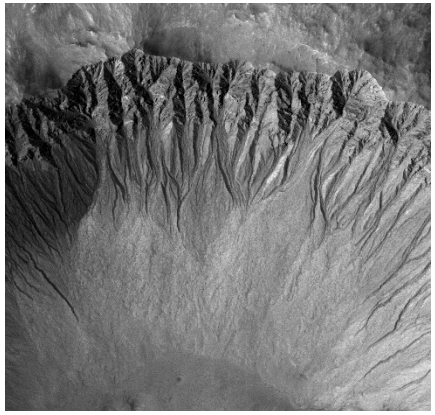
2021年10月11日（月）　14:40-15:00

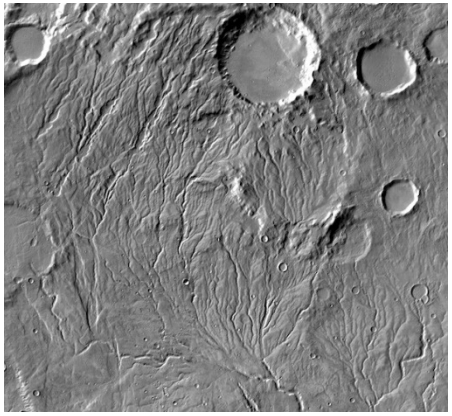
火星での生命兆候探査のための、物体検知法の検討・開発

吉村義隆（玉川大学）、
奥平恭子、遠藤真輝、矢次勇葵、出村裕英（会津大学）

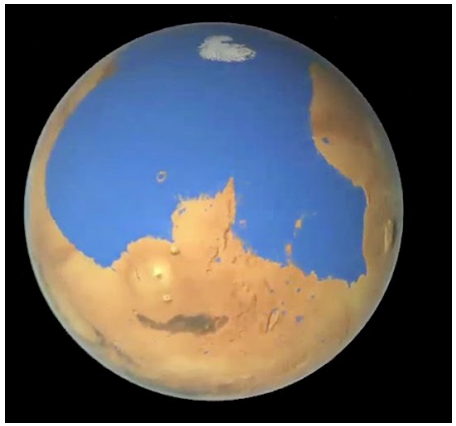
Past water activities and habitable environments



Gullies



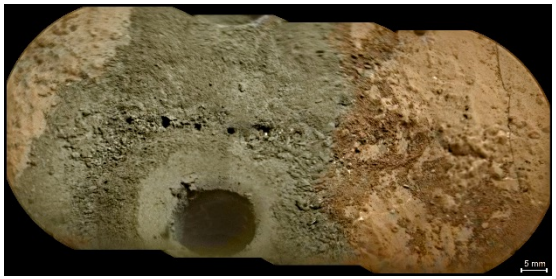
Valley networks



Ancient Mars

NASA/JPL

MSL Curiosity rover also found ancient aqueous environments at Gale Crater:



- Neutral pH
- Low salinity
- Microbial energy sources
- Biogenic elements (C, H, N, O, P)

J. P. Grotzinger, et al. *Science*, 2014

Life could have appeared and flourished on ancient Mars

Microorganisms could be extant?

Limits for terrestrial microorganisms versus conditions on Mars surface

Factor	Limits for terrestrial microorganisms	Mars surface
Temperature	-20～122℃(Metabolism) -18℃（Cell division）	-130～20℃
Pressure	700 Pa (Cell division)	600～800 Pa (6/1000 of the Earth's)
pH	-0.06～12.5	7.7±0.5
Ionizing radiation	1,440 Gy day^{-1}	0.4 mGy day^{-1}
Redox potential	Limits undefined (tolerant or use perchlorate)	Highly oxidizing (0.6% perchlorate)
UV radiation	～5,000 J m^{-2}	20 W m^{-2}

UV radiation is shielded by thin soil layers.

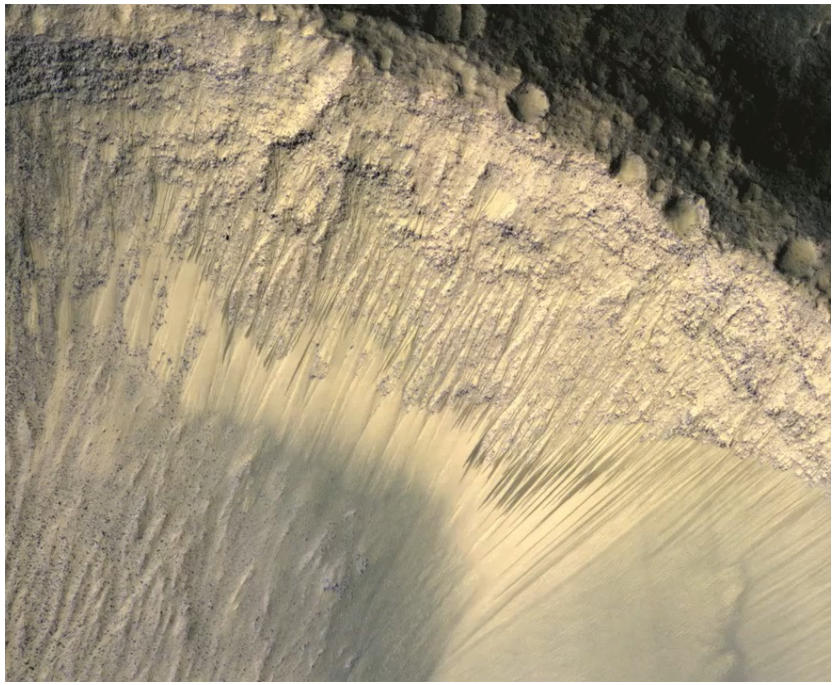
Microorganisms could be found near the surface at present

Potential energy sources

Electron donors (Energy sources)	Electron acceptor	Microbes
H_2	CO_2	Methanogen
H_2	ClO_4^-, NO_3^-, $Fe(OH)_3$, SO_4^{2-}, etc.	Hydrogen oxidizing microbes
CH_4	NO_3^-, MnO_2, $Fe(OH)_3$, SO_4^{2-}, etc.	Methane oxidizing microbes
Fe (II)-sulfides	NO_3^-, MnO_2, etc.	Iron oxidizing microbes
S^0	ClO_4^-, NO_3^-, MnO_2, etc.	Sulfur oxidizing microbes

(Adapted from Rummel et al. 2014; Cockell 2014; Westall et al. 2015)

Potential presence of liquid water



NASA / JPL-Caltech / University of Arizona.

Water or sand flow ?
at the slope of craters
appears in spring and summer
(Recurring Slope Lineae, RSL).

Recurring slope lineae (RSL); narrow dark streaks on steep slopes. Although the formation process of RSL remains unclear. If liquid water exists, it would be saltwater, which lowers the freezing point and keeps in a liquid state in Martian environments.

Significance of searching life on Mars

- If life is found, we will have another example of life form.
- Comparing Martian life and terrestrial life may reveal what is essential for the birth and evolution of life.
  - DNA, RNA, amino acids, proteins etc.
- Evaluate the risk of human contact with extraterrestrial life in future manned exploration.
  - If living microbes is found, it must be done carefully since it may be harmful to humans.
  - If not found, it would be safe.

We will find living microbes on Mars

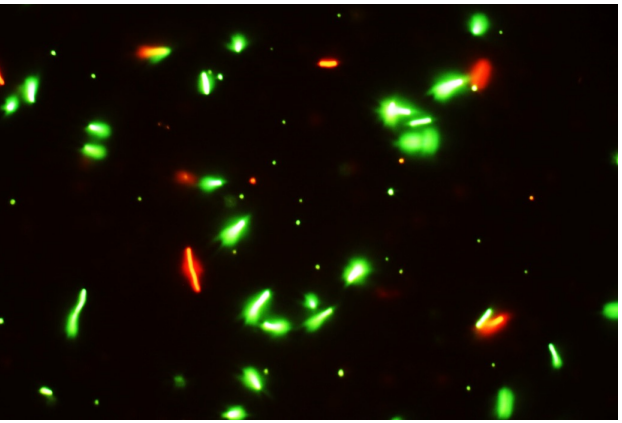
- What is life?
  1. Life utilizes carbon-based organic compounds such as amino acids, proteins, nucleic acids, lipids etc.
  2. Life is separated by an envelope like a cell membrane to reduce the influence of outer environments.
  3. Life possesses metabolic activities by catalysts like enzymes to make organic compounds and energy.

The fundamental framework of life is a “cell”.

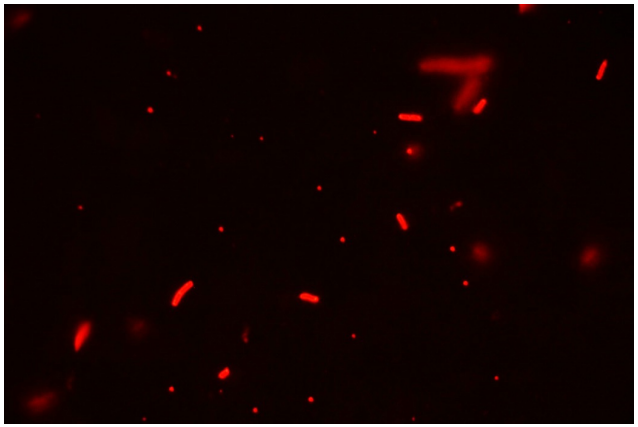
Life-signature Detection Microscope (LDM)

1. Based on a fluorescence microscope.
2. Detect organic compounds, membranes structures, and catalytic activities stained by fluorescent pigments.
3. Identify cell-like structure in micron scale.

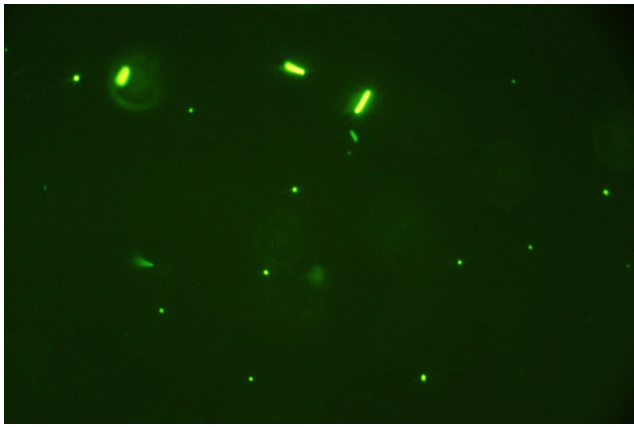
SYTO24 & Propidium Iodide



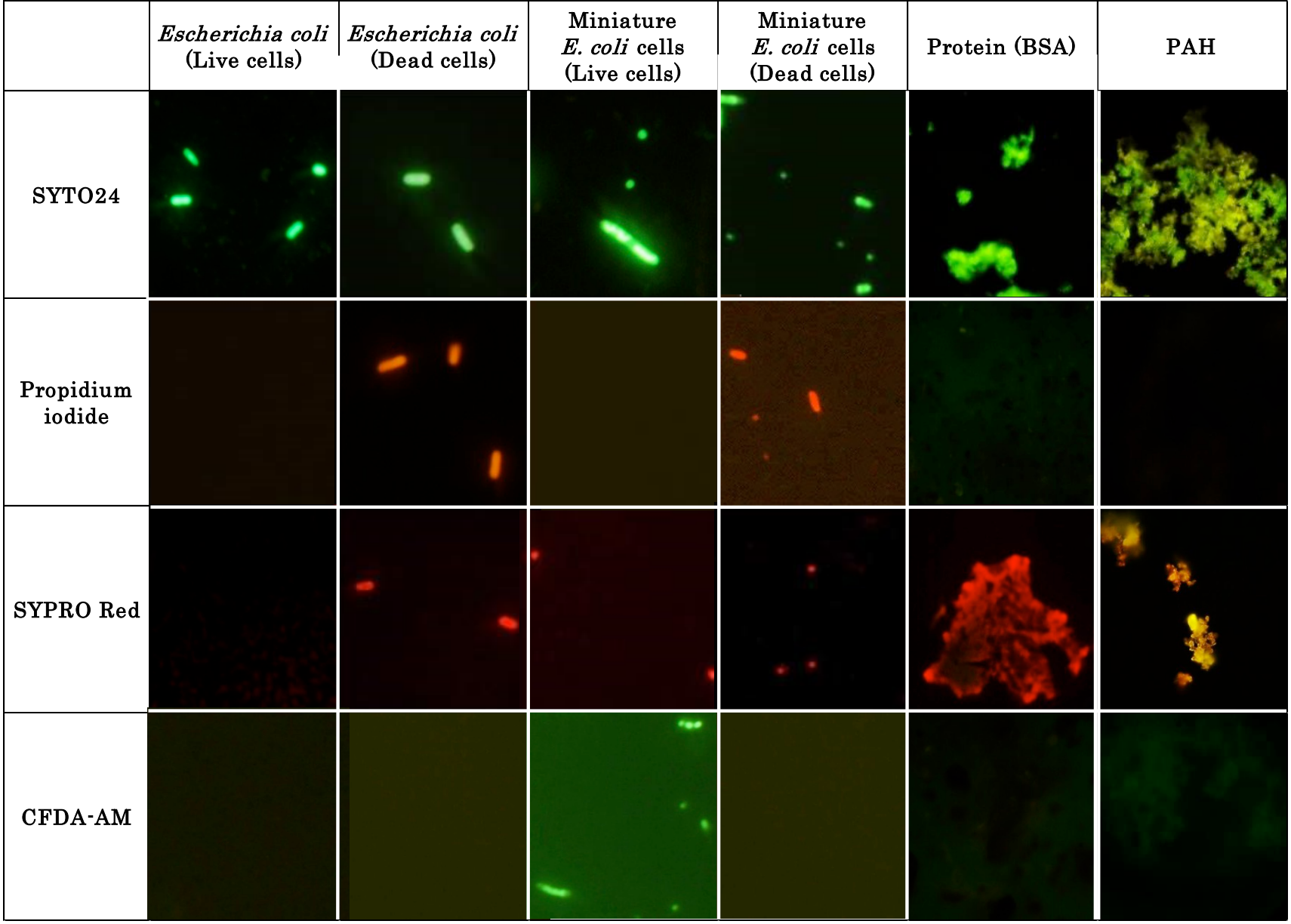
SYPRO Red



CFDA-AM

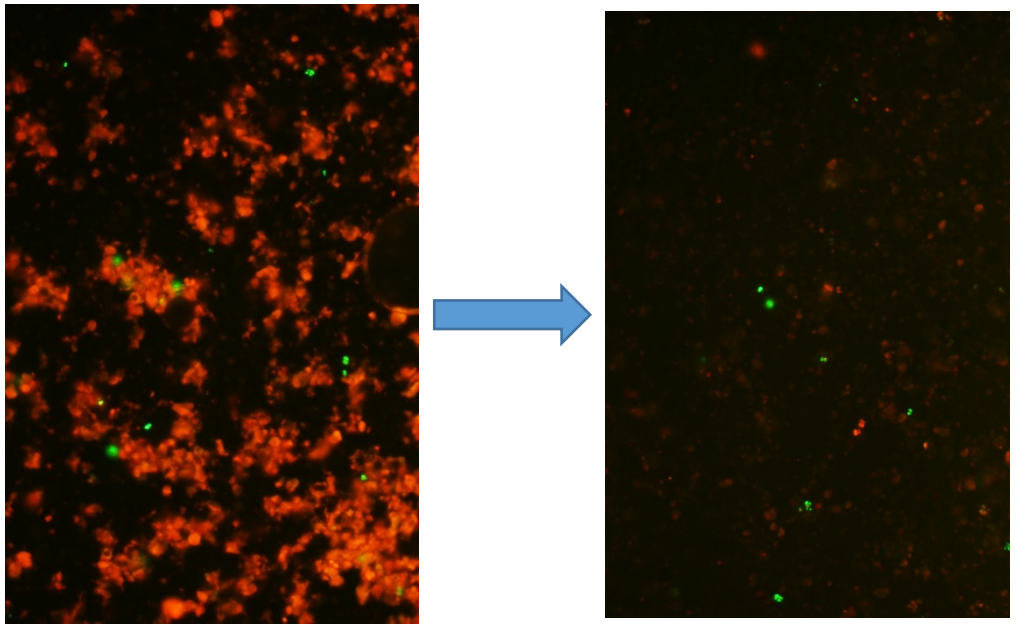


適切な蛍光色素を用いると、生菌は緑に、死菌は赤に発光する

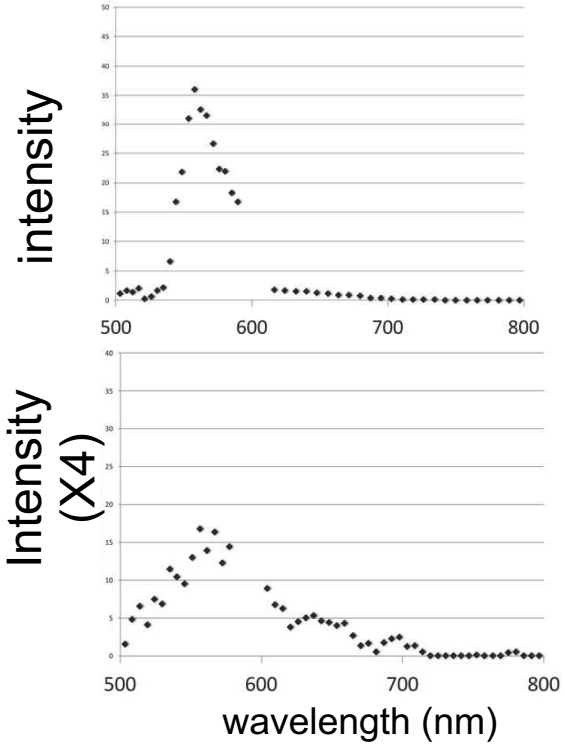


Microbes, miniature cells, proteins, polycyclic aromatic hydrocarbons (PAHs) can be detected.

Possible false positive signals



Deinococcus radiodurans in Montmorillonite stained by SYTO 24 and PI.



Spectrum of SYTO 24 (a) and a mineral particle (b).

Nonspecific binding of pigments

→ Masking detergents

Autofluorescence of mineral particles

→ Difference of Spectrum, fluorescent intensity

etc.

Characteristics of LDM

1. Imaging system

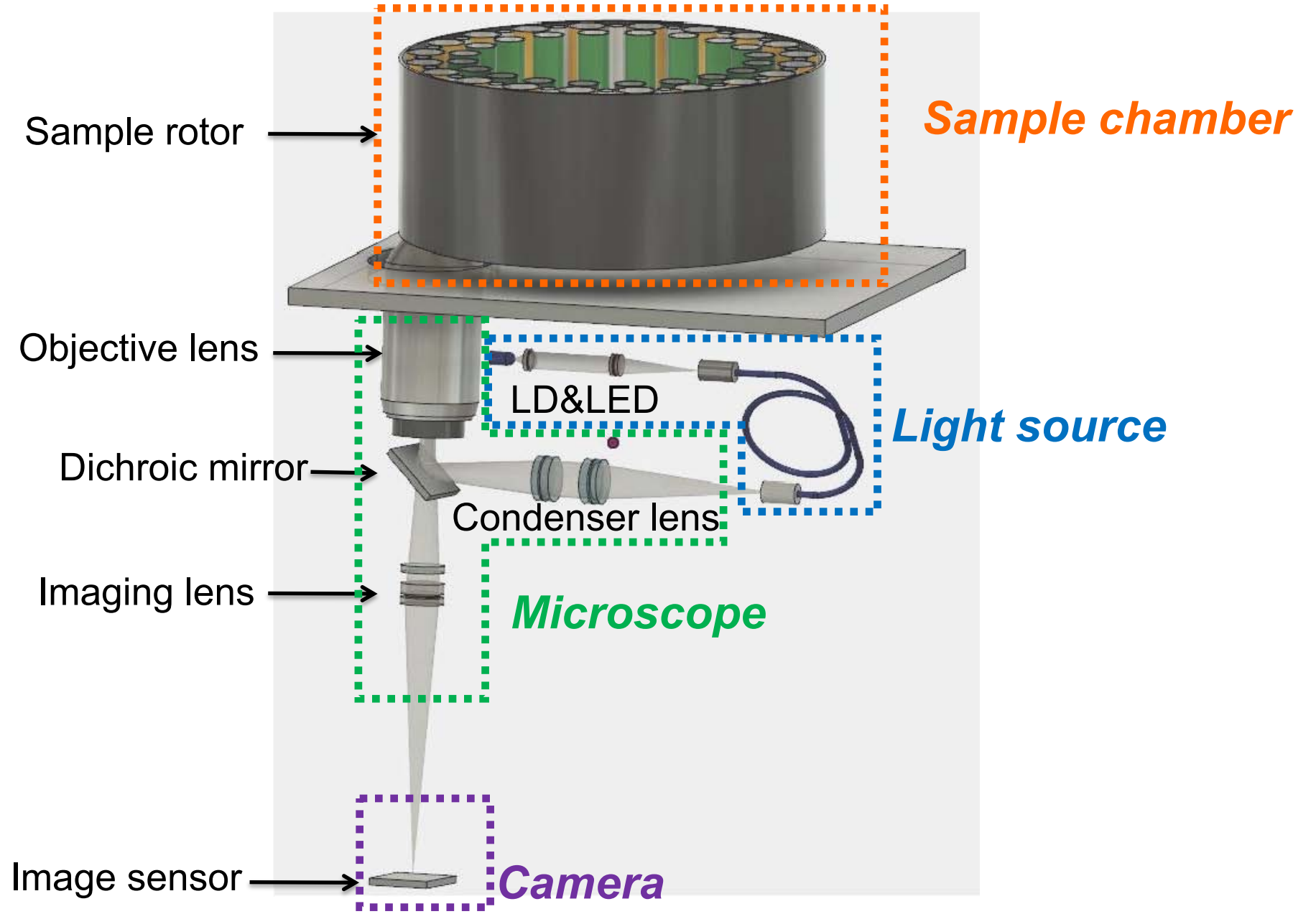
   1 μm/pixel that is 10 times higher than optical imagers used in space, to visualize cell forms and other particles.

2. Sensitivity

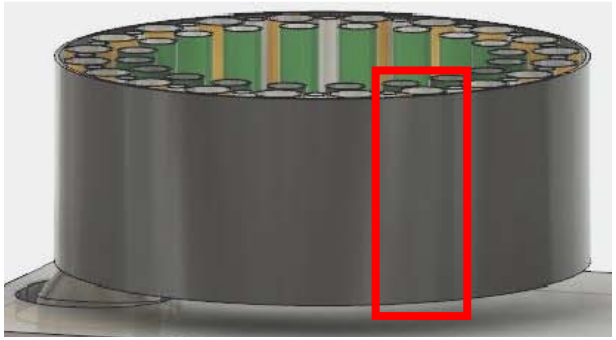
   Detection limit: 10^4 cells/g soil,

   that is comparable to the least populated area of terrestrial environments on Earth.

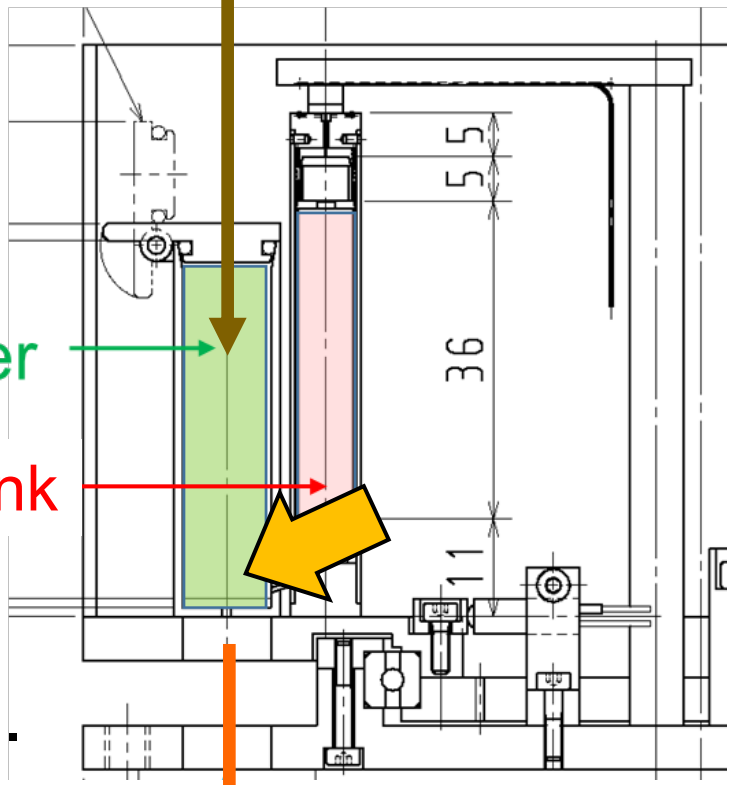
The bread board model (BBM)



The sample chamber



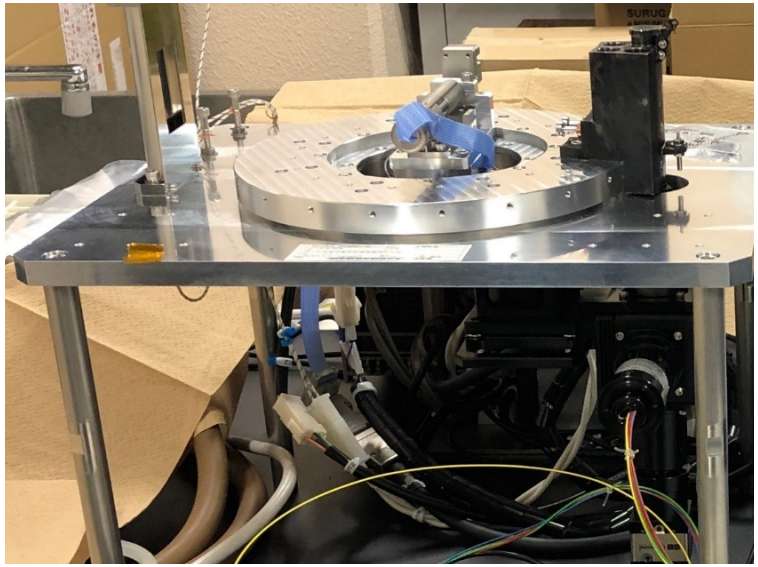
A sample unit

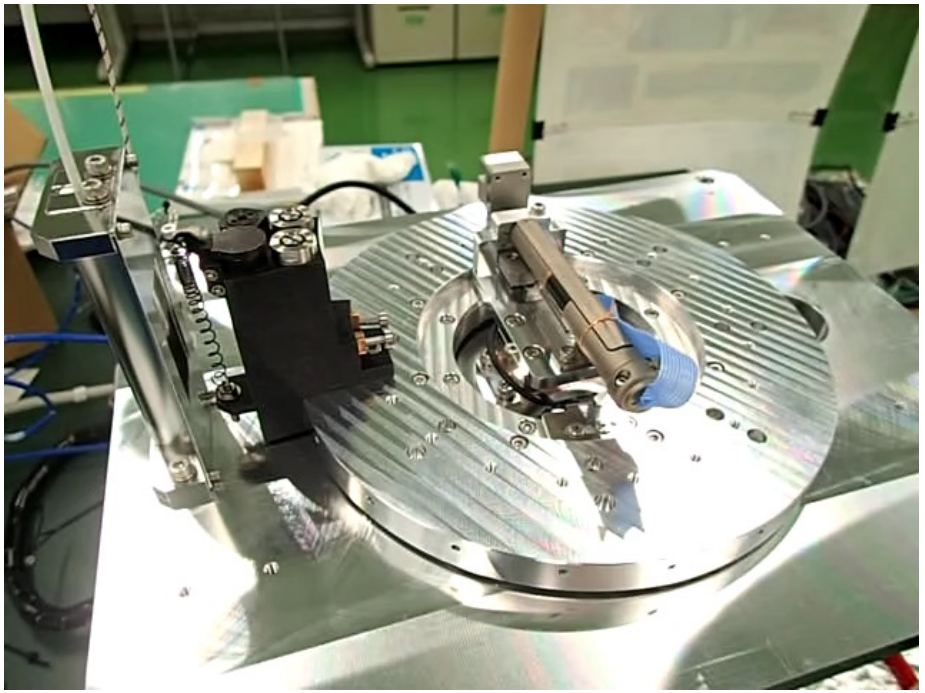


20 sample units can be attached.
One unit have a sample holder,
2 pigment solution tanks, a filter unit.

Observed volume: about 1 mm^3

The bread board model

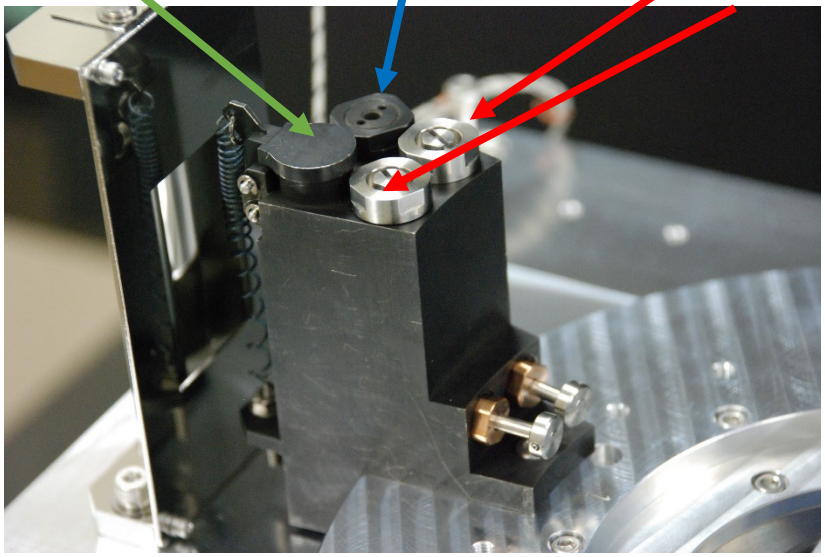


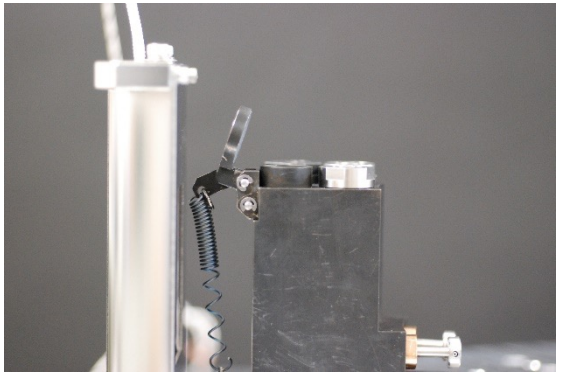


Sample holder

Filter unit

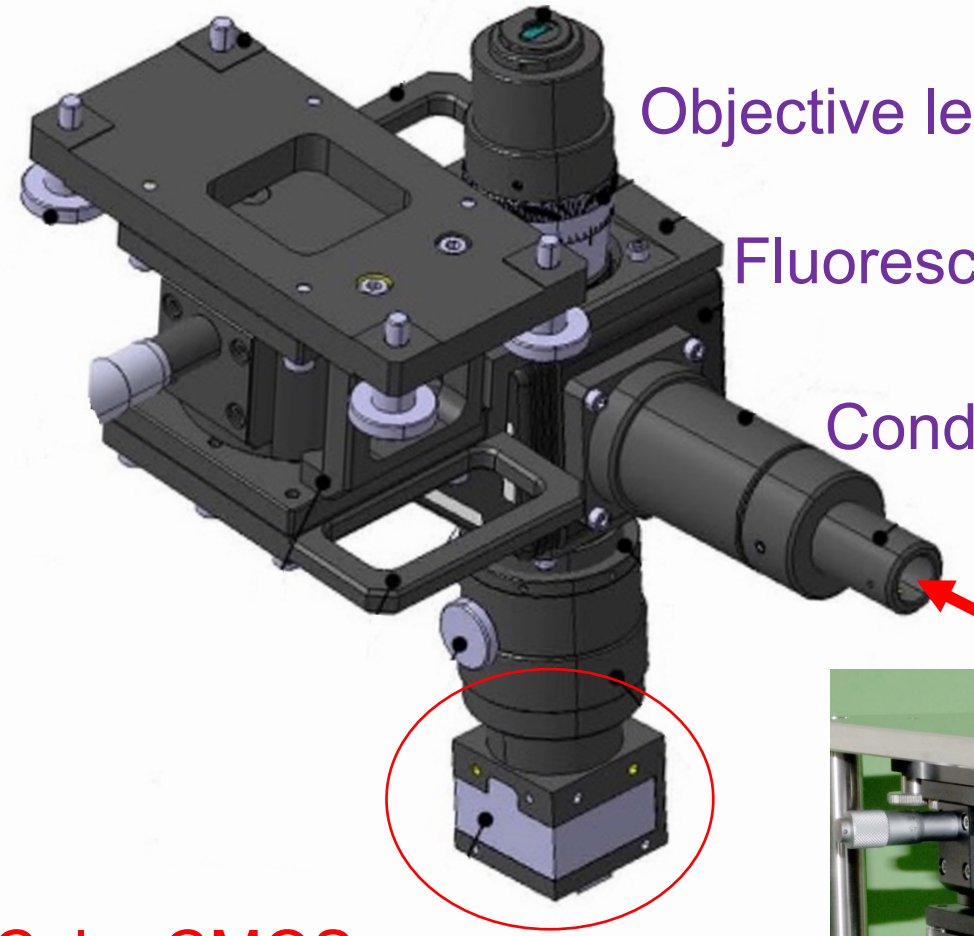
Pigment solution tanks

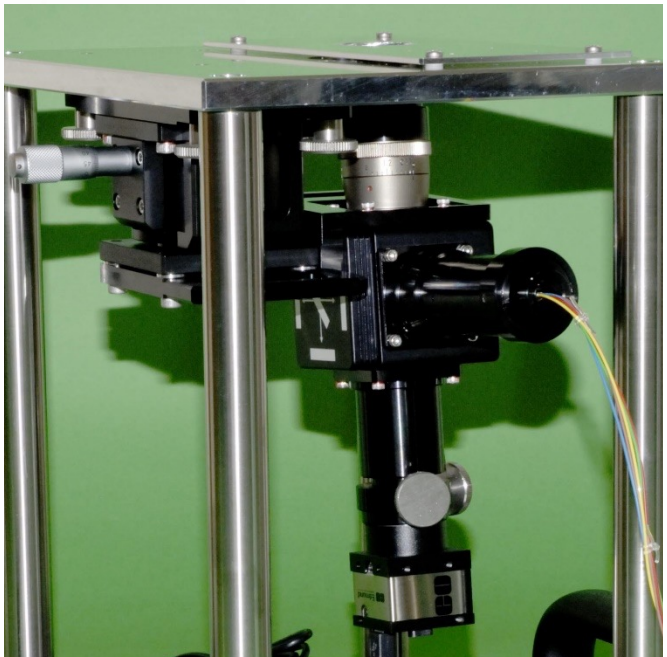




Sample holder

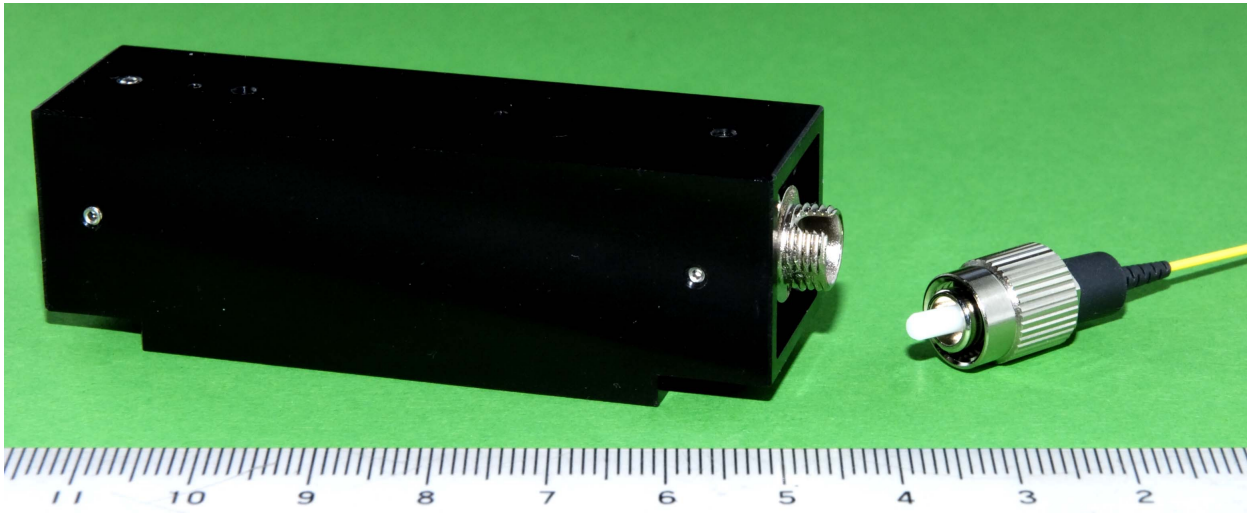
Microscope and camera





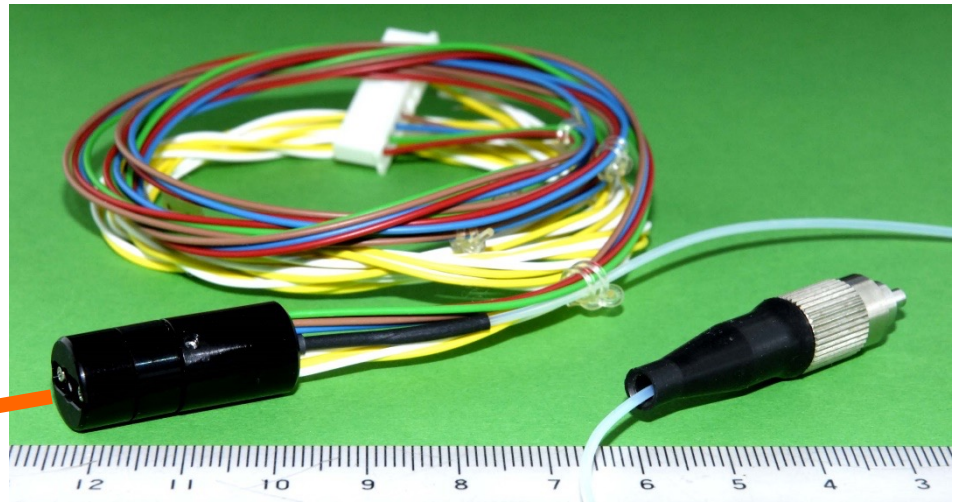
Weight: 1.7 kg

Light source



Weight: 83.0 g

Light box containing a laser diode (488 nm) for fluorescent imaging



Microscope

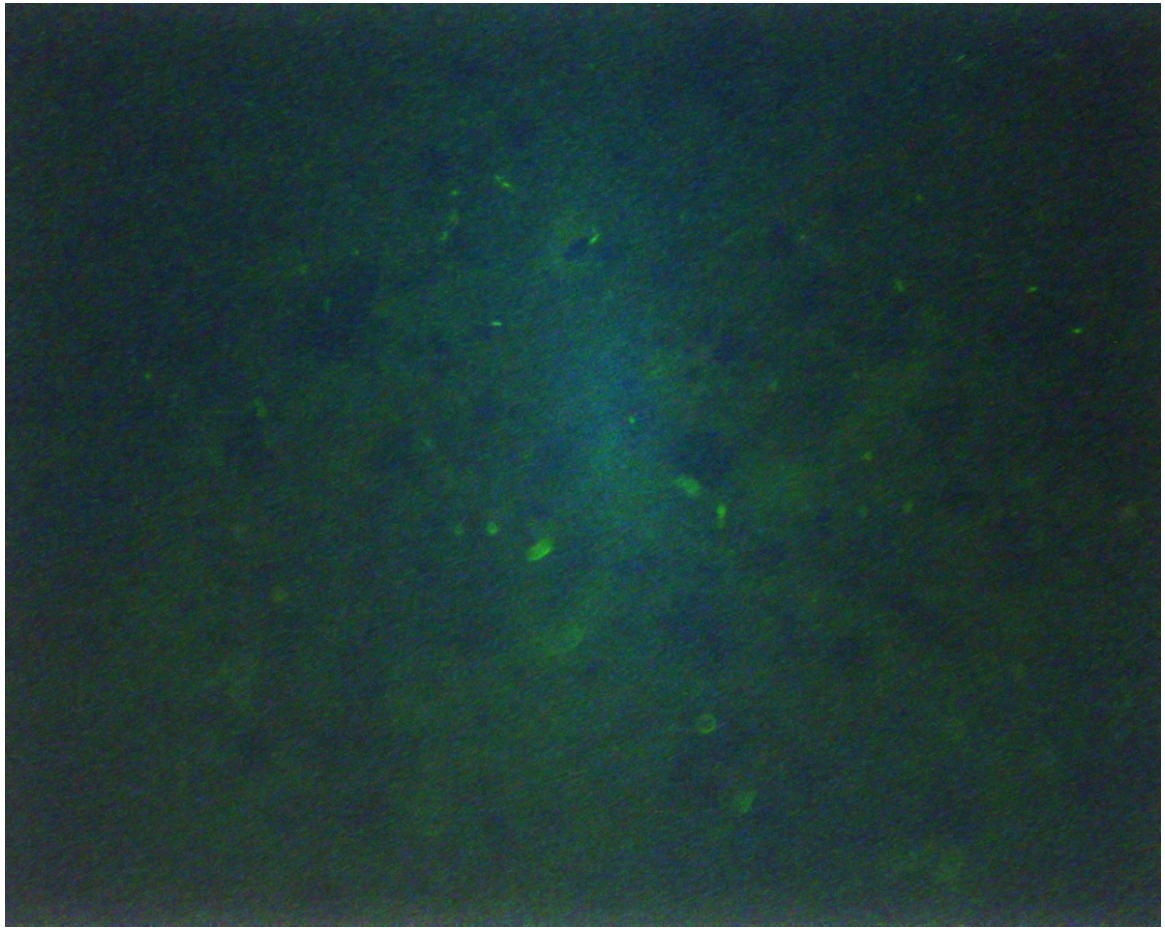
Three colored LEDs for bright field imaging are set at the end of optical fiber.

Conclusions

1. Life-signature Detection Microscope (LDM) is an instrument to search biosignatures of life, especially living microbes on Mars, based on fluorescence microscopy.
2. Biosignatures detected by LDM are organic compounds, membrane structures, and catalytic activities, which are characteristics of “cells”.
3. The bread board model of the LDM has been completed.

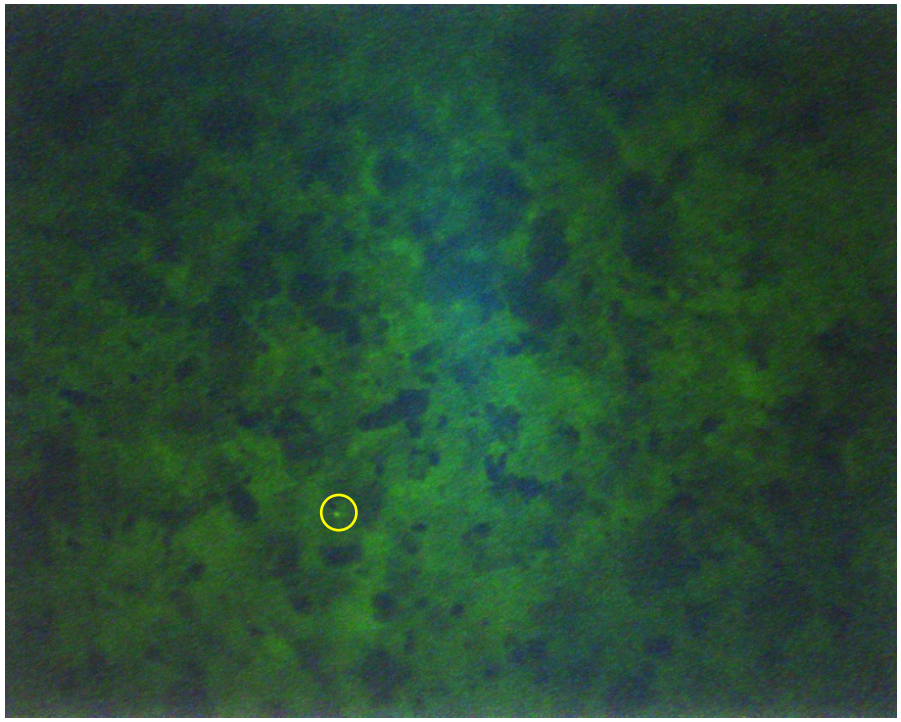
微生物撮像試験（BBM使用）

火星模擬土（MGS-1）に、バクテリア（*Bacillus subtilis*）を添加し、蛍光色素（CFDA-AM）で染色

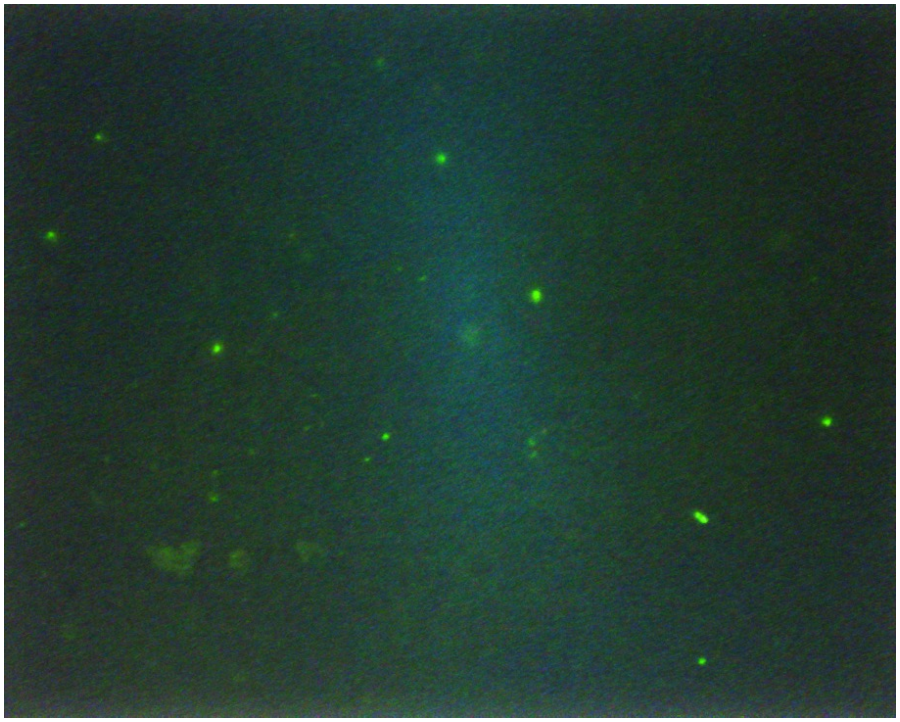


視野範囲：1.12mm × 0.894mm
画素数：1280 × 1024 pixel
撮像サイズ：0.87 μm/pixel
分解能：3 μm
輝度分解能：8 bit
励起波長：488 nm

Imaging and sensitivity tests



Sample holder



Filter unit

Viable bacteria (*Bacillus subtilis*) inoculated into Mars soil simulant (MGS-1) in the density of 10^4 cells g^{-1} were successfully detected by CFDA-AM in both the sample holder and the filter unit.

継続研究報告（2021年9月末現在）

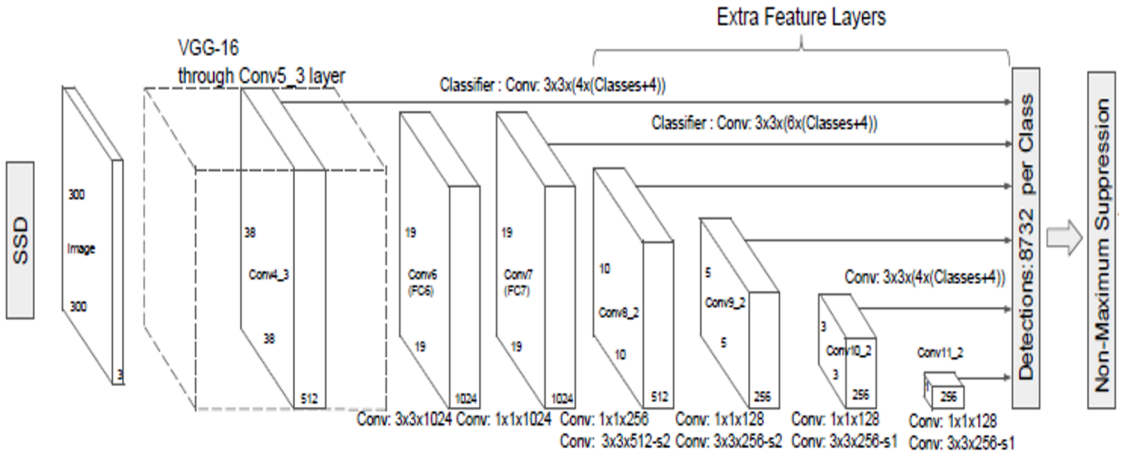
物体検知結果

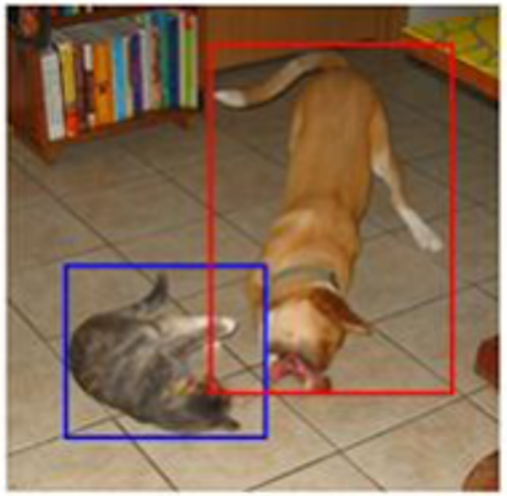
（遠藤 真輝）

Deep Learning モデル

SSD300

Liu *et al.*



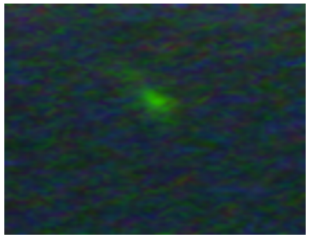


source: https://blog.negativemind.com/2019/02/26/general-object-recognition-single-shot-multibox-detector/
https://superbc.dev/index.php/2020/05/17/investigatenvidiassd300/

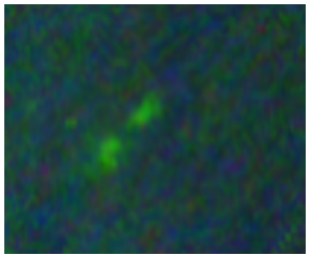
使用データ

LDM試料筒から撮影された、以下３種類のデータセットを使用

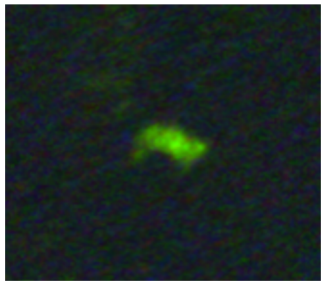
1,　火星模擬土にバクテリアを混合し,蛍光色素で染色した画像
　　火星模擬土のみを蛍光色素染色した画像



2,　火星模擬土にバクテリアを混合し、蛍光色素で染色した画像
　　１と比べ、色素濃度を低く設定



3,　火星模擬土に4種の鉱物を混合し、蛍光色素で染色した画像



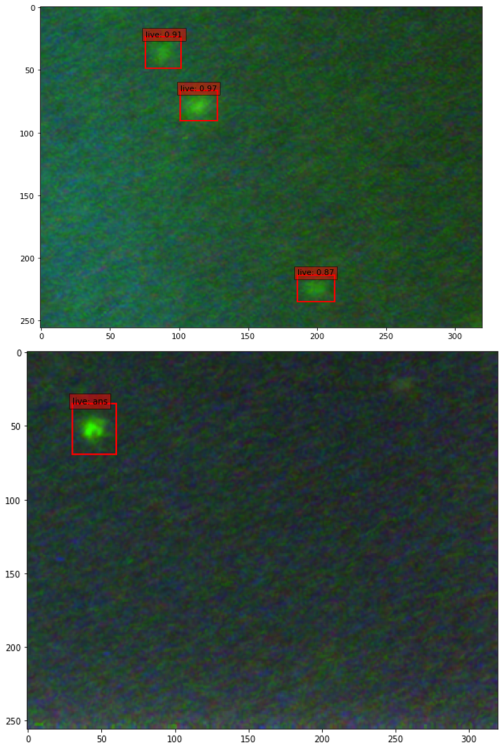
結果:

特徴がはっきりした生菌、鉱物を識別、検知することができた

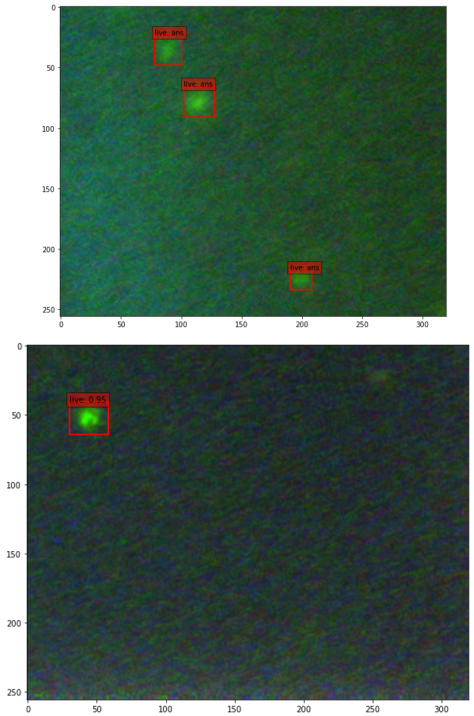
- **ある程度の大きさ、　高い輝度、しっかりした輪郭等**

検知した物体が生菌なら’live’, 鉱物なら’material’と表示される。
また、一緒に示されている数字は検知した物体の信頼度を表している。

予測画像

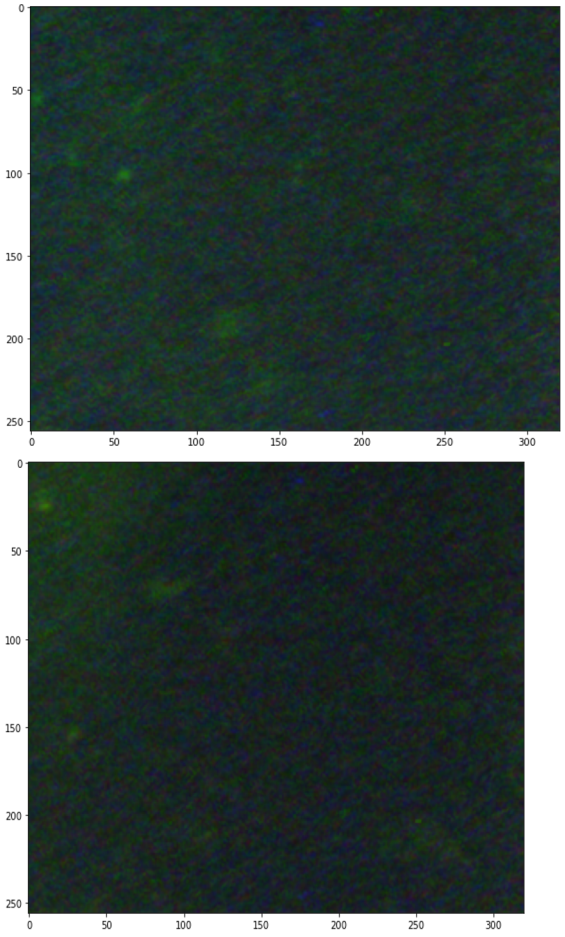


ラベル画像

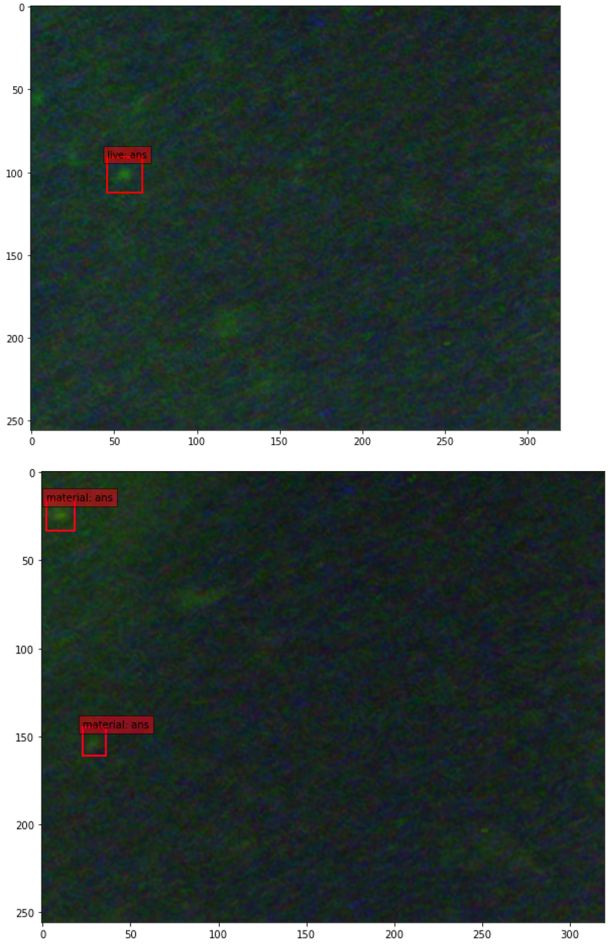


結果：検知失敗例

予測画像



ラベル画像



現状：

検知結果は学習途中のAIを使った結果である。学習に時間をかければさらに精度が向上すると思われる

作成したモデルの性能をはかるための指標が複雑で、まだプログラムの実装はできていない

今後も研究・開発を継続していく

Acknowledgments

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