1. Widely used in environmental microbiology
2. Detect biomolecules such as DNA, proteins labelled with fluorescent dyes.
3. Various types of fluorescent dyes are commercially available

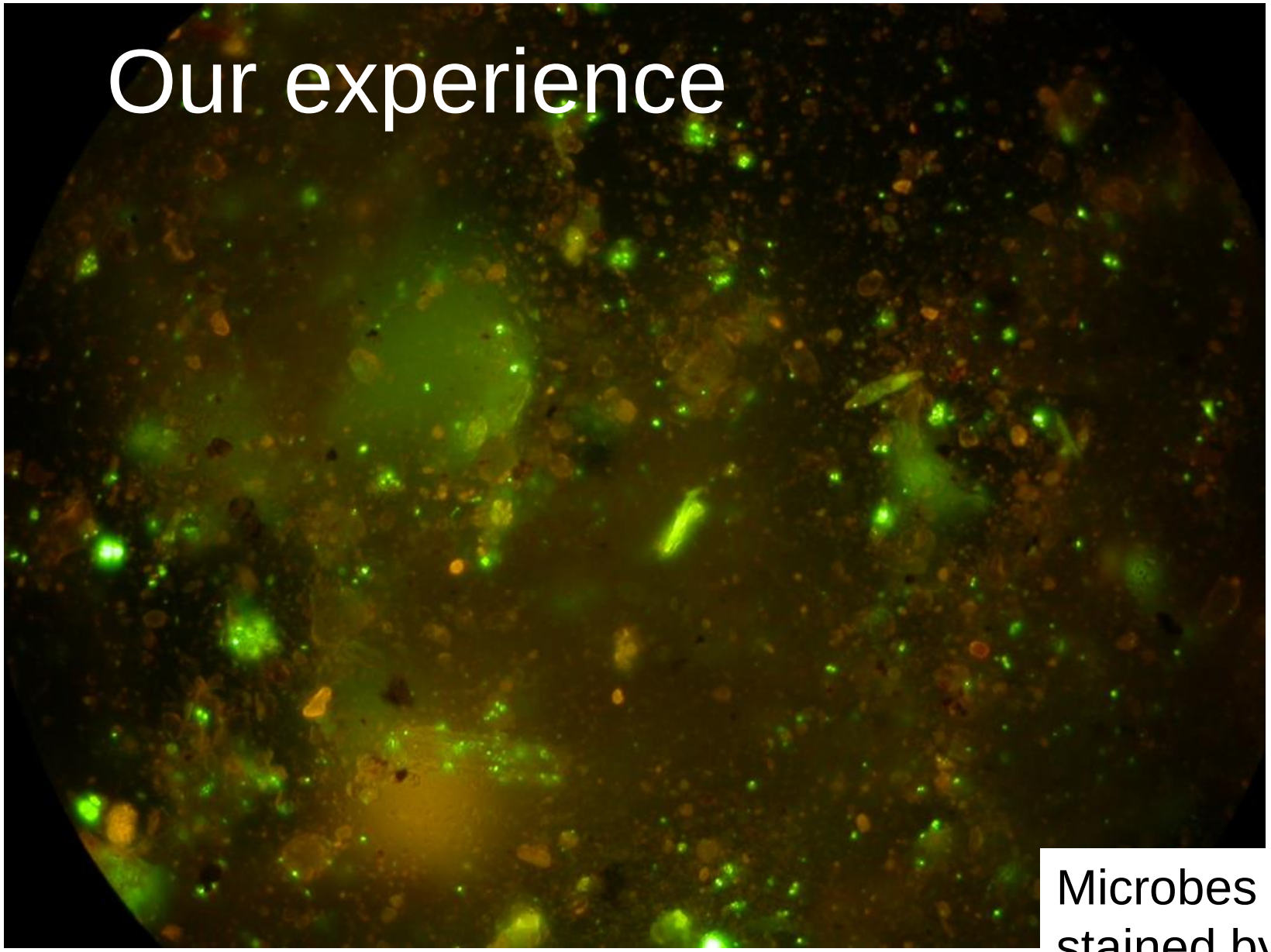
Using the combination of dyes, particles surrounded by membrane and catalytic activities can be imaged (possibly “cells”?)



Lower limit of detection of our LDM: less than 10^4 cells / g of clay

Yamagishi, A., et al., *Biol. Sci. Space,* **24** (2010), pp. 67-82.

Fluorescence microscope



Microbes in Atacama desert stained by SYBR Green

Fluorescence microscope

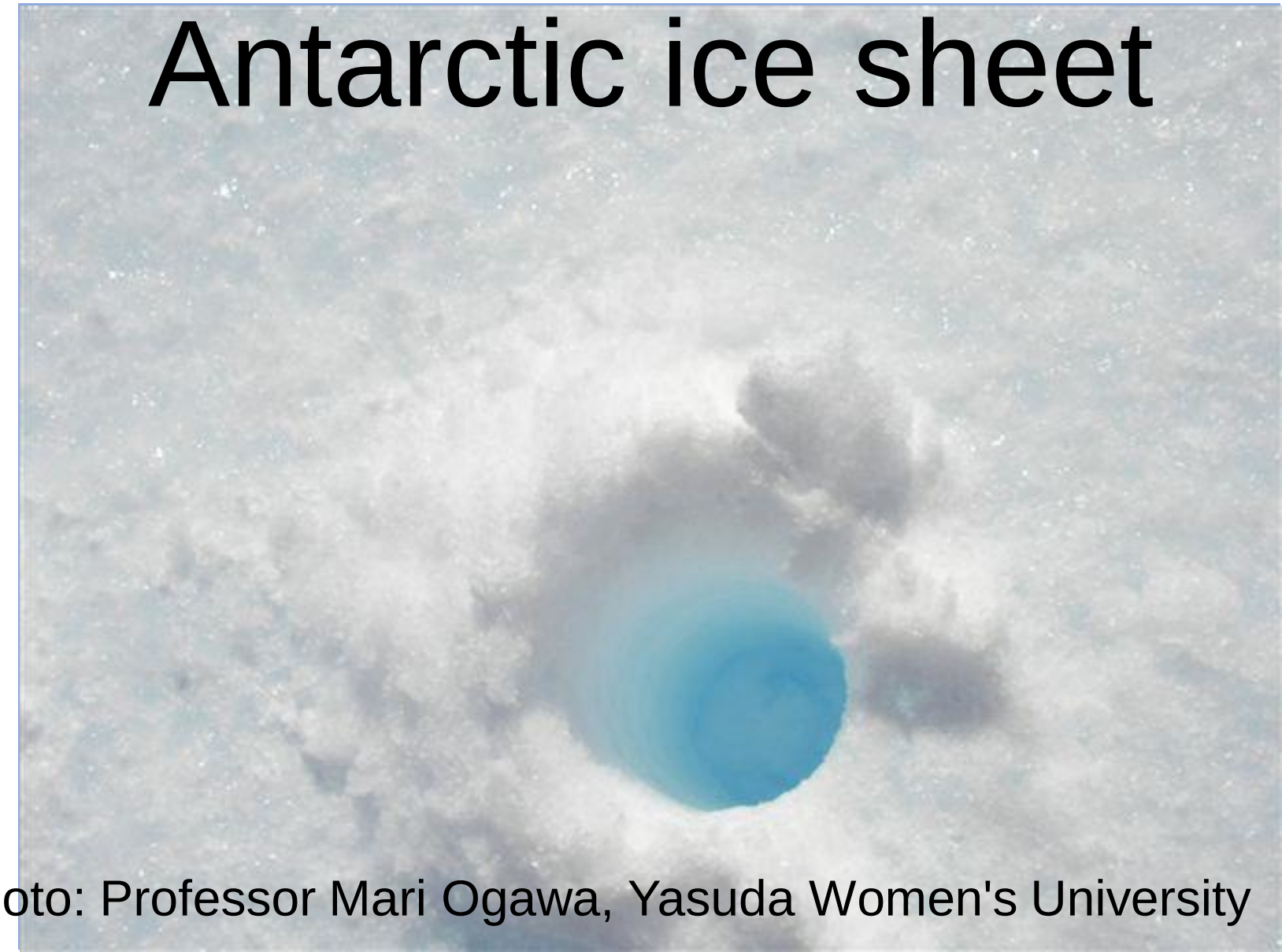
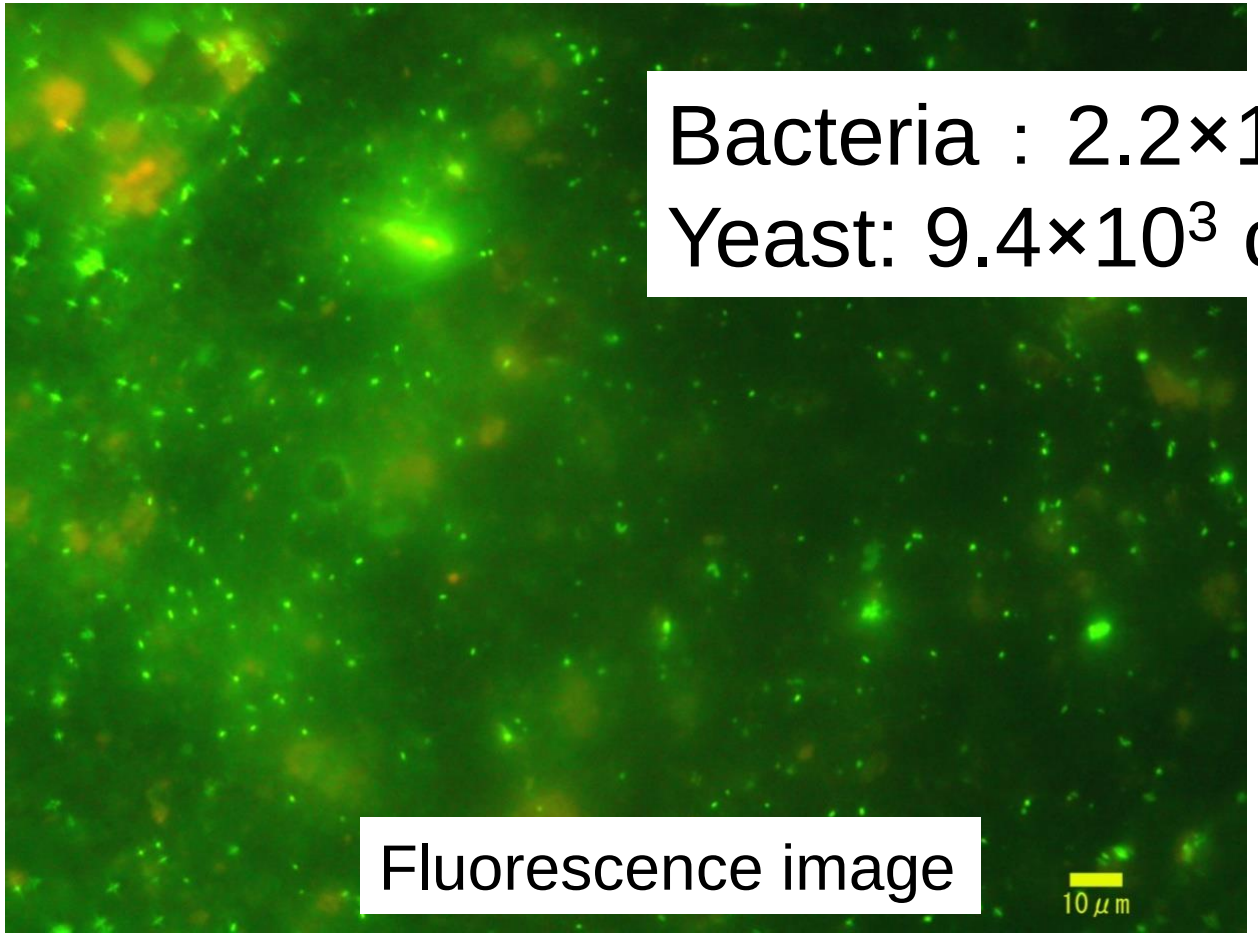


Photo: Professor Mari Ogawa, Yasuda Women's University

Microbes in Antarctic ice

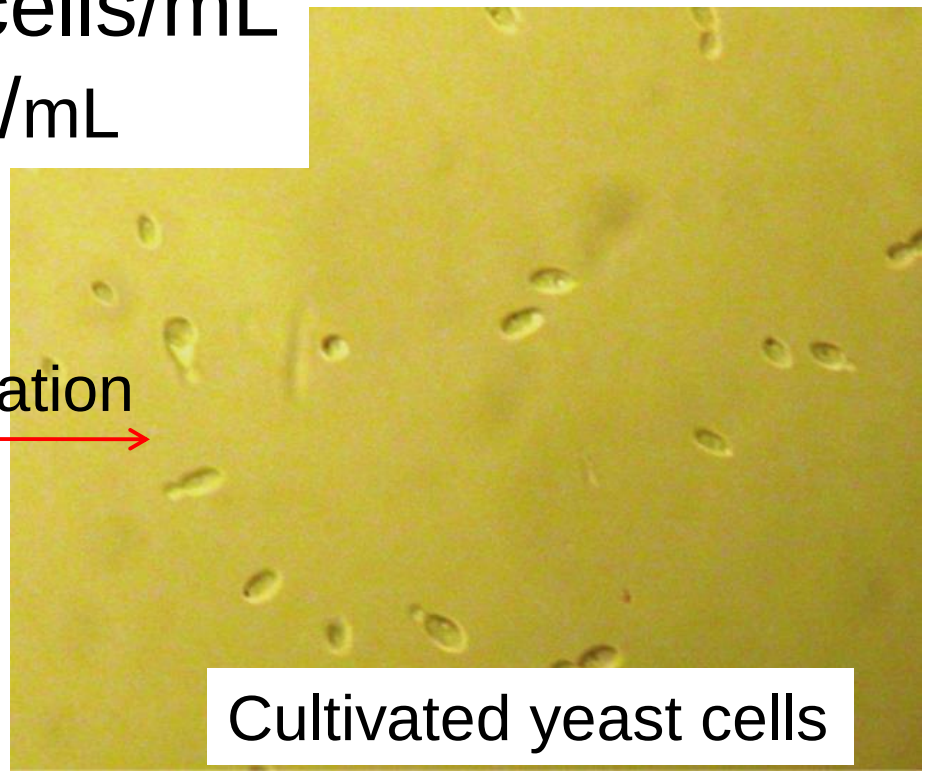


Bacteria : 2.2×10^5 cells/mL

Yeast: 9.4×10^3 cells/mL

Fluorescence image

cultivation →



Cultivated yeast cells

Microorganisms stained by SYBR GOLD

Fluorescence microscope

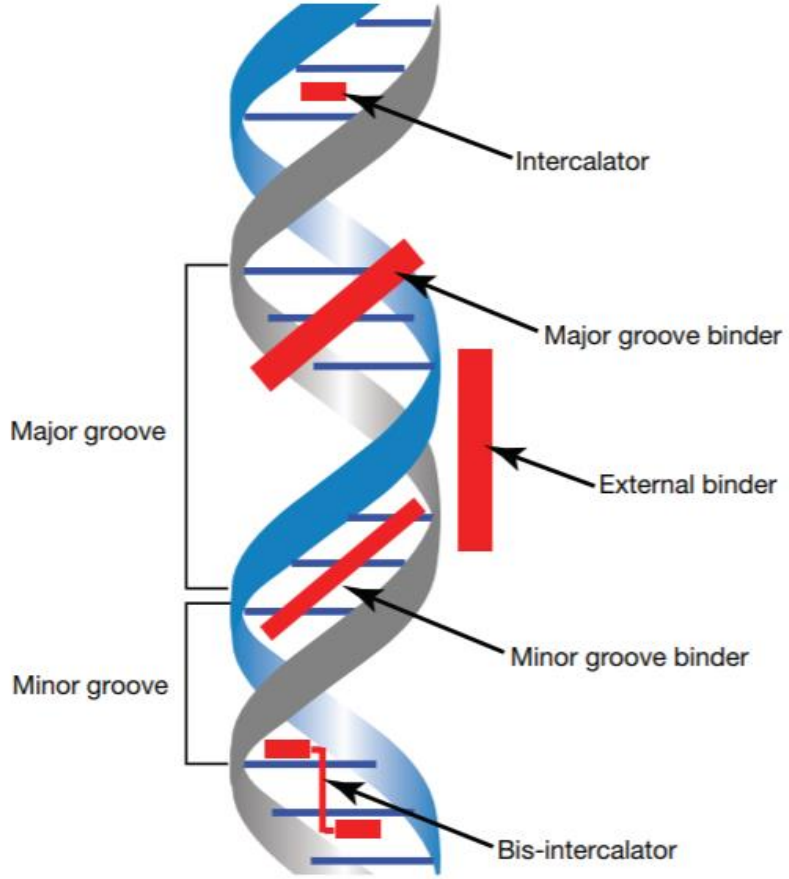


Figure 8.1.1 Schematic diagram showing the different binding modes of dyes (and other ligands) to DNA.

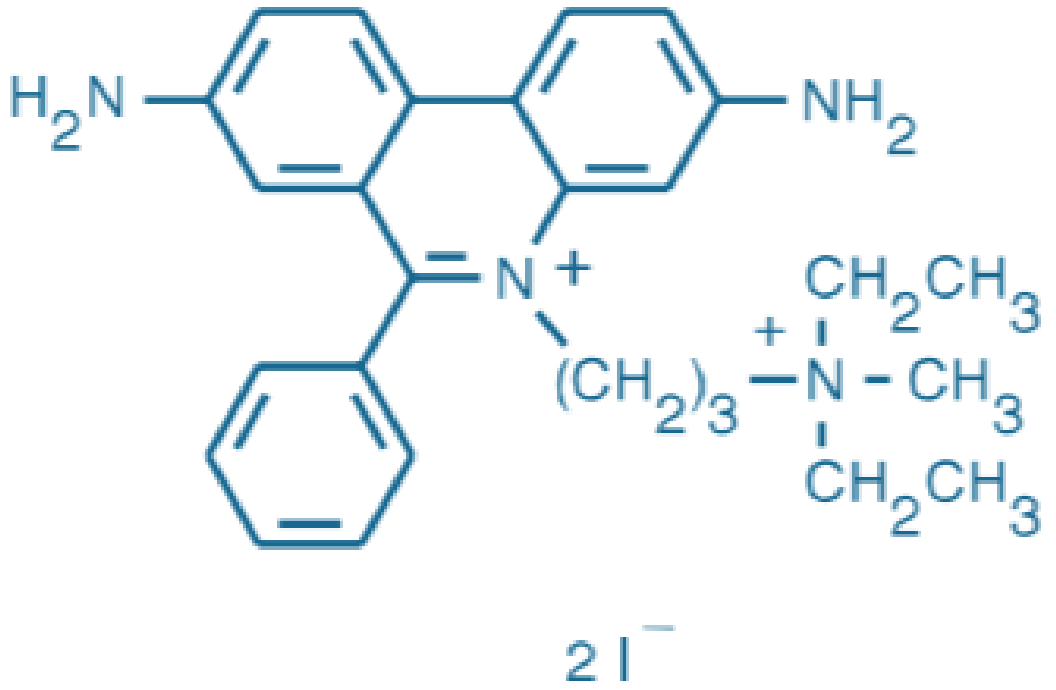
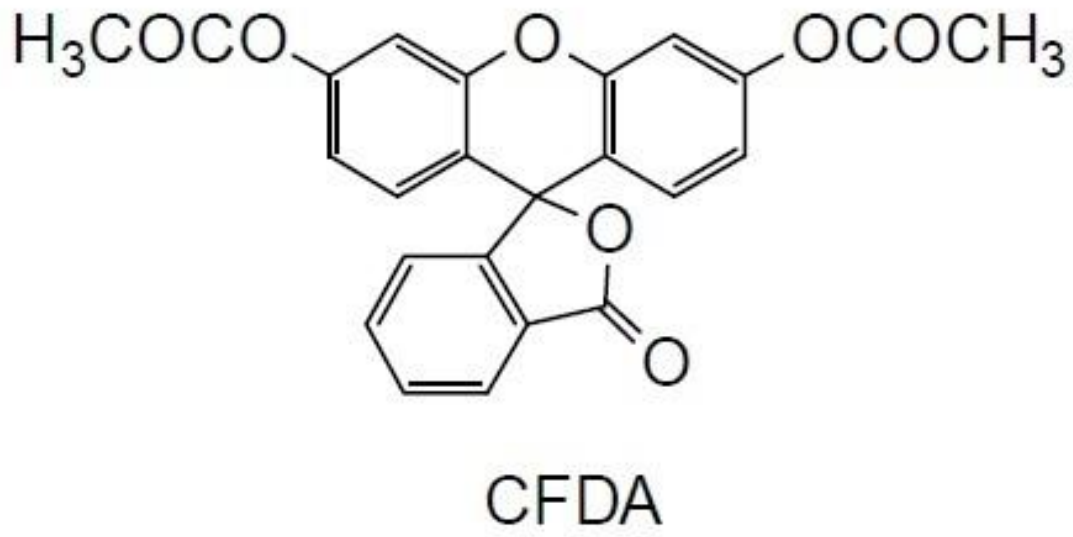


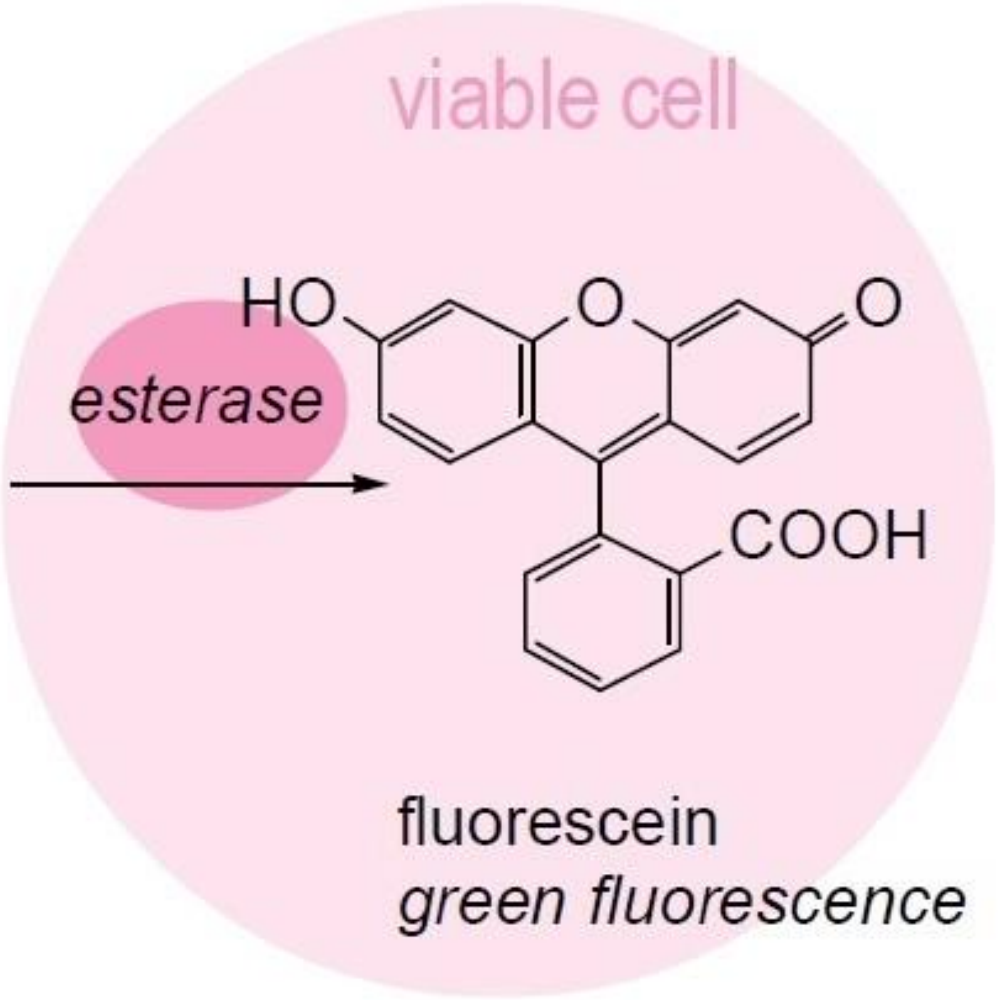
Figure 8.1.20 Propidium iodide (P1304MP).

https://www.thermofisher.com/content/dam/LifeTech/global/technical-reference-library/Molecular%20Probes%20Handbook/chapter-pdfs/Ch-8-Nucleic-Acid-Detection-Analysis.pdf?icid=WE216841

Fluorescence microscope



CFDA

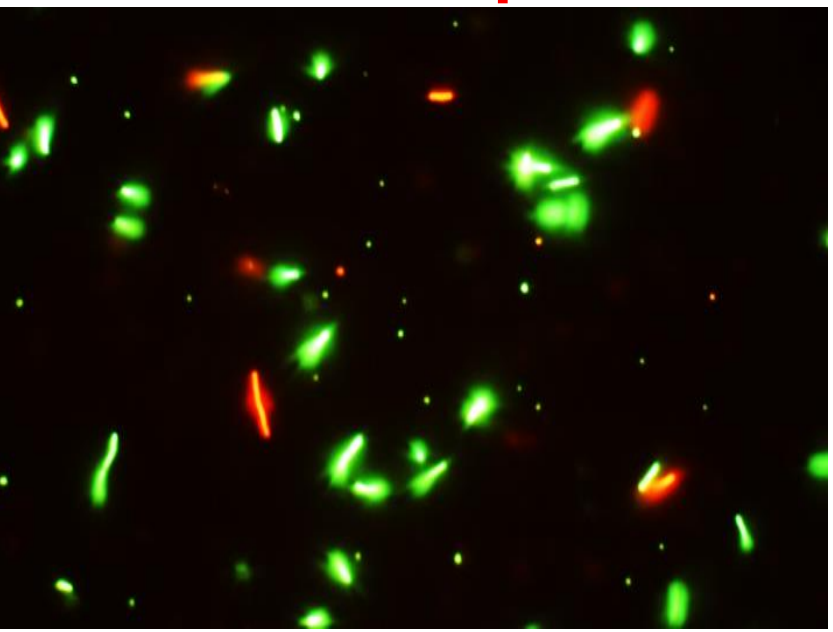


https://www.dojindo.com/store/p/149-Bacstain-CFDA-Solution.html

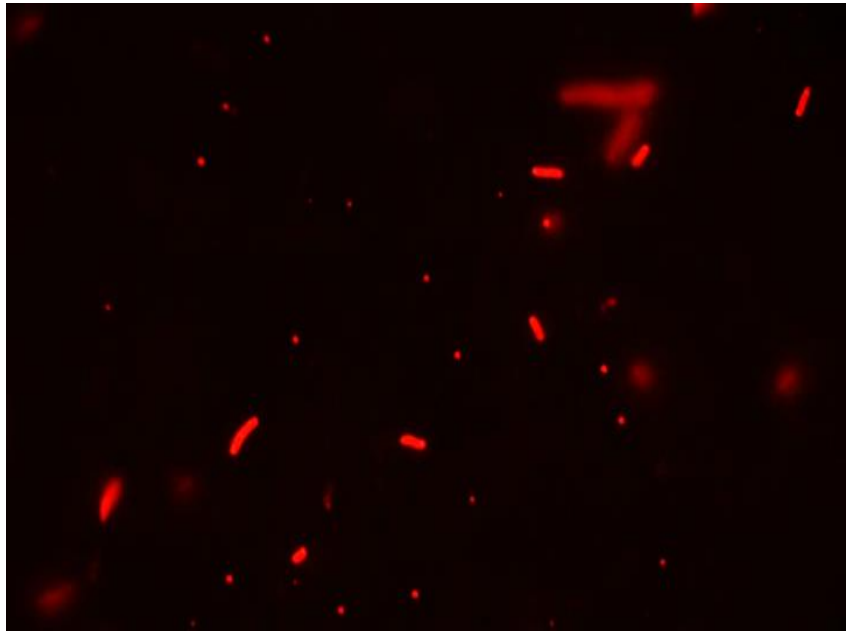
Fluorescence microscope

Fluorescent dyes

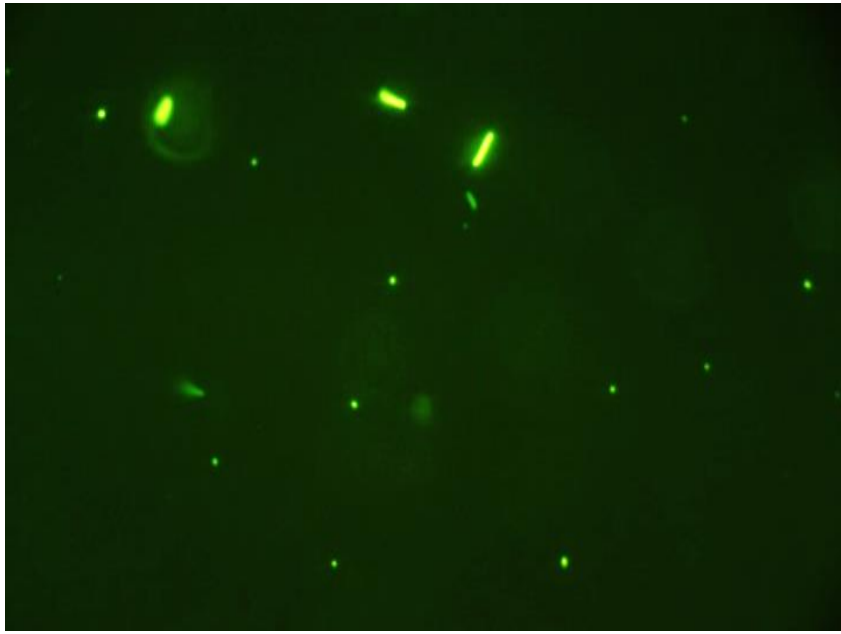
Membrane structures ～
live / dead
SYTO24 & Propidium Iodide



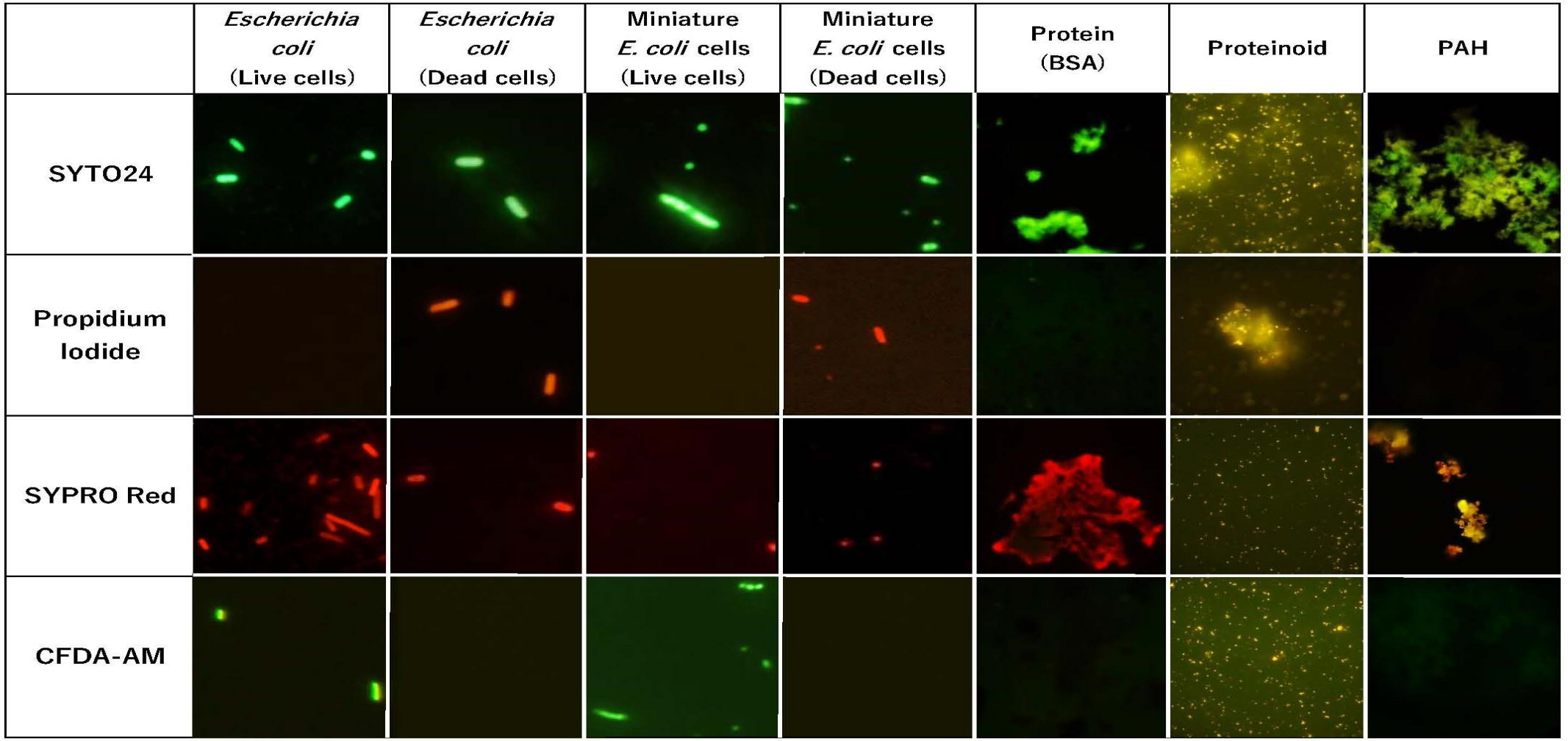
Organic compounds
SYPRO Red



Catalytic activities
CFDA-AM



Fluorescence microscope



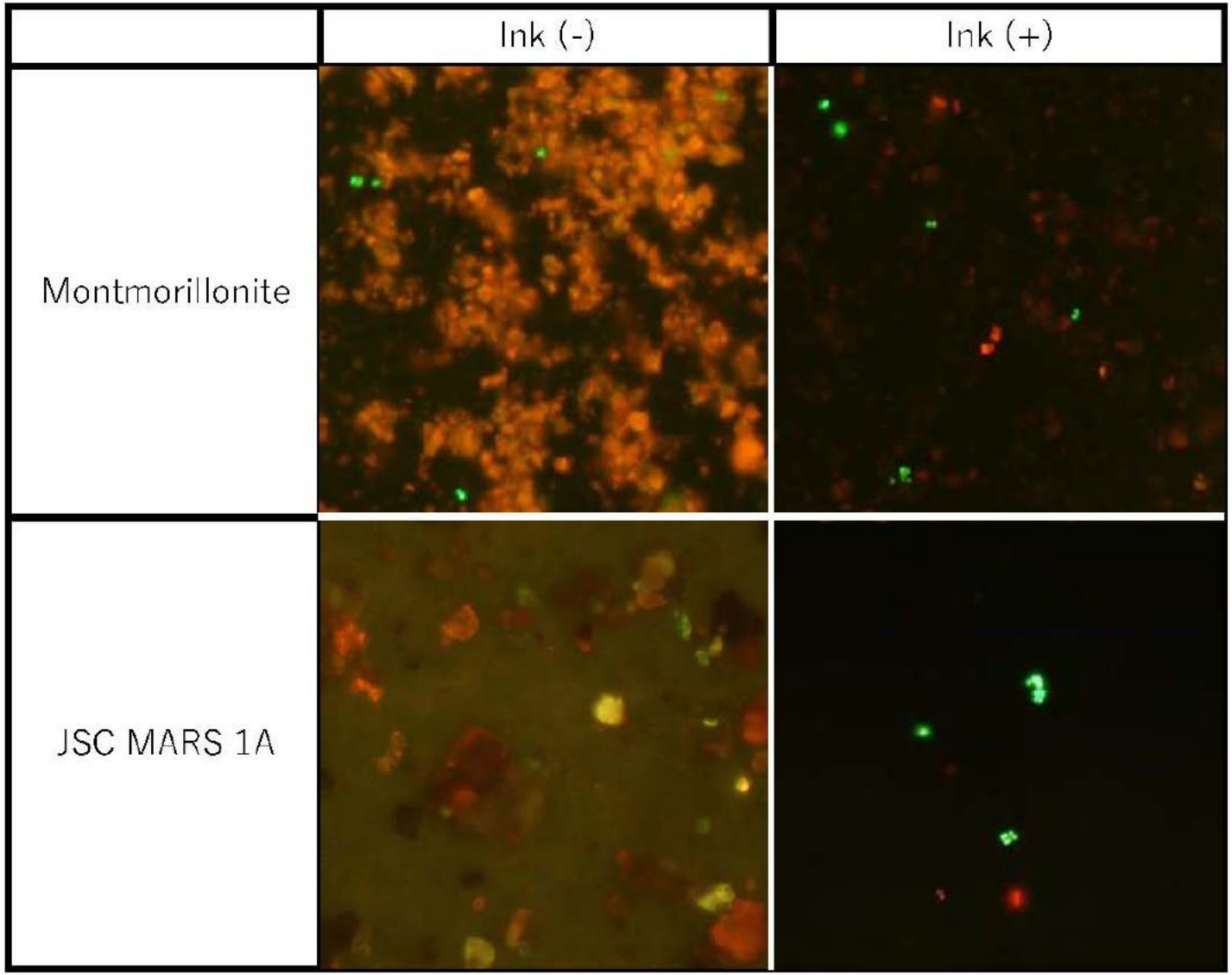
Microbes, miniature cells, proteins, proteinoid, polycyclic aromatic hydrocarbons (PAH) can be detected.

Fluorescent microscope

	Live cells (membrane with selectivity)	Dead cells (membrane w/o selectivity)	Protein (certain molecule)	PAH (certain molecule)
(1) SYTO24	○	○	○	○
(2)Propidium iodide		○		
(3) SYPRO Red	○	○	○	○
(4) CFDA-AM	○			

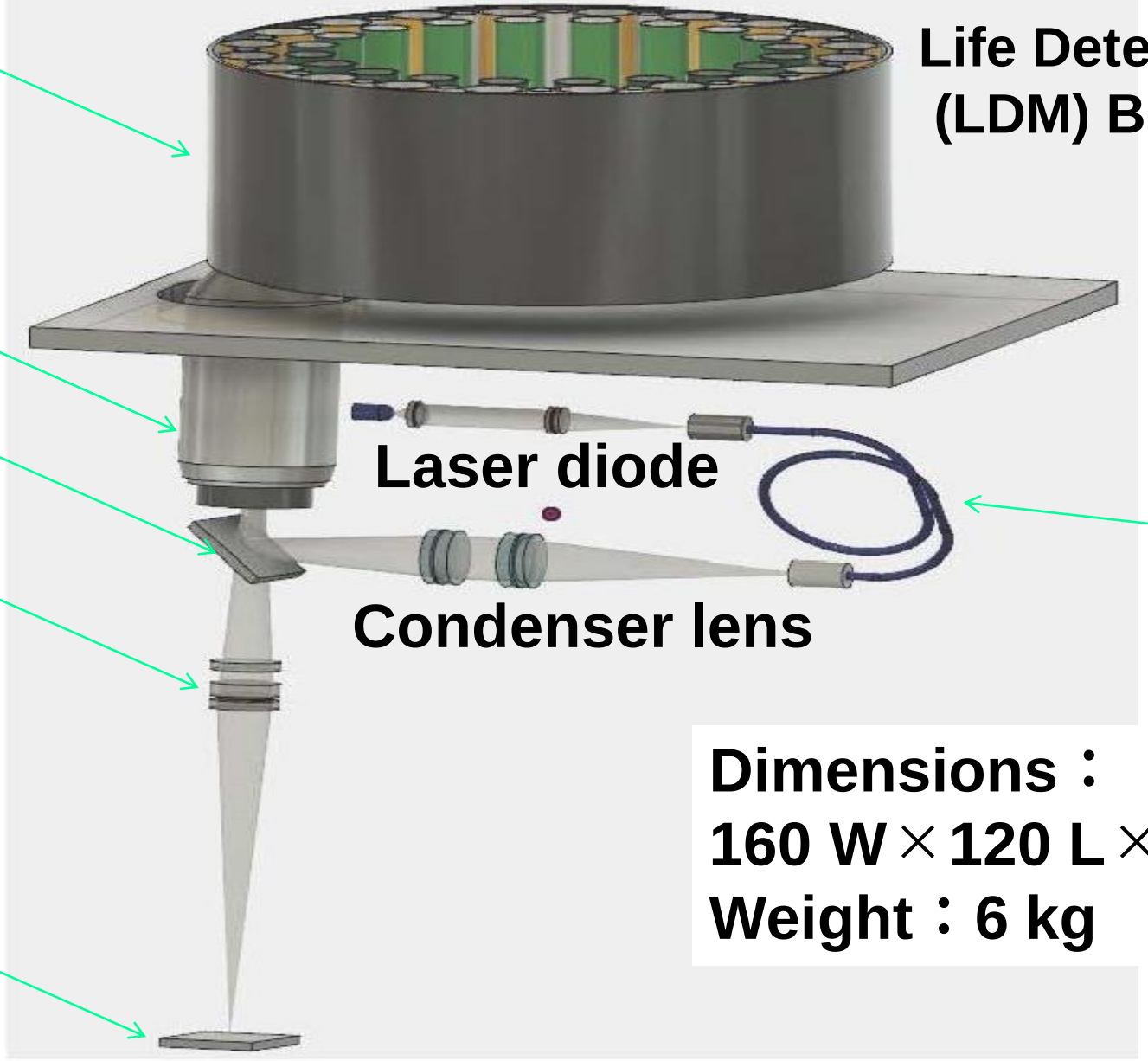
Fluorescence microscope

Fales positive signal can be reduced by black ink.



A. Yamagishi et al., Trans. JSASS Aerospace Tech. Japan
Vol. 16, No. 3, pp. 299-305, 2018

Fluorescence microscope



Dimensions：
160 W × 120 L × 240 H (mm),
Weight：6 kg

A. Yamagishi et al., Trans. JSASS Aerospace Tech. Japan Vol. 16, No. 3, pp. 299-305, 2018

Our focus

■Design of turret impactor sampler to fit the microscope (S. Ohno)

■Development of tiny microscope for space application (K. Enya)

■Search for the effective fluorescent dyes

20～ dyes were tried for *E. coli* suspension in 75% H_2SO_4.

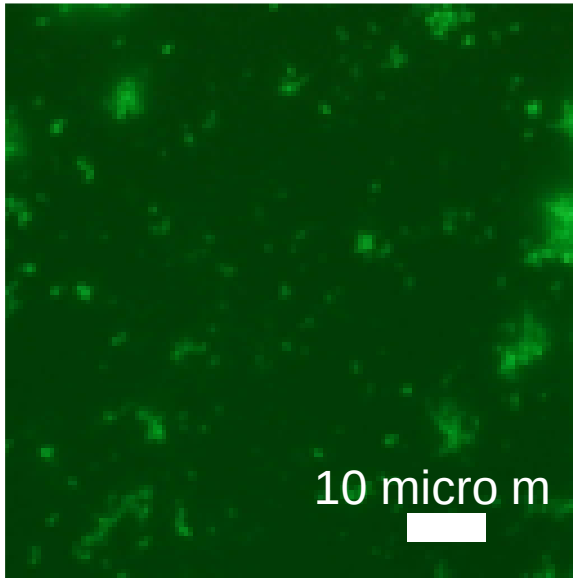
4-5 dyes were effective for cell imaging (what information?)

■Search for the model microbe (S. Sasaki)

Thermoplasma acidophilum (was too difficult for me!)

Sulfolobus, Thiobacillus ferrooxidans (will be my friends)

5 Mega pixels
USB powered
～100 g



S. Sasaki, *in prep*

Oct 10 (India), Oct 31 (Russia), early 2020 (USA, Poland, Austria)

https://www.danshihack.com/2019/07/22/junp/tenki-no-ko-2.html