Developing technologies for biological experiments in deep space

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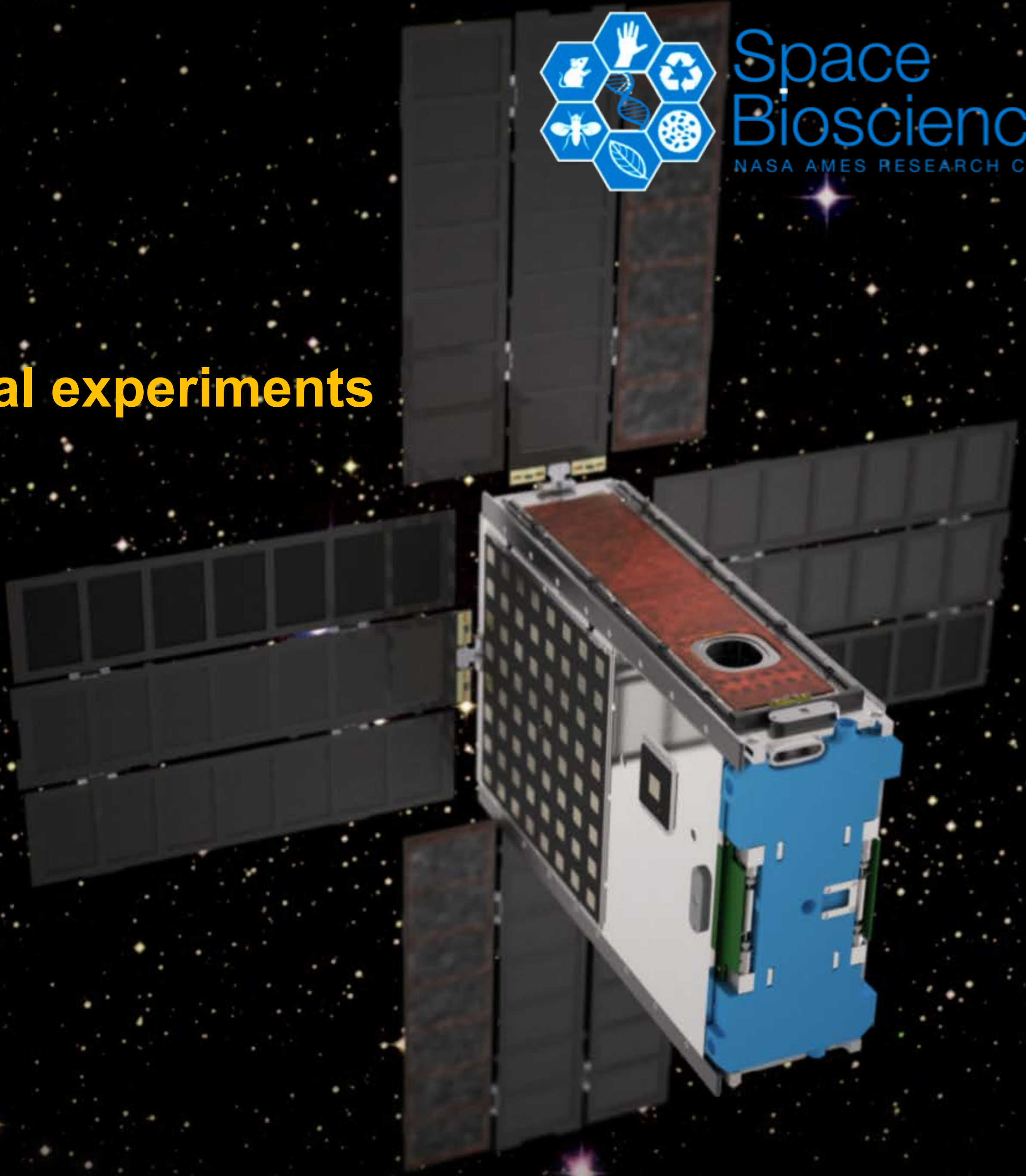
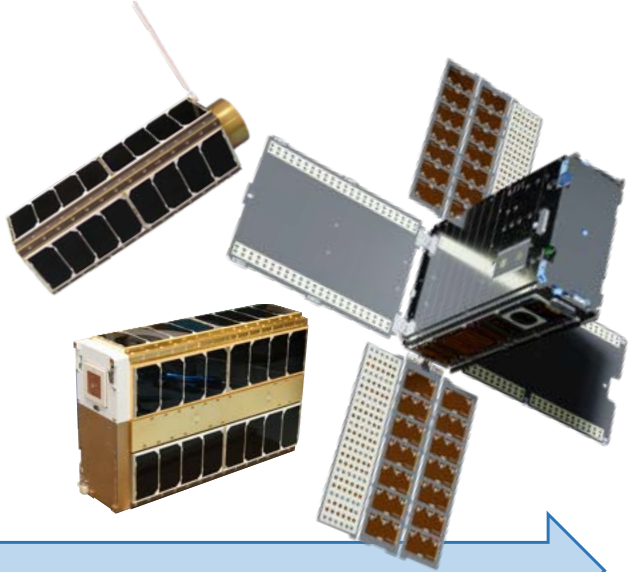


NASA’s life science programs



1973 – 1974
Skylab

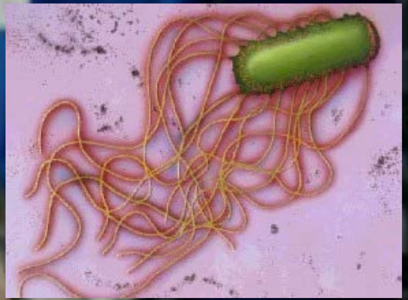
1981 - 2011
Space Shuttle Program

2000 –
International Space Station

2006 –
Bio CubeSats

Micrography effects

- Nausea / vomit
- Disorientation & sleep loss
- Body fluid redistribution
- Muscle & bone loss
- Cardiovascular deconditioning
- Increase pathogenicity in microbes



Interplanetary space radiation

What type of radiation are we going to encounter beyond low Earth orbit (LEO)?

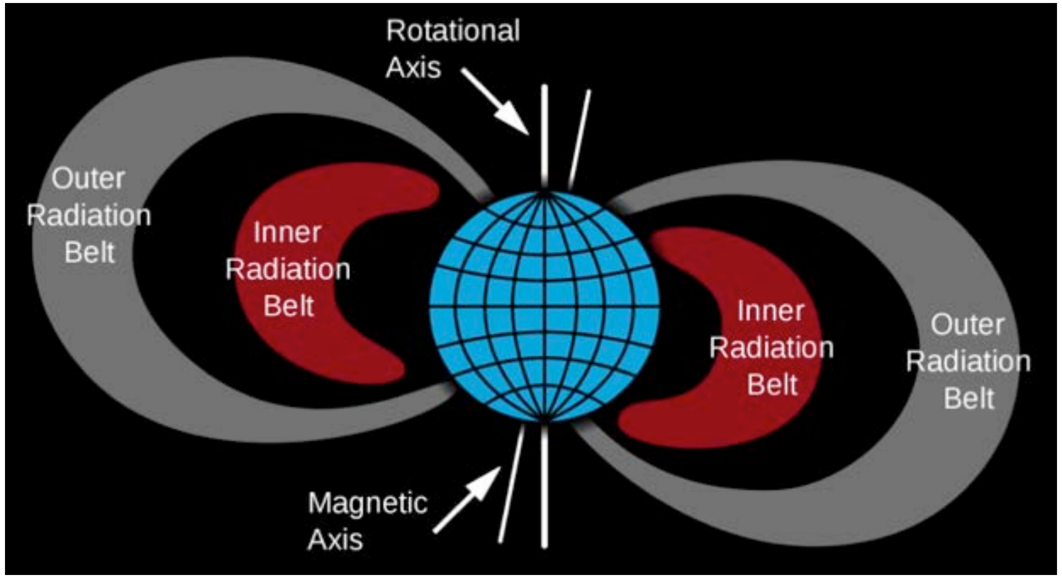
Galactic Cosmic Rays (GCRs):

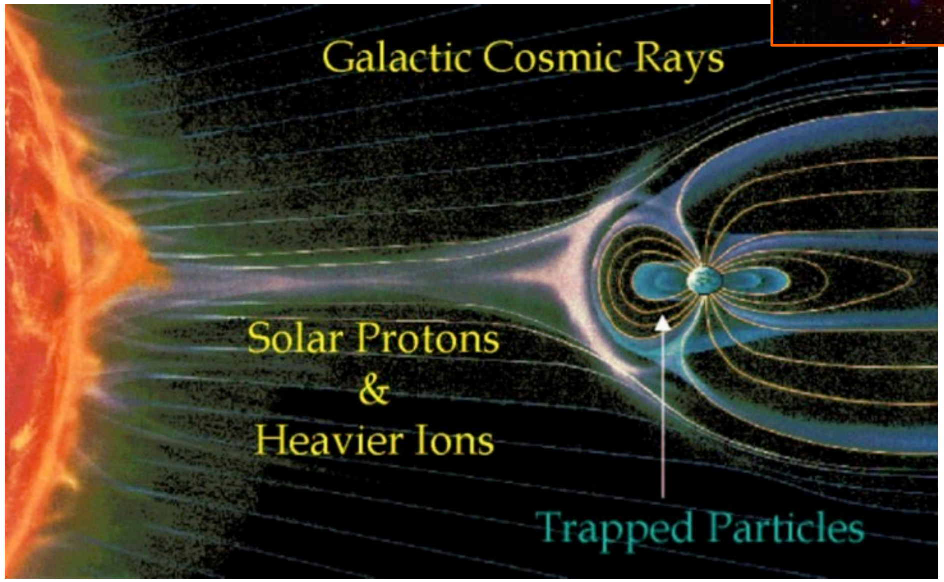
– Interplanetary, continuous, modulated by the 11-year solar cycle

– High-energy protons and highly charged, energetic heavy particles (Fe-56, C-12)

– Not effectively shielded; can break up into lighter, more penetrating pieces

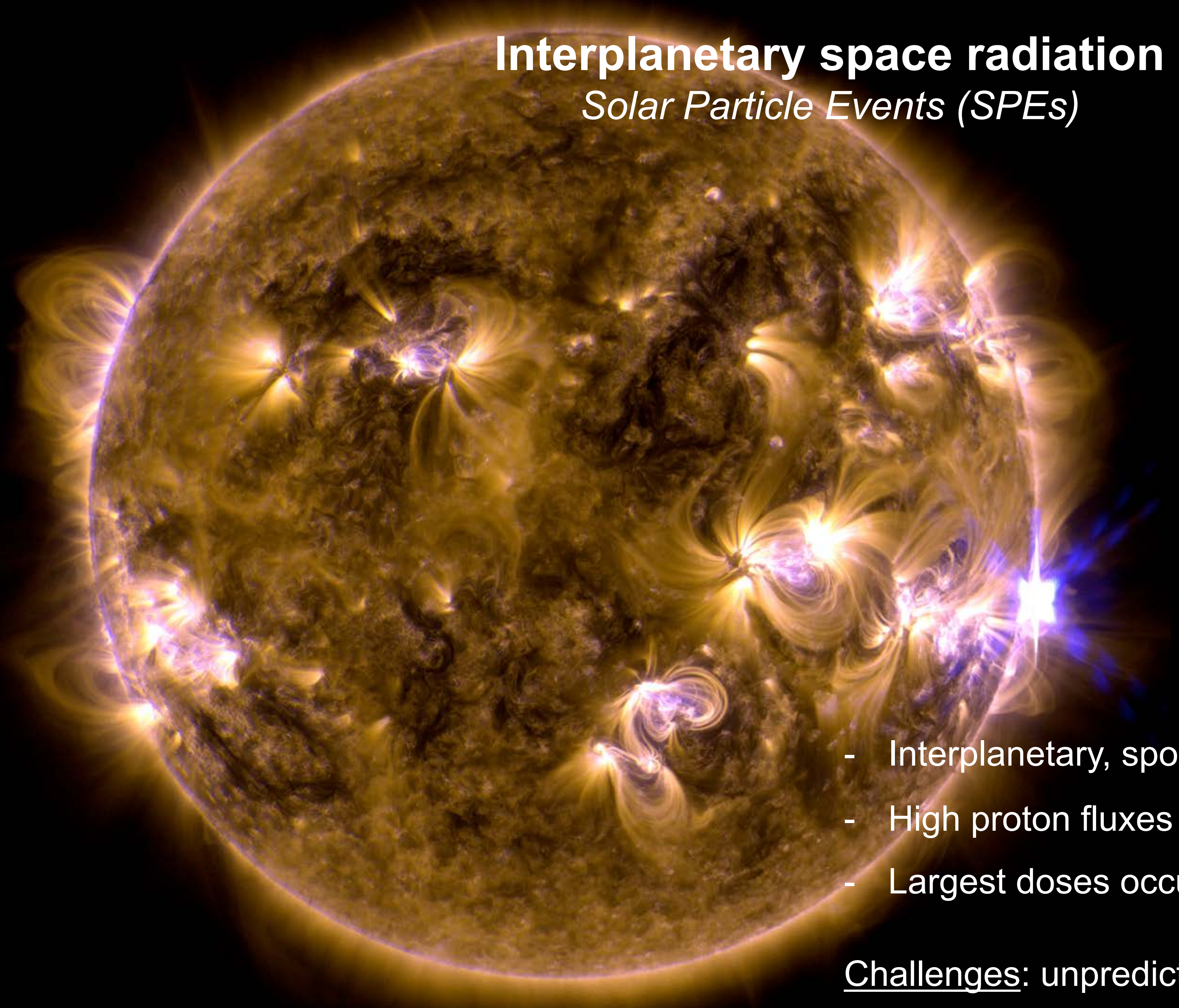
Challenges: biology effects poorly understood (but most hazardous)

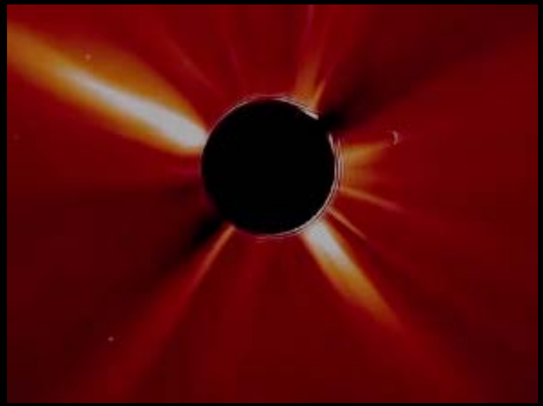




Interplanetary space radiation

Solar Particle Events (SPEs)

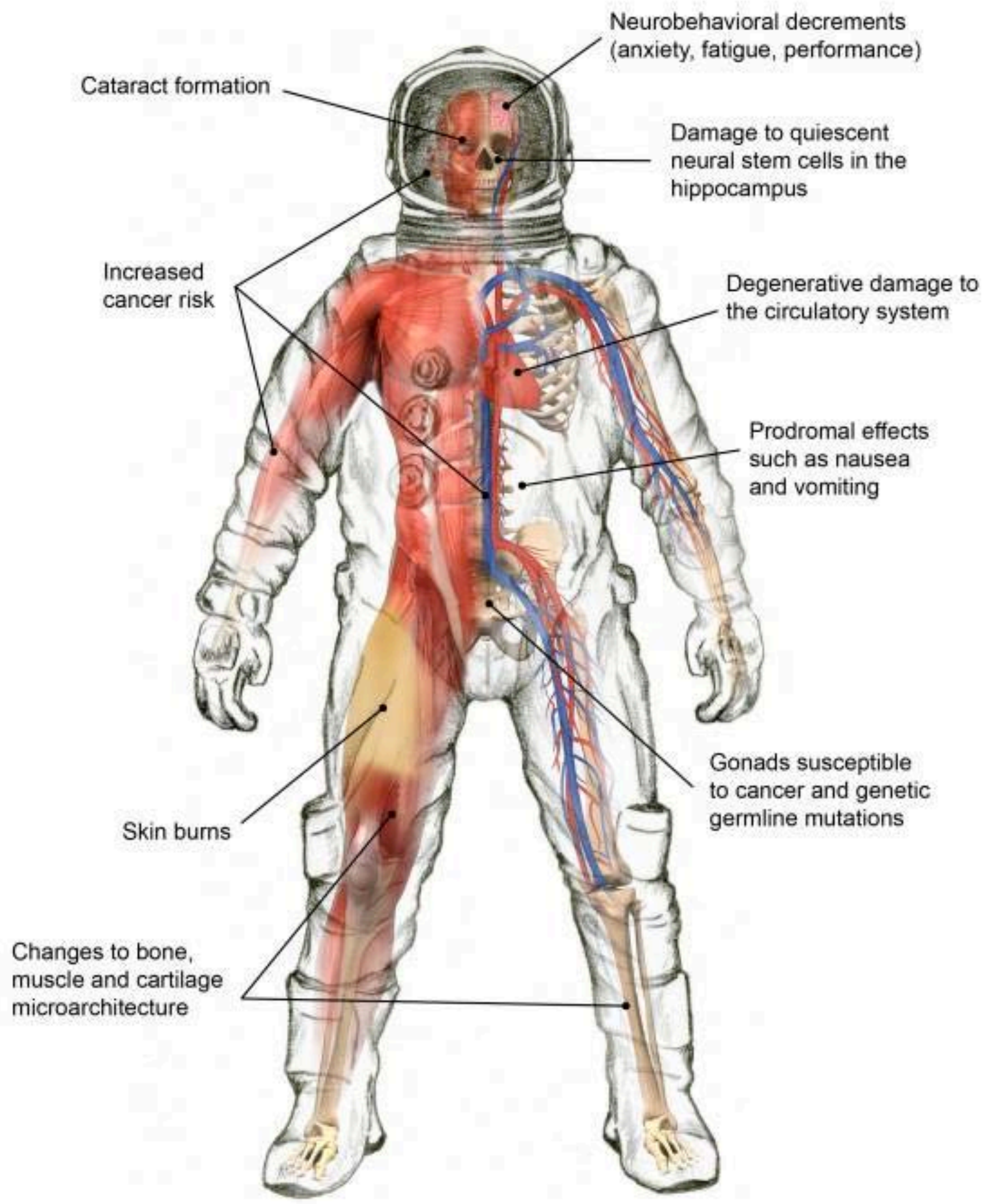




- Interplanetary, sporadic, transient (several min to days)
- High proton fluxes (low and medium energy)
- Largest doses occur during maximum solar activity

<u>Challenges</u>: unpredictable; large doses in a short time

Space radiation effects

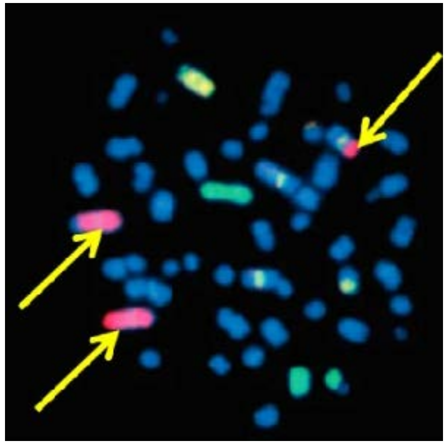


Space radiation is the # 1 risk to astronaut health on extended space exploration missions beyond the Earth's magnetosphere

- Immune system suppression, learning and memory impairment have been observed in animal models exposed to mission-relevant doses (Kennedy et al. 2011; Britten et al. 2012)
- Low doses of space radiation are causative of an increased incidence and early appearance of cataracts in astronauts (Cuccinota et al. 2001)
- Cardiovascular disease mortality rate among Apollo lunar astronauts is 4-5-fold higher than in non-flight and LEO astronauts (Delp et al. 2016)

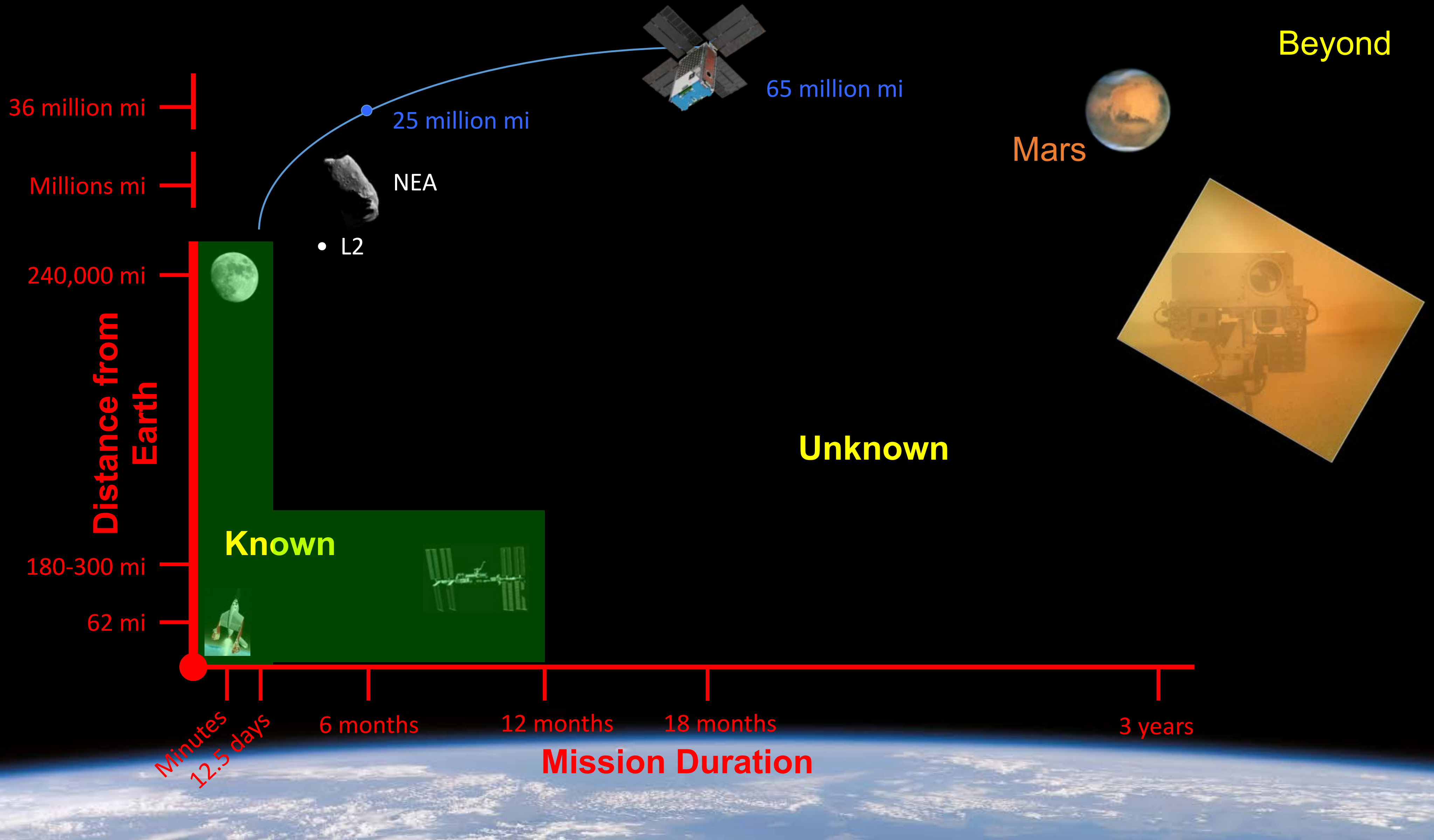
- Astronauts have shown an increase in chromosomal abnormalities, even in LEO, during ISS, Mir & STS (Hubble shuttle) missions
- GCR will be much more abundant as astronauts go to higher orbits beyond Earth's protective magnetic field

Cucinotta et al. 2008



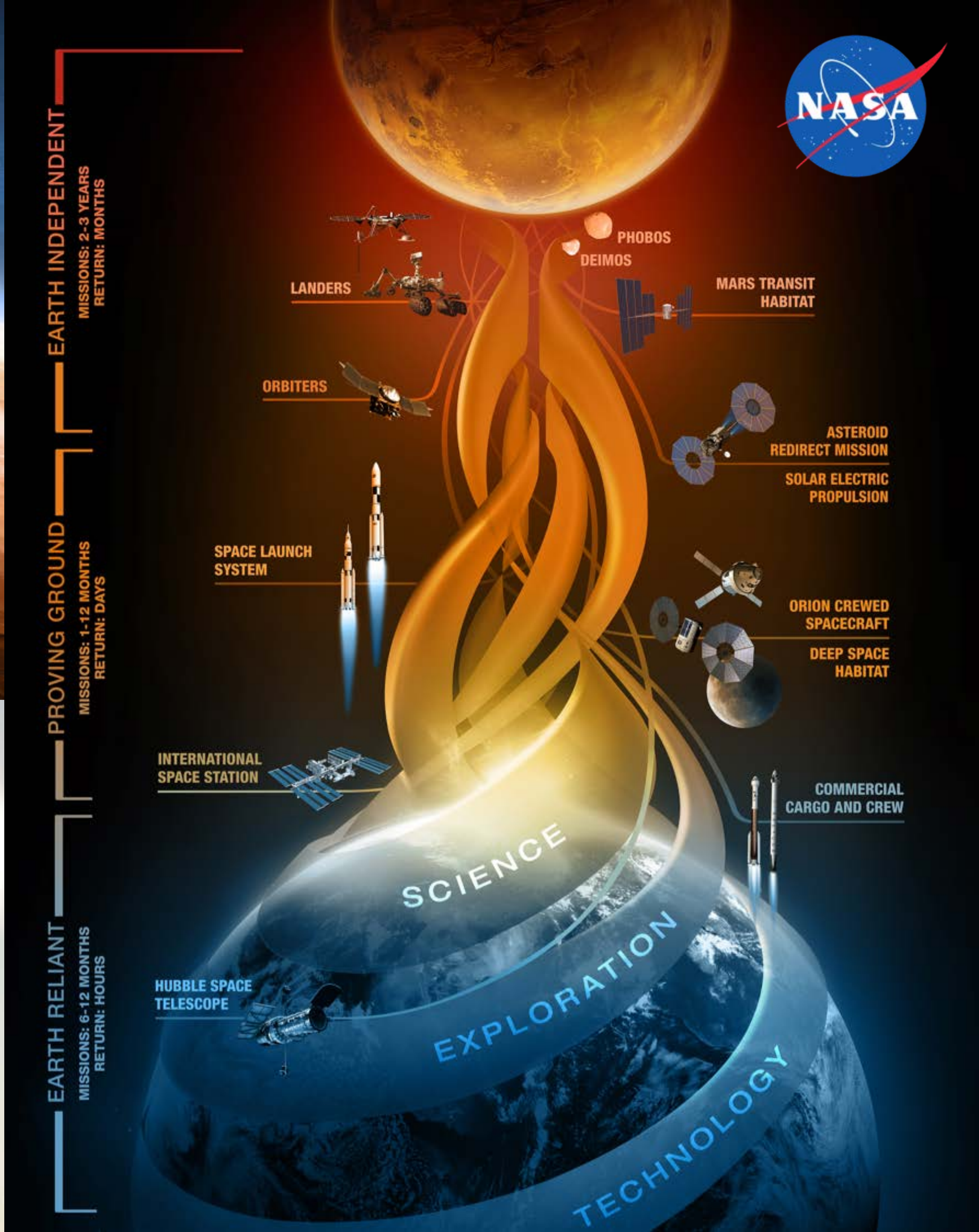
Chromosomes 1, 2, 4 in red, green & yellow (ISS)

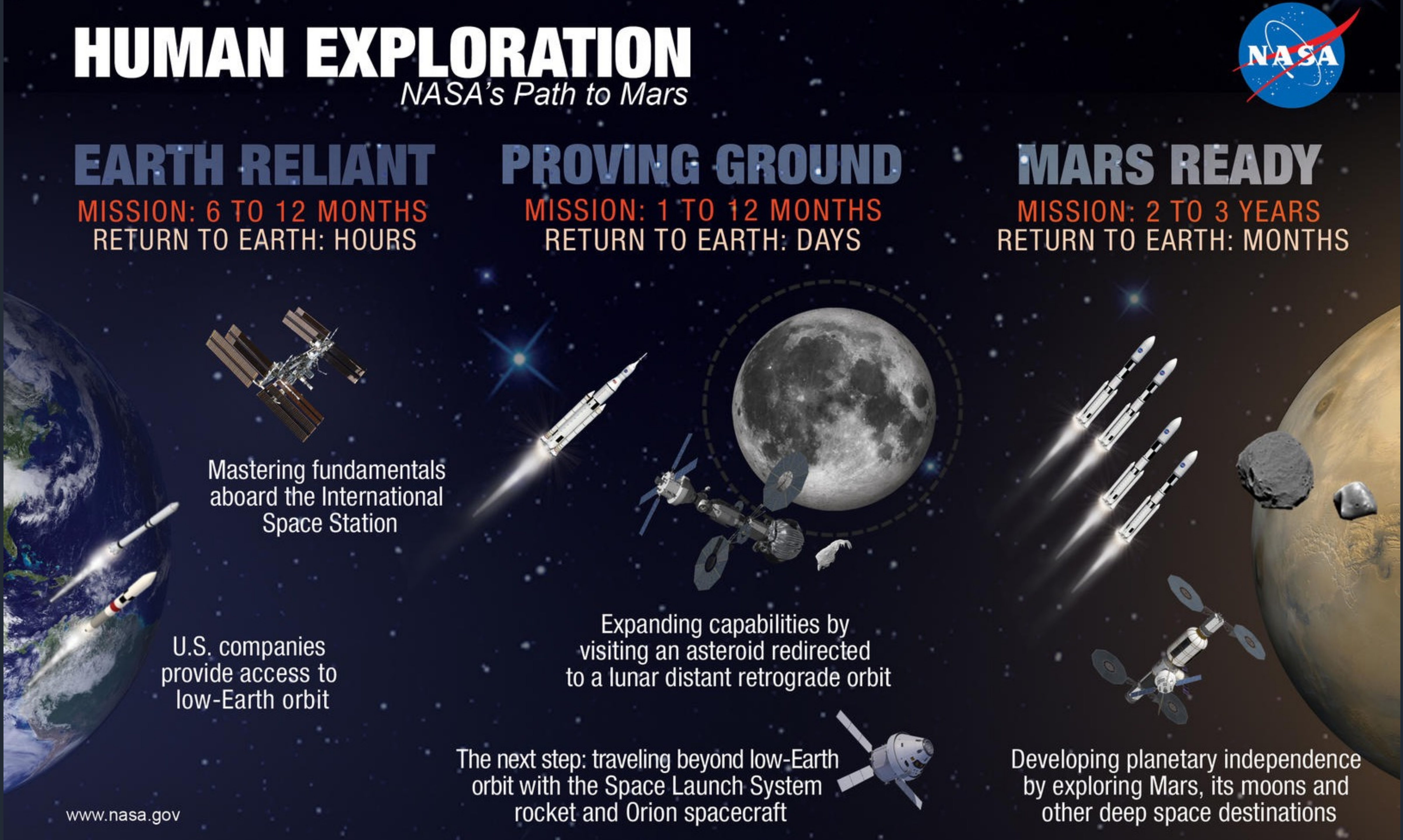
The limits of life in space – as we know it – is 12.5 days on a lunar round trip or 1.5 years in LEO. As we send people further into space, we can use model organisms and/or biosensors to understand the biological risks and how they can be addressed



What’s next for NASA?

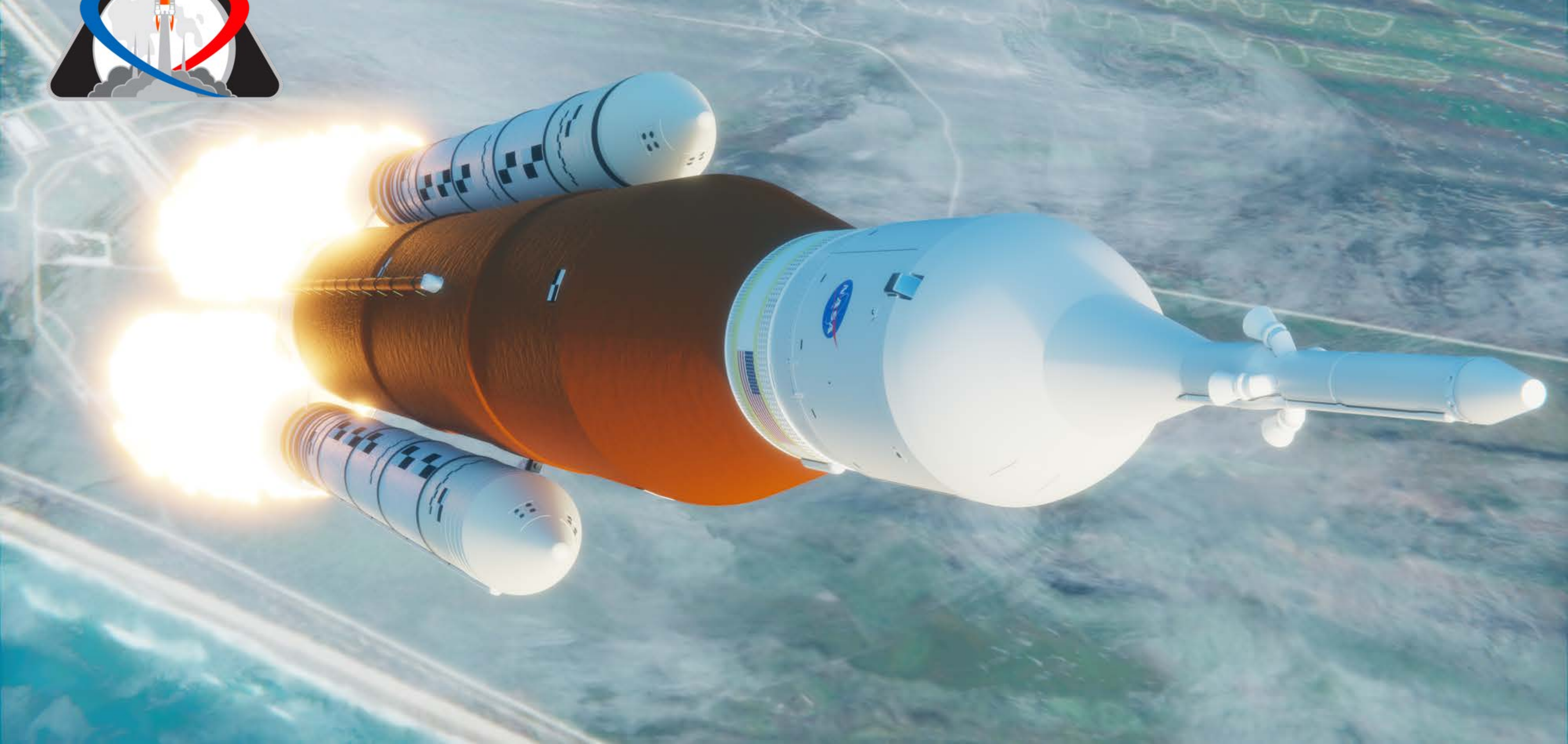

NASA'S JOURNEY TO
MARS

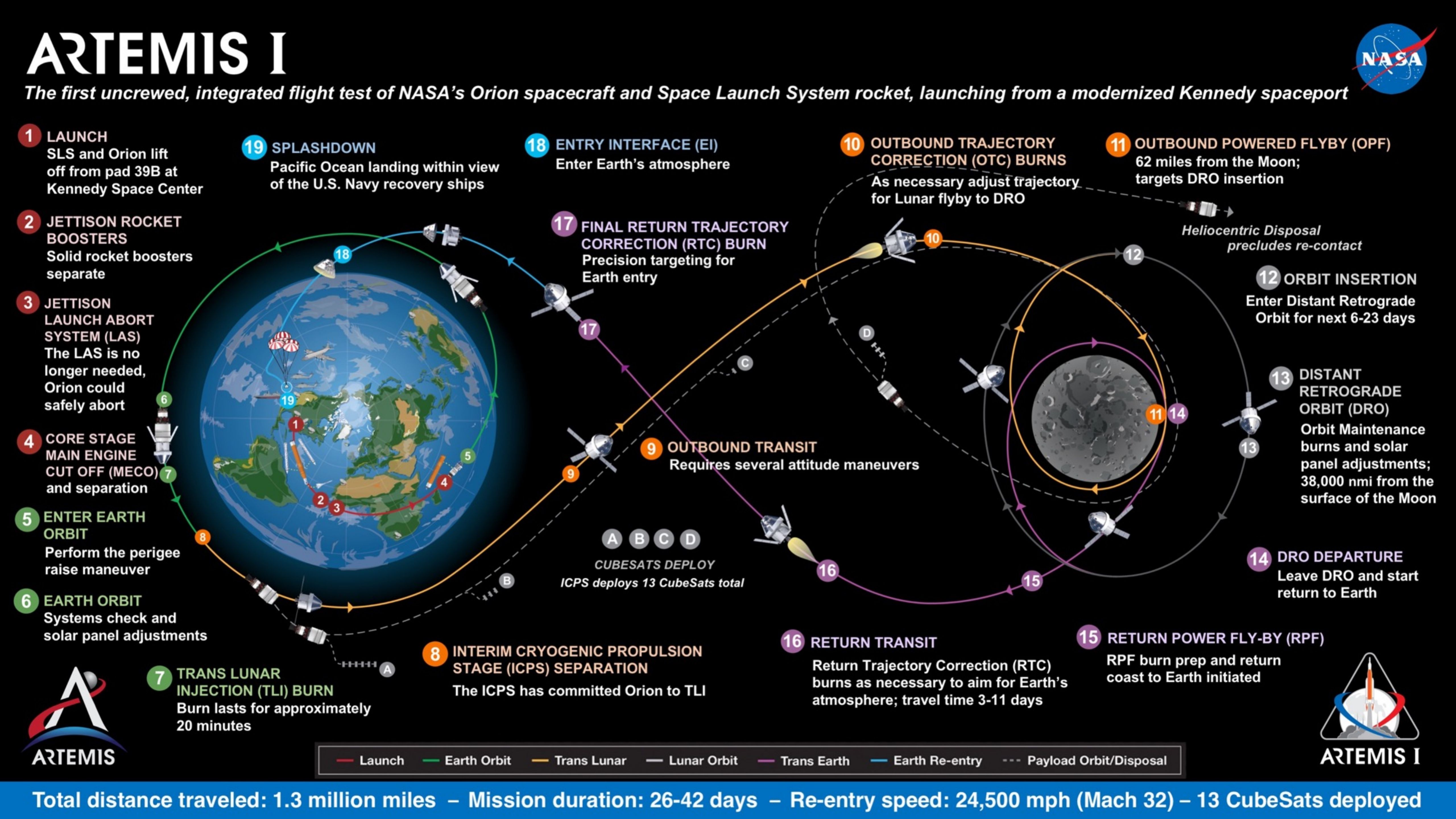

NASA
EARTH INDEPENDENT
MISSIONS: 2-3 YEARS
RETURN: MONTHS
PROVING GROUND
MISSIONS: 1-12 MONTHS
RETURN: DAYS
EARTH RELIANT
MISSIONS: 6-12 MONTHS
RETURN: HOURS
PHOBOS
DEIMOS
LANDERS
MARS TRANSIT
HABITAT
ORBITERS
ASTEROID
REDIRECT MISSION
SOLAR ELECTRIC
PROPULSION
SPACE LAUNCH
SYSTEM
ORION CREWED
SPACECRAFT
DEEP SPACE
HABITAT
INTERNATIONAL
SPACE STATION
COMMERCIAL
CARGO AND CREW
HUBBLE SPACE
TELESCOPE
SCIENCE
EXPLORATION
TECHNOLOGY


HUMAN EXPLORATION
NASA's Path to Mars
NASA
EARTH RELIANT
MISSION: 6 TO 12 MONTHS
RETURN TO EARTH: HOURS
PROVING GROUND
MISSION: 1 TO 12 MONTHS
RETURN TO EARTH: DAYS
MARS READY
MISSION: 2 TO 3 YEARS
RETURN TO EARTH: MONTHS
Mastering fundamentals aboard the International Space Station
U.S. companies provide access to low-Earth orbit
Expanding capabilities by visiting an asteroid redirected to a lunar distant retrograde orbit
The next step: traveling beyond low-Earth orbit with the Space Launch System rocket and Orion spacecraft
Developing planetary independence by exploring Mars, its moons and other deep space destinations
www.nasa.gov

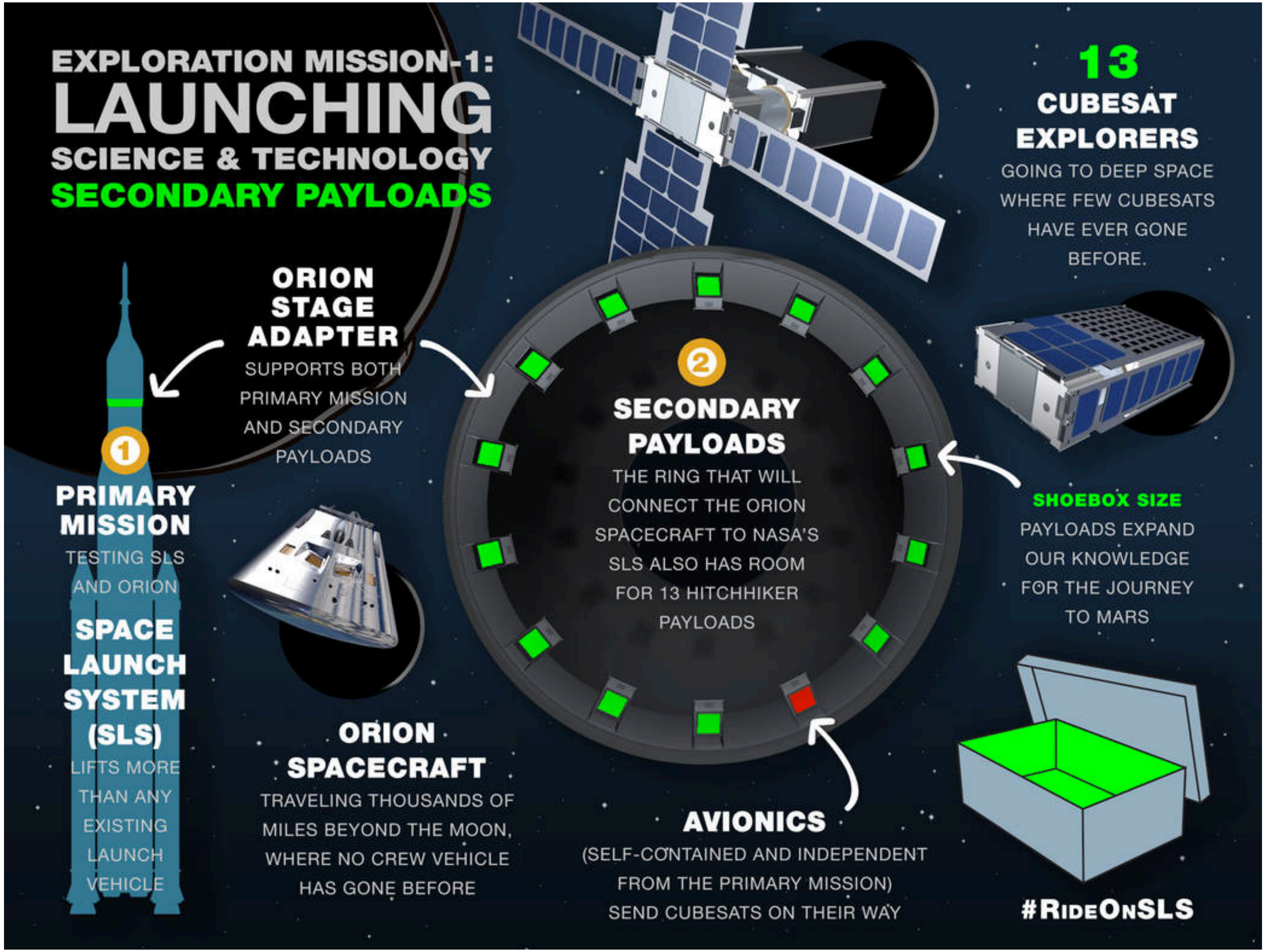
Artemis-1 mission & BioSentinel

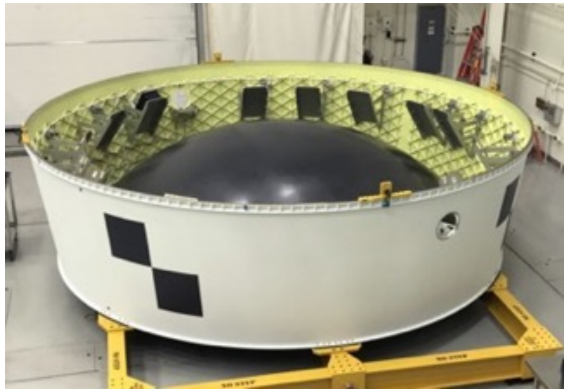
(launch 2021/2022)




ARTEMIS I
The first uncrewed, integrated flight test of NASA's Orion spacecraft and Space Launch System rocket, launching from a modernized Kennedy spaceport
NASA
1 LAUNCH
SLS and Orion lift off from pad 39B at Kennedy Space Center
2 JETTISON ROCKET BOOSTERS
Solid rocket boosters separate
3 JETTISON LAUNCH ABORT SYSTEM (LAS)
The LAS is no longer needed, Orion could safely abort
4 CORE STAGE MAIN ENGINE CUT OFF (MECO) and separation
5 ENTER EARTH ORBIT
Perform the perigee raise maneuver
6 EARTH ORBIT
Systems check and solar panel adjustments
7 TRANS LUNAR INJECTION (TLI) BURN
Burn lasts for approximately 20 minutes
8 INTERIM CRYOGENIC PROPULSION STAGE (ICPS) SEPARATION
The ICPS has committed Orion to TLI
9 OUTBOUND TRANSIT
Requires several attitude maneuvers
10 OUTBOUND TRAJECTORY CORRECTION (OTC) BURNS
As necessary adjust trajectory for Lunar flyby to DRO
11 OUTBOUND POWERED FLYBY (OPF)
62 miles from the Moon; targets DRO insertion
Heliocentric Disposal precludes re-contact
12 ORBIT INSERTION
Enter Distant Retrograde Orbit for next 6-23 days
13 DISTANT RETROGRADE ORBIT (DRO)
Orbit Maintenance burns and solar panel adjustments; 38,000 nmi from the surface of the Moon
14 DRO DEPARTURE
Leave DRO and start return to Earth
15 RETURN POWER FLY-BY (RPF)
RPF burn prep and return coast to Earth initiated
16 RETURN TRANSIT
Return Trajectory Correction (RTC) burns as necessary to aim for Earth's atmosphere; travel time 3-11 days
17 FINAL RETURN TRAJECTORY CORRECTION (RTC) BURN
Precision targeting for Earth entry
18 ENTRY INTERFACE (EI)
Enter Earth's atmosphere
19 SPLASHDOWN
Pacific Ocean landing within view of the U.S. Navy recovery ships
A B C D
CUBESATS DEPLOY
ICPS deploys 13 CubeSats total
Launch
Earth Orbit
Trans Lunar
Lunar Orbit
Trans Earth
Earth Re-entry
Payload Orbit/Disposal
ARTEMIS
ARTEMIS I
Total distance traveled: 1.3 million miles – Mission duration: 26-42 days – Re-entry speed: 24,500 mph (Mach 32) – 13 CubeSats deployed

Artemis-1: secondary payloads (6U CubeSats)

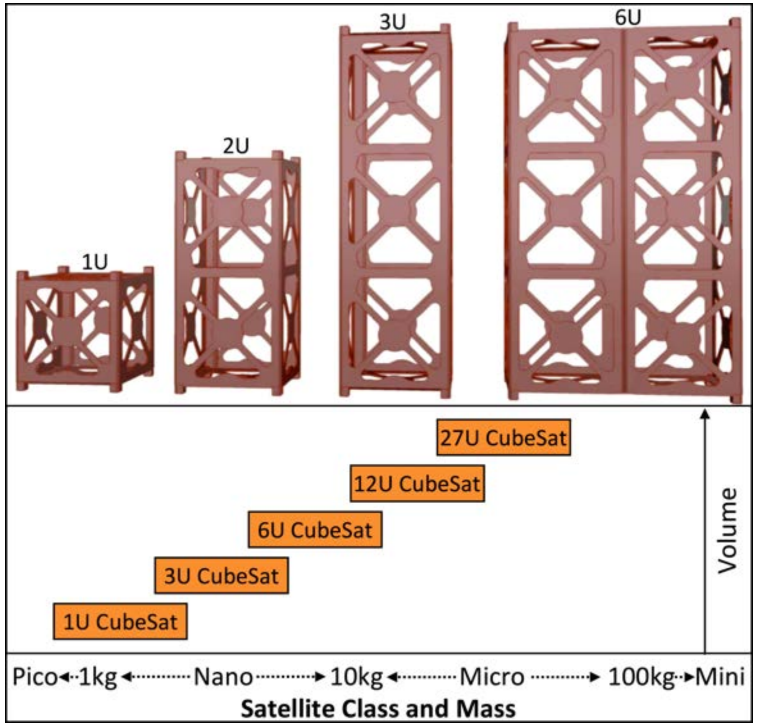




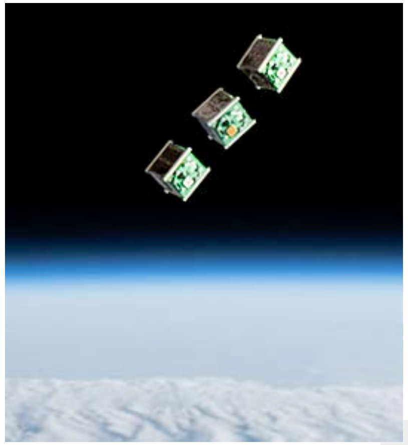
CubeSats

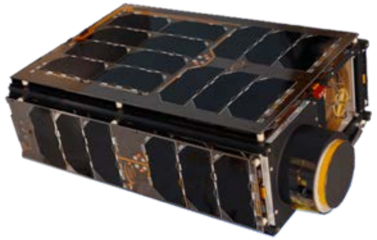
CubeSats

CubeSat configurations

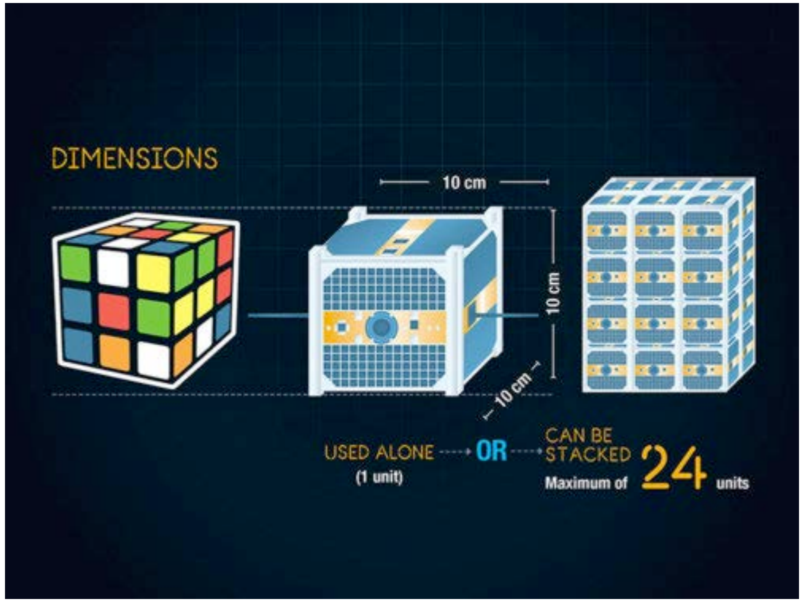


Poghosyan & Golkar, 2017

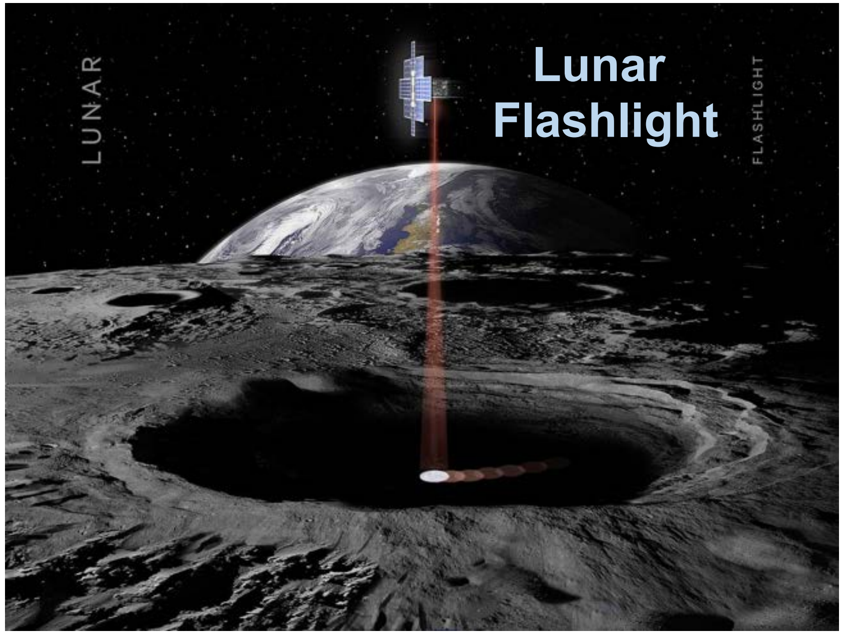


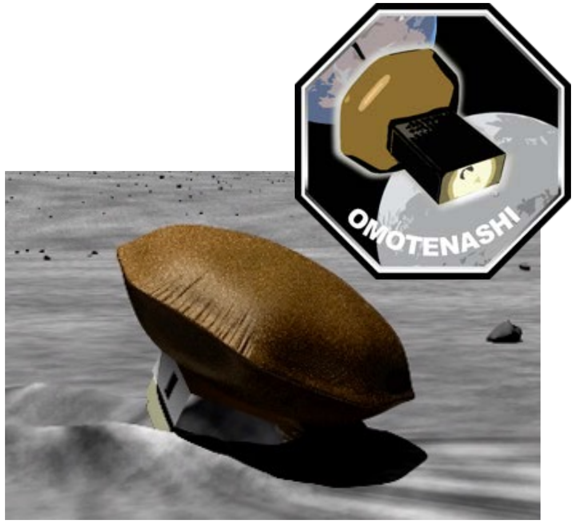


CubeSats: toys, tools, or debris cloud?

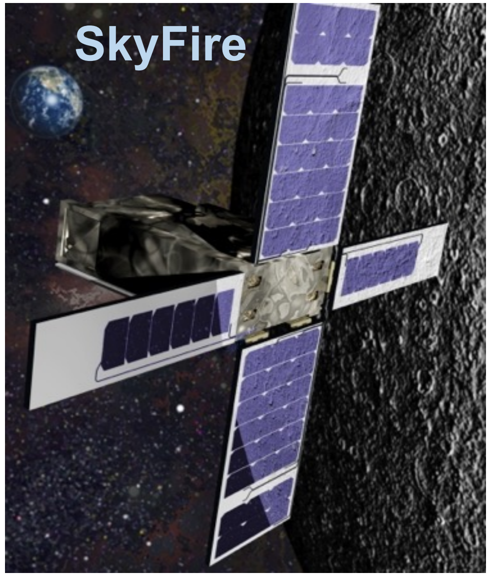


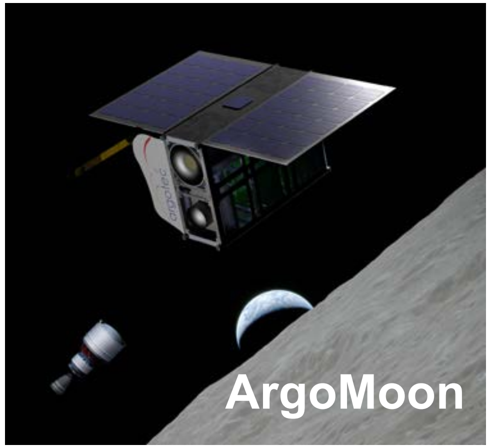
CubeSat technologies (Artemis-1)

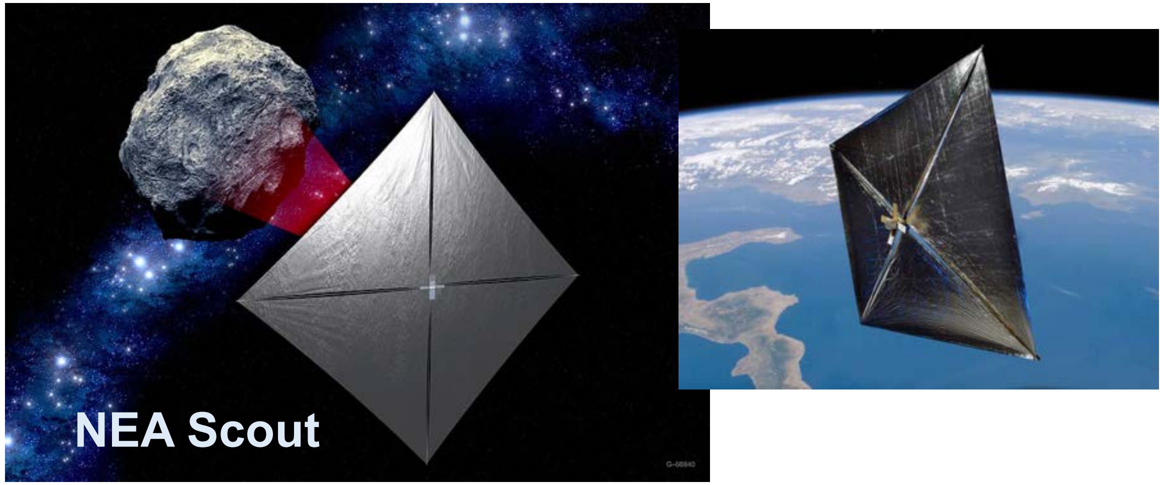




Cislunar Explorers

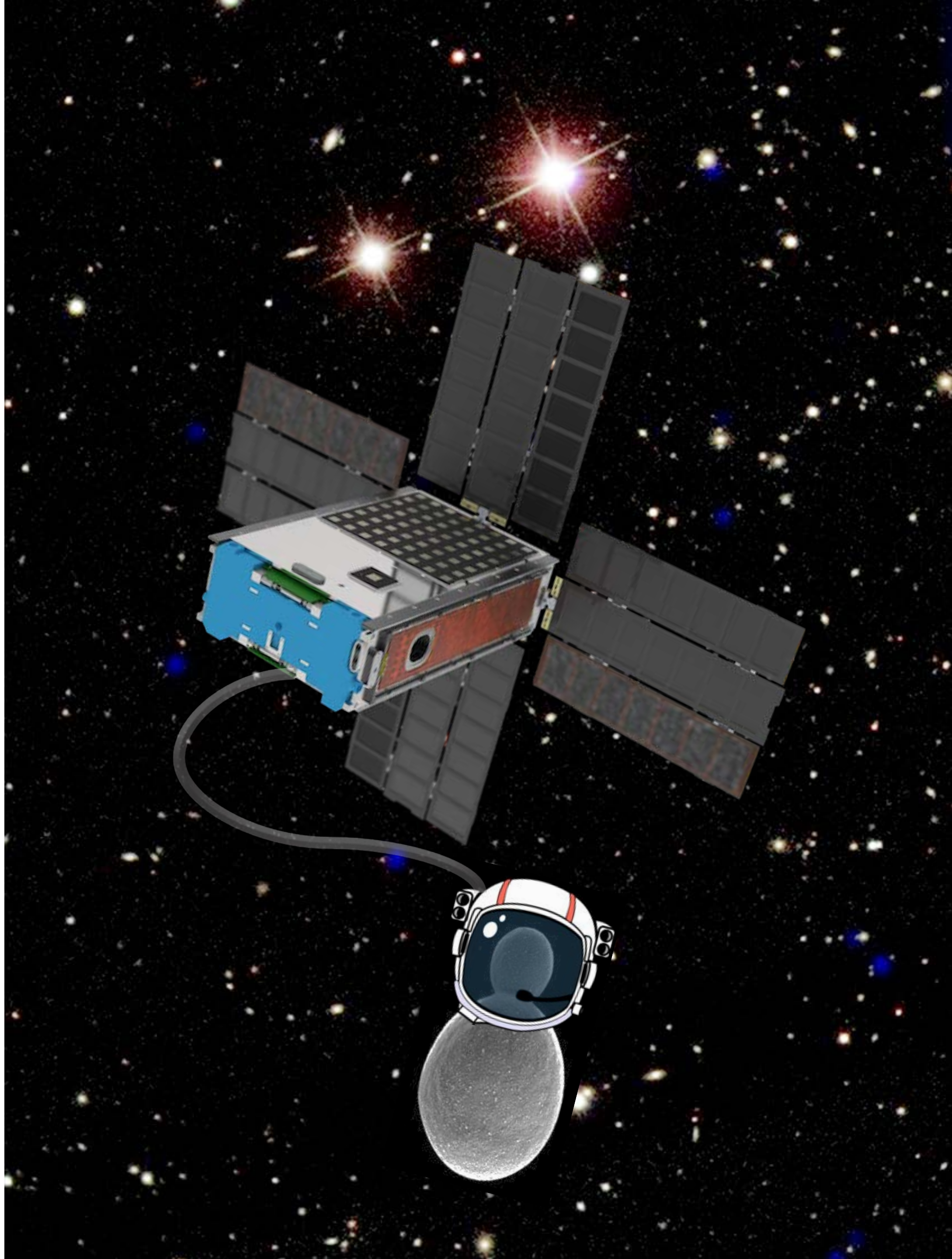






Biological missions using CubeSats

(NASA Ames Research Center, 2006 – 2022)

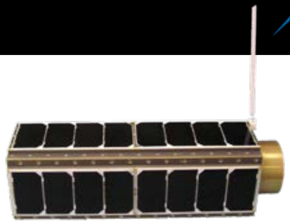


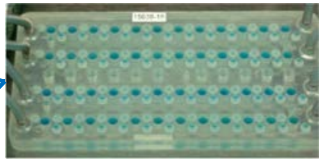
NASA Ames pioneering biological space missions

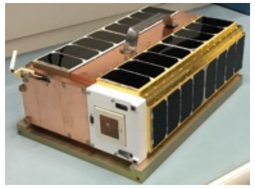
E. coli

GeneSat-1 (2006 / 3U): ***gene expression***

EcAMSat (2017 / 6U): ***antibiotic resistance***



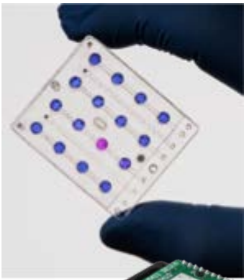


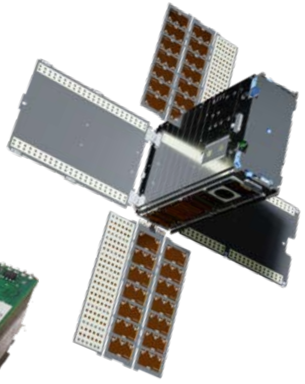


S. cerevisiae

PharmaSat (2009 / 3U): ***drug dose response***

BioSentinel (~2022 / 6U): ***DNA damage response***

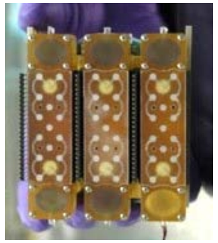


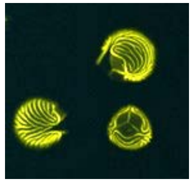


B. subtilis

O/OREOS* (2010 / 3U): ***survival, metabolism***

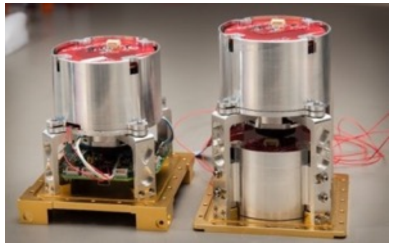
**Organism/Organic Response to Orbital Stress*





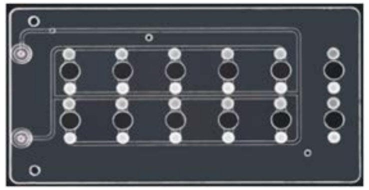
C. richardii

SporeSat-1 (2014 / 3U): ***ion channel sensors, microcentrifuges***

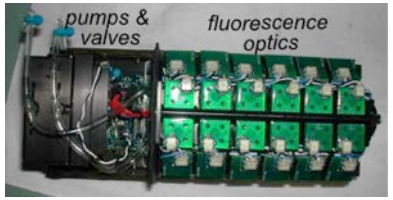


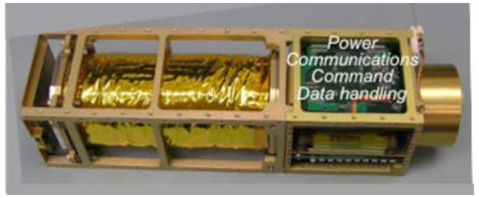
GeneSat mission: NASA's 1st CubeSat

1st bio nanosatellite in Earth's orbit, 1st real-time, in-situ gene expression measurement in space

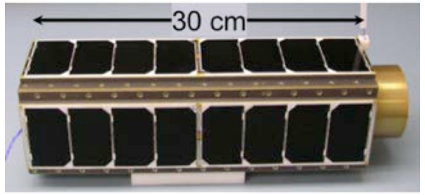


12-well fluidic card

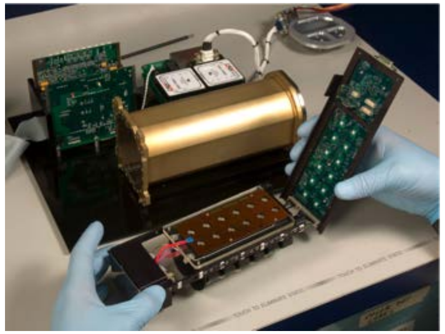


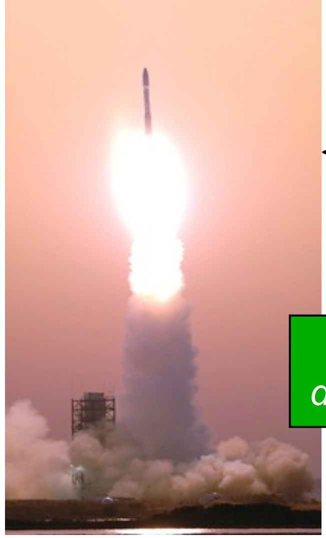


3U CubeSat



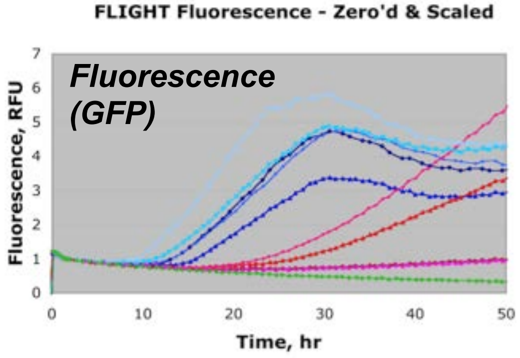
- Model organism: *E. coli* (~ 0.5 x 2 μm bacteria)
- Nutrient deprivation in dormant state (6 weeks)
- Launch: Dec 2006 to low Earth orbit (440 km)
- Nutrient solution feed upon orbit stabilization, grow *E. coli* in microgravity
- Monitor gene expression via GFP
- Monitor optical density: cell population



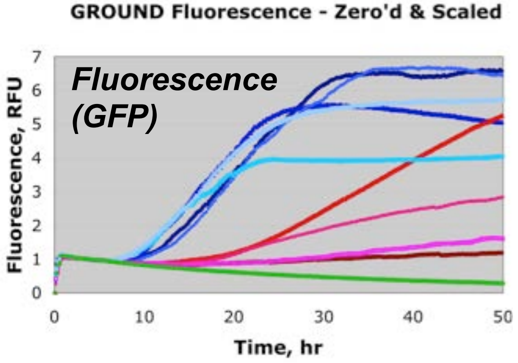


Dec 16, 2006

Telemeter data to Earth

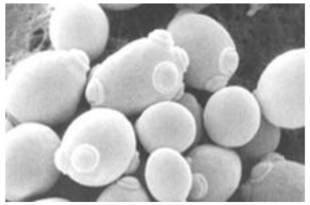


Compare to ground data



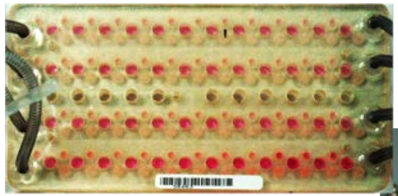
PharmaSat mission

Effect of microgravity on yeast susceptibility to antifungal drug



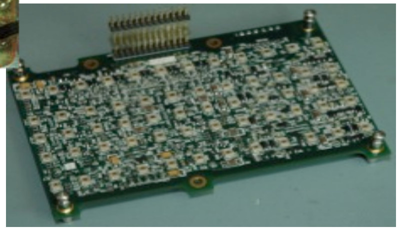
S. cerevisiae

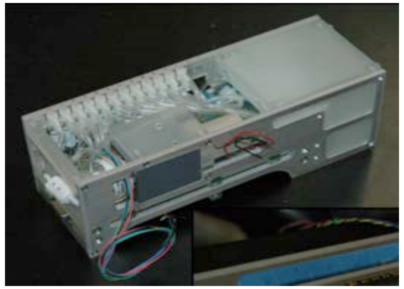
- Launch: May 2009 to LEO (~450 km)
- Grow yeast in multiwell fluidics card in microgravity
- Measure inhibition of growth by antifungal
- Optical absorbance (turbidity: cell density)
- Metabolism indicator dye: alamarBlue (3-LED optical detection)
- Control + 3 concentrations of antifungal



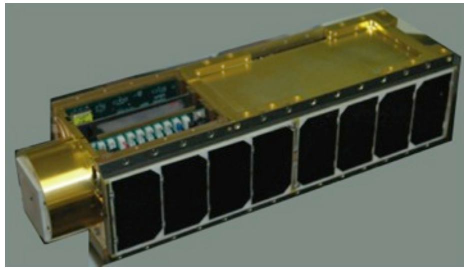
48-well fluidic card

+

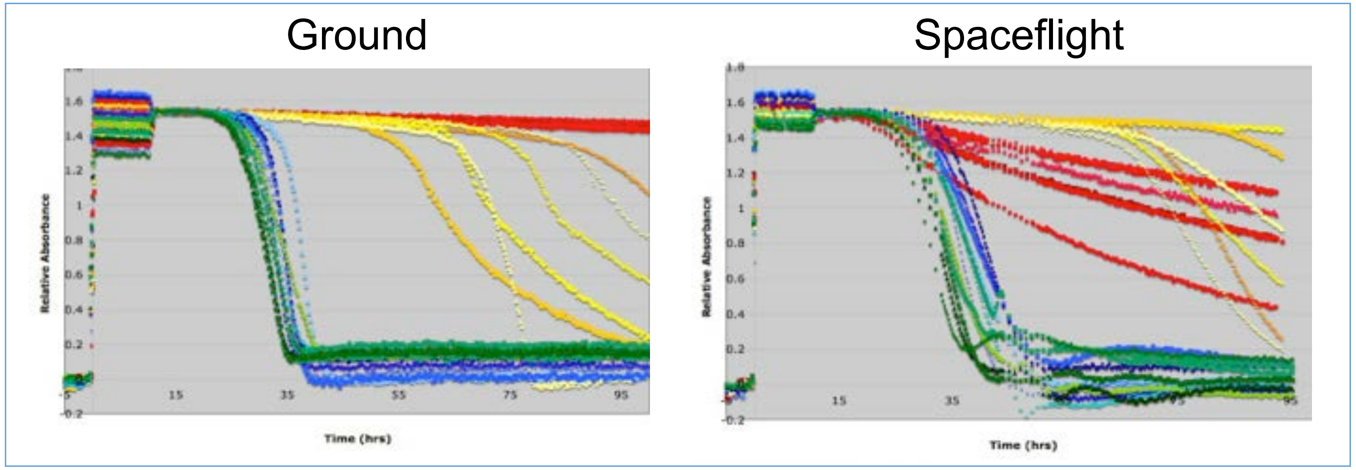




3U CubeSat



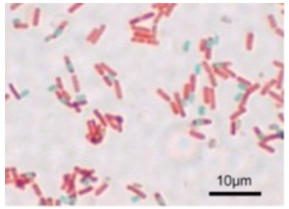
May 19, 2009



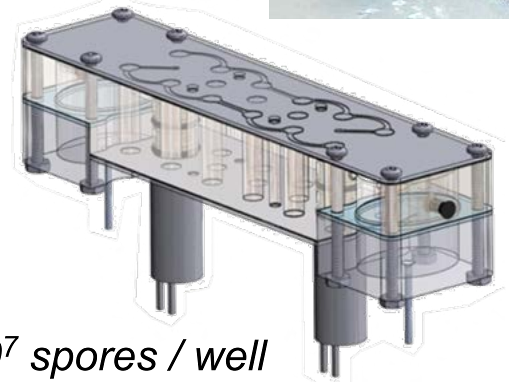
O/OREOS mission

Organism / Organic Response to Orbital Stress (1st astrobiology CubeSat)

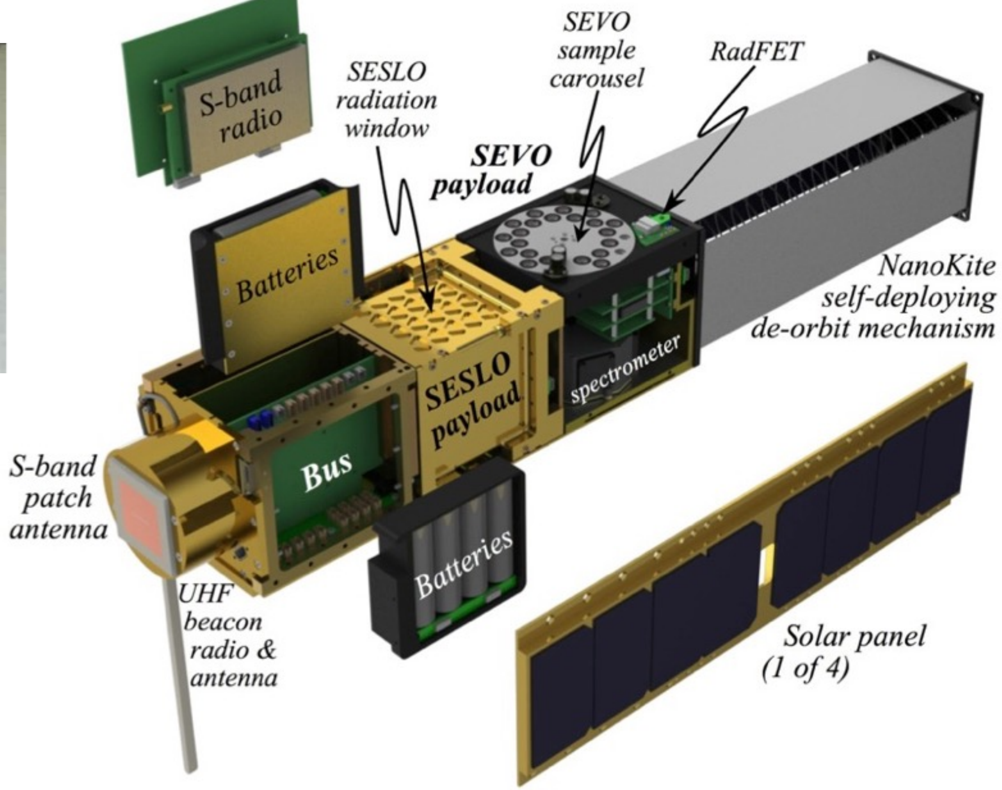
- Effects of space exposure on biological organisms (6 mo) & organic molecules (18 mo)
- SESLO (Space Environment Survival of Living Organisms): monitor survival, growth, and metabolism of *B. subtilis* using *in-situ* optical density /colorimetry
- SEVO (Space Environment Viability of Organics): track changes in organic molecules and biomarkers: UV / visible / NIR spectroscopy

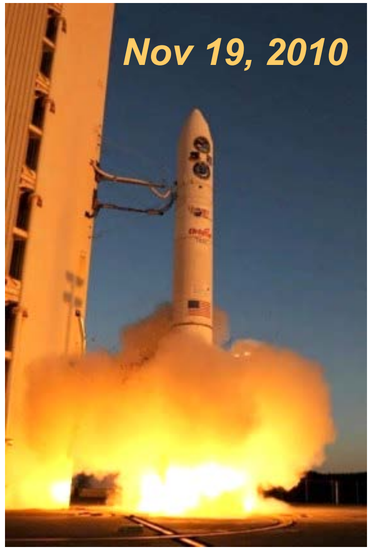


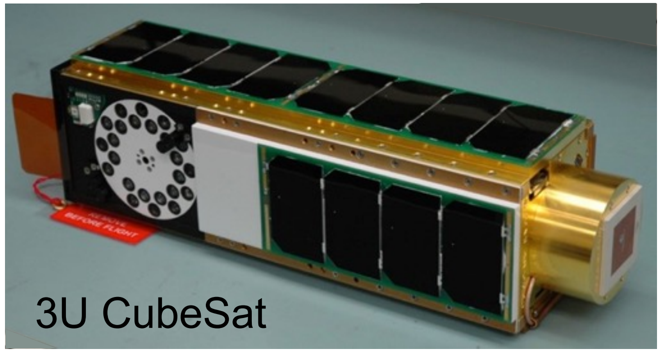
B. subtilis



10^7 spores / well
(75 μL per well)



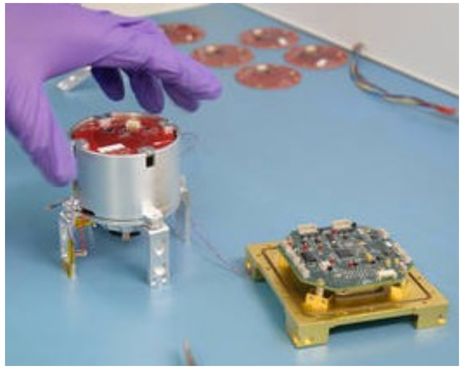


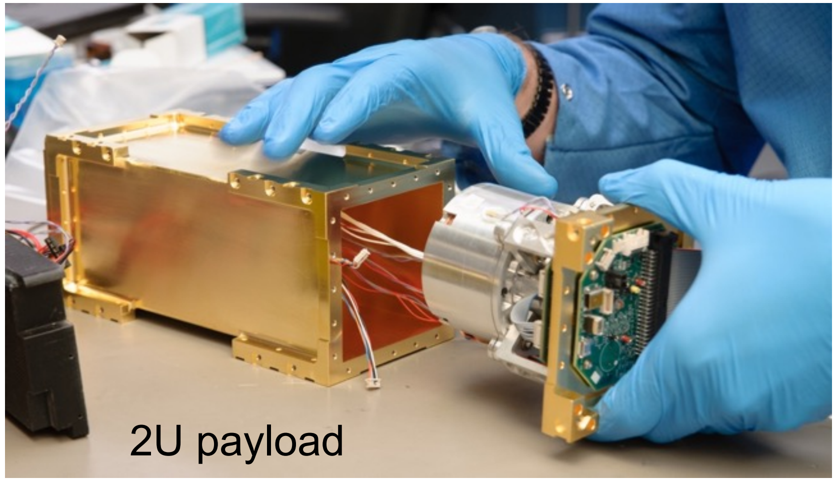


Gravitational response of fern spores via Ca^{2+} ion channel response

- Model organism: *Ceratopteris richardii* (aquatic fern spores)
- 2U payload (3U total)
- Launch: April 18, 2014 to low Earth orbit
- Variable gravity in microgravity environment using 50-mm microcentrifuges
- 32 ion-specific [Ca^{2+}] electrode pairs

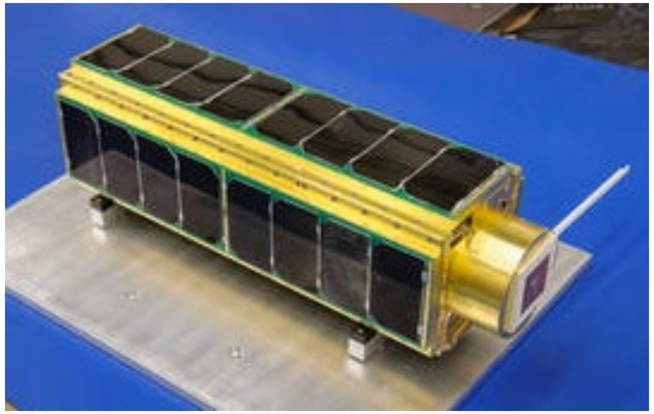
BioCDs





2U payload

SpaceX CRS-3



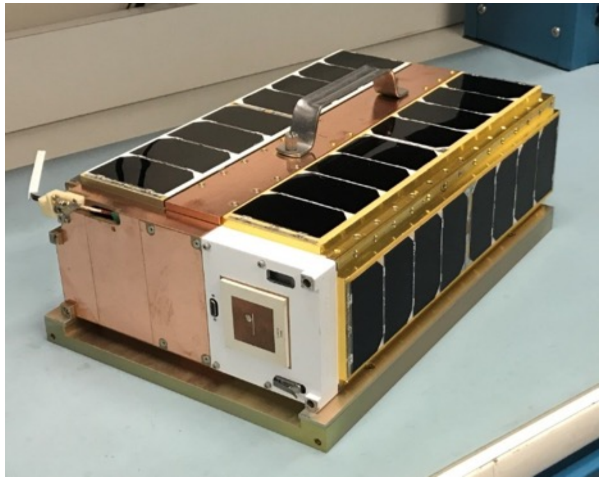
3U CubeSat

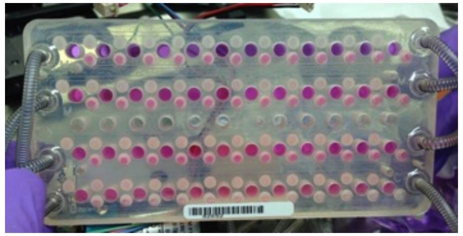
EcAMSat mission

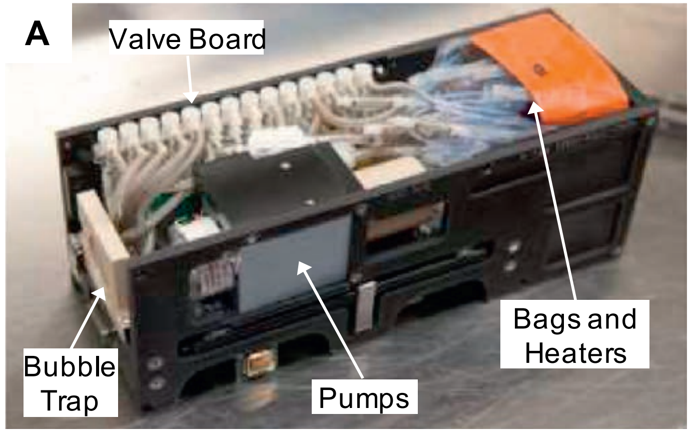
E. coli AntiMicrobial Satellite mission

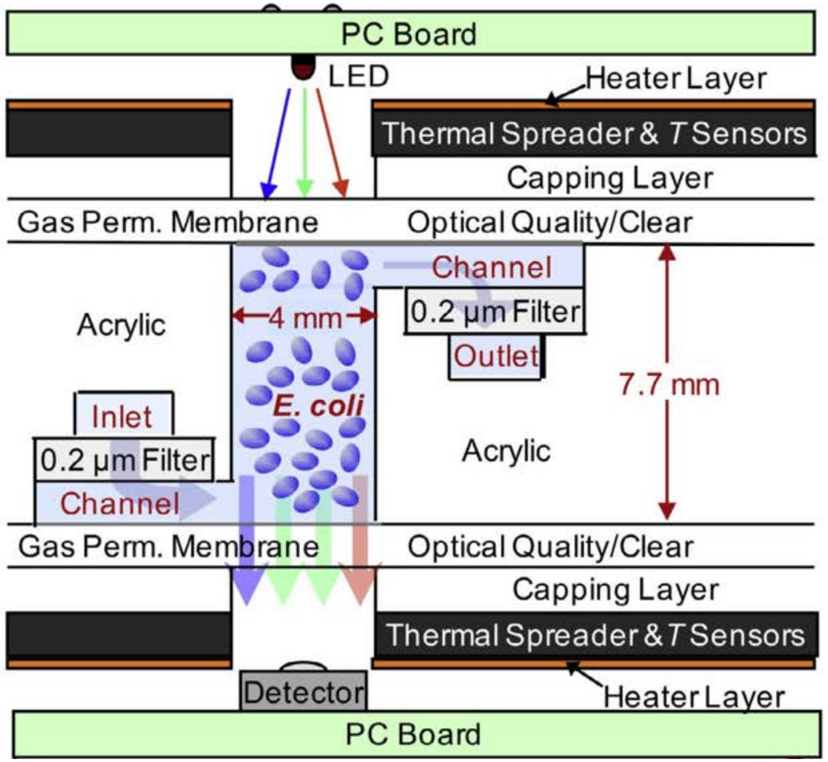
- Antibiotic resistance in microgravity vs. dose in uropathogenic *E. coli*
- 6U format provided 50% more solar-panel power to keep payload experiment at 37 °C for extended durations
- Launch: Nov 12, 2017 (ISS deployment: Nov 20, 2017)
- 1st 6U bio CubeSat and 1st bio satellite to be deployed from ISS

6U CubeSat



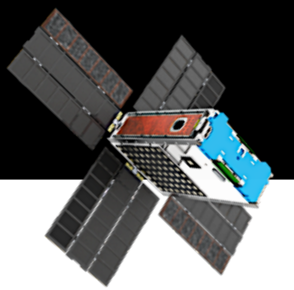






Deployment from ISS

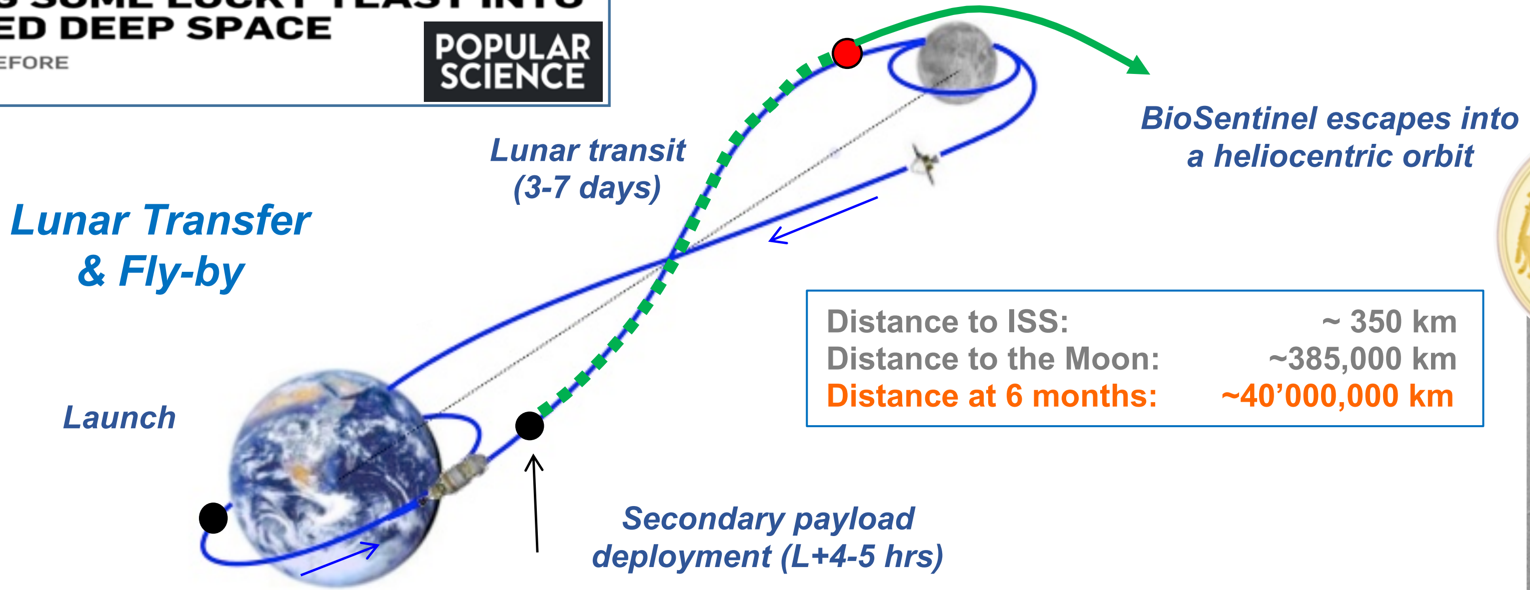
BioSentinel mission: NASA's 1st interplanetary bio satellite



<u>Main objective</u>: develop a tool with autonomous life support technologies to study the biological effects of the space radiation environment at different orbits

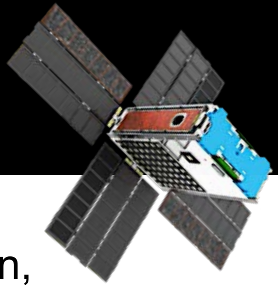
- First biological study beyond low Earth orbit (LEO) in 50 years
  - First biological 6U CubeSat to fly beyond LEO
  - First CubeSat to combine biological studies with autonomous capability & physical dosimetry beyond LEO
  - Secondary payload in SLS ARTEMIS-1 (launch in 2021/2022) – 13 payloads
  - Far beyond the protection of Earth's magnetosphere (~0.3 AU from Earth at 6 months; ~40 million km)
  - BioSentinel will allow to compare different radiation and gravitational environments (free space, ISS, Lunar surface…)


SPACE
NASA IS SENDING SOME LUCKY YEAST INTO RADIATION-FILLED DEEP SPACE
WHERE NO YEAST HAS GONE BEFORE
By Shannon Stirone May 15, 2015
POPULAR SCIENCE



Distance to ISS:	~ 350 km
Distance to the Moon:	~385,000 km
Distance at 6 months:	~40'000,000 km

What is BioSentinel?



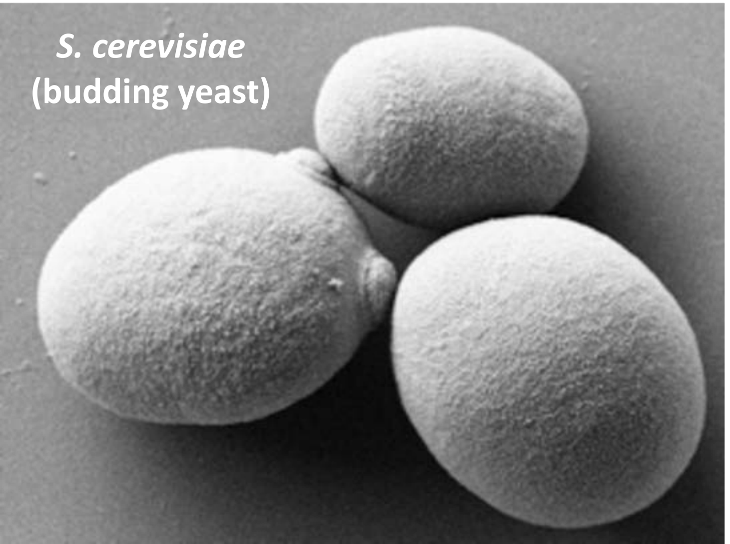
BioSentinel is a yeast radiation biosensor that will measure the DNA damage response caused by space radiation, and will provide a tool to study the true biological effects of the space environment at different orbits.

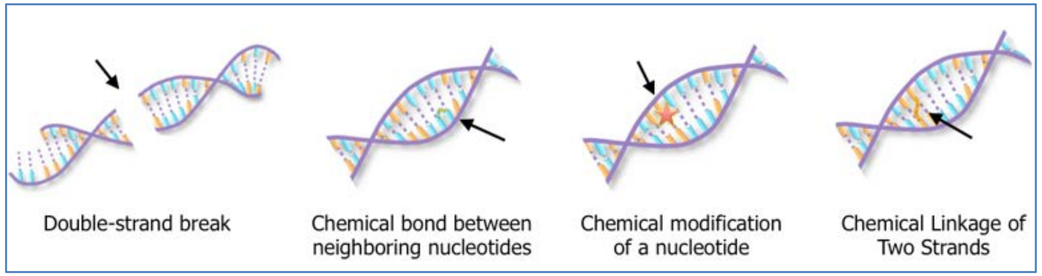
Why?

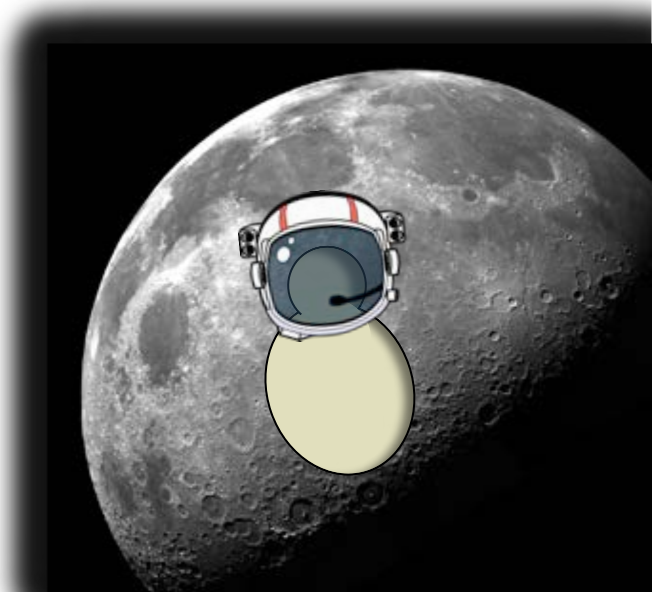
Space radiation environment's unique spectrum cannot be duplicated on Earth. It includes high-energy particles, is omnidirectional, continuous, and of low flux.

How?

Lab-engineered *S. cerevisiae* cells will sense & repair direct (and indirect) damage to their DNA. Yeast cells will remain dormant until rehydrated and grown using a microfluidic and optical detection system.







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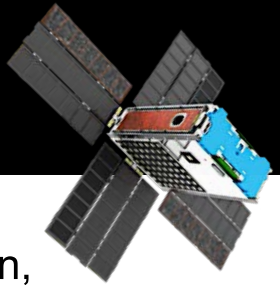

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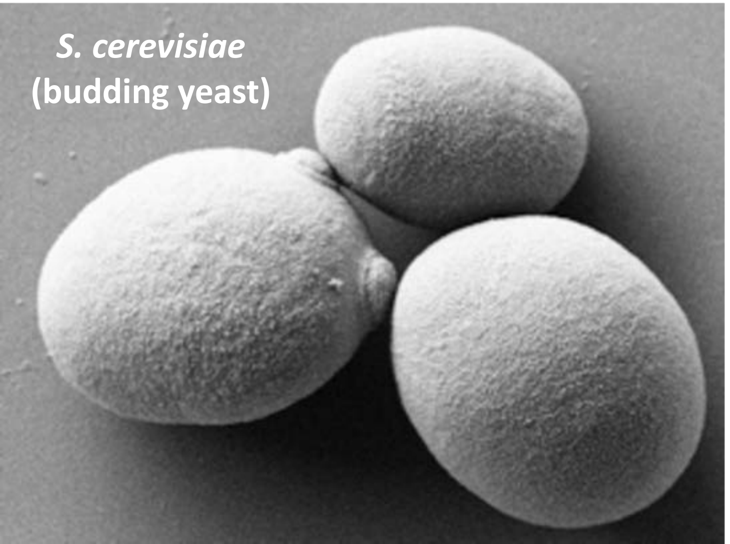
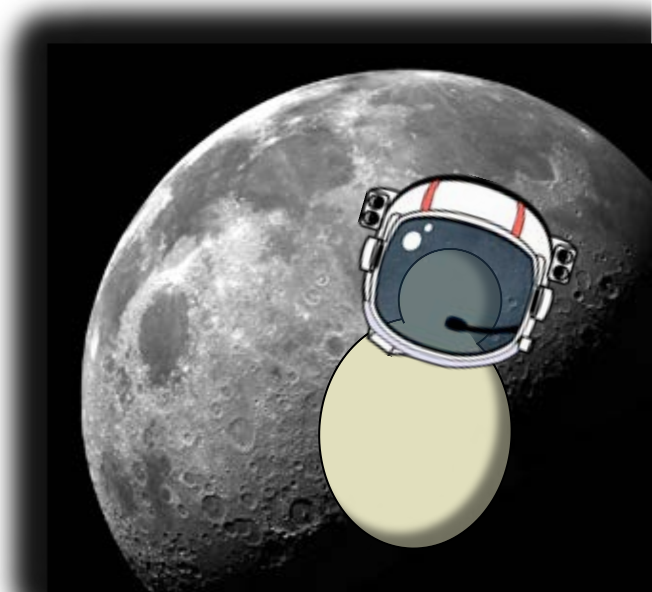


Why budding yeast?

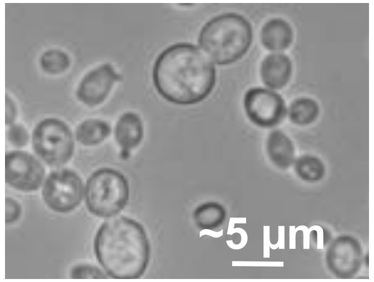
It is an eukaryote; easy genetic & physical manipulation; assay availability; flight heritage; ability to be stored in dormant state

While it is a simple model organism, yeast cells are the best for the job given the limitations & constraints of spaceflight

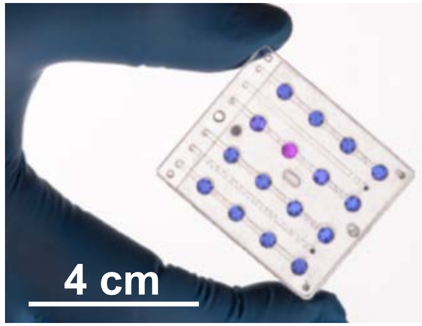


BioSentinel: a 6U nanosatellite for deep space

Budding yeast

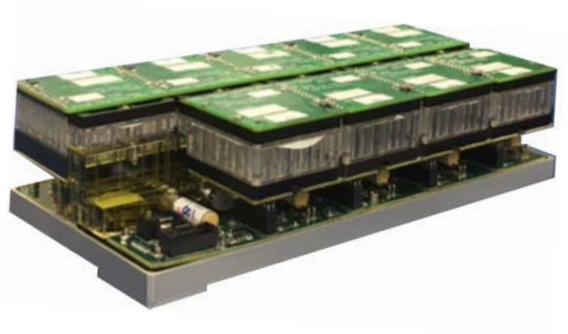


16-well fluidic card (x18)

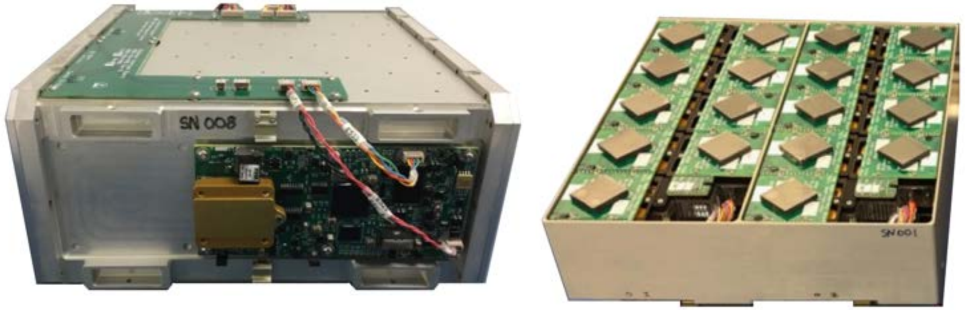


Card stack

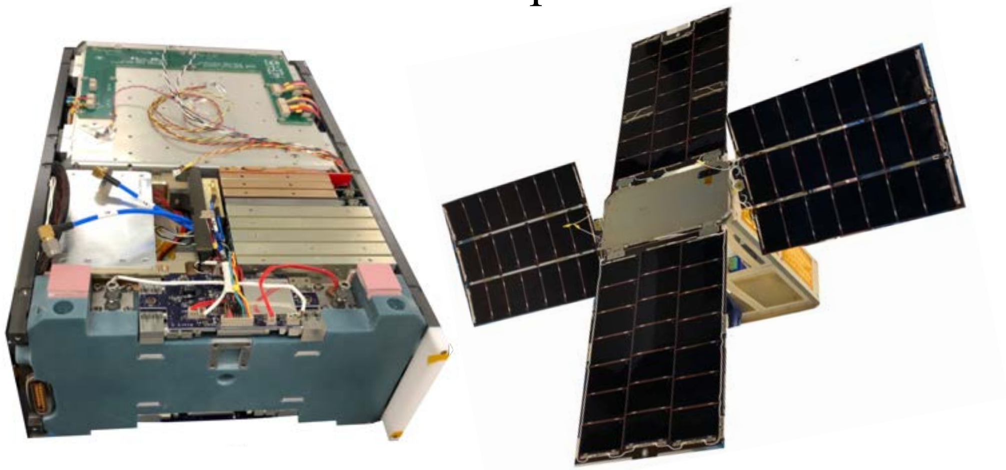
9-card fluidic manifold (x2)



4U BioSensor payload

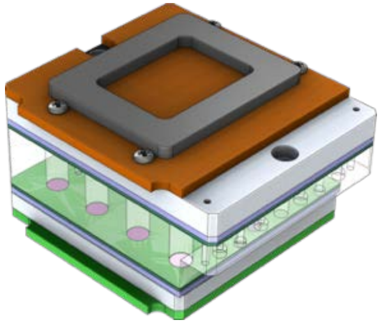


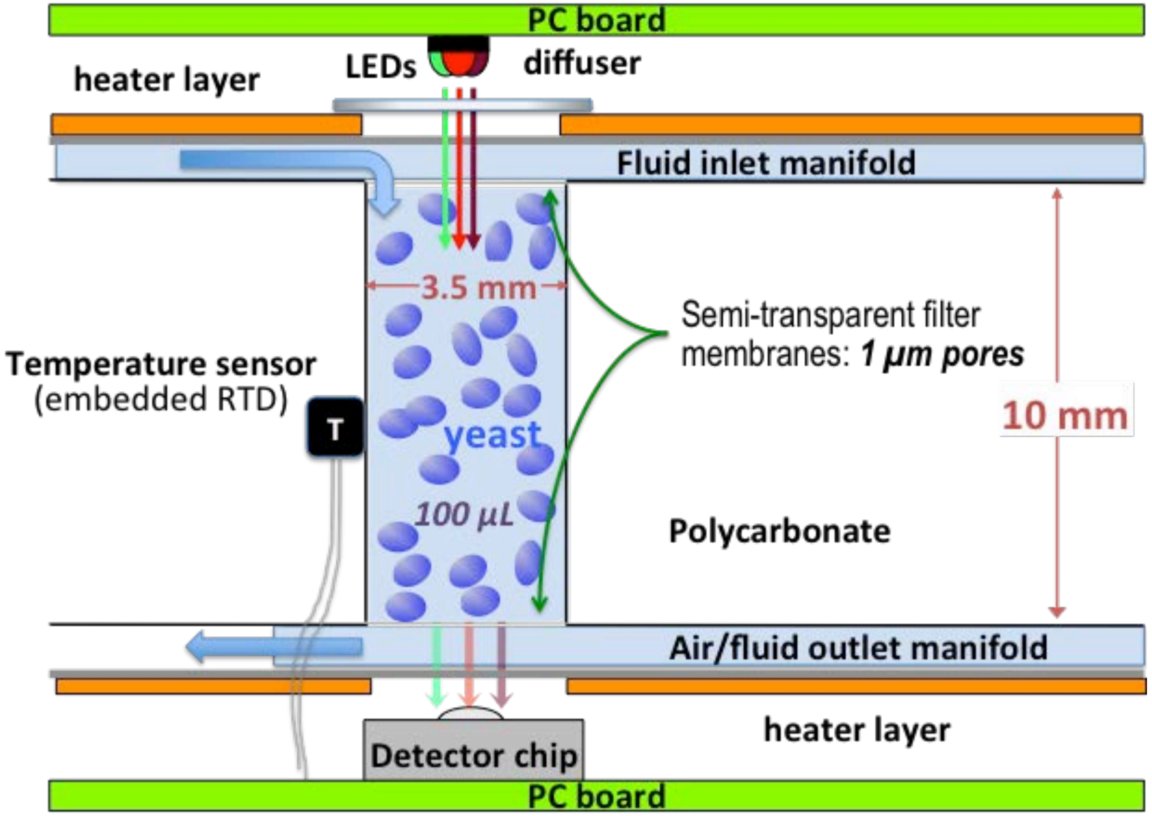
6U BioSentinel spacecraft



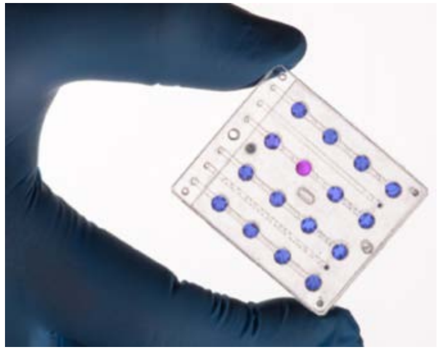
BioSentinel: microfluidics card

Microfluidic card (x18)

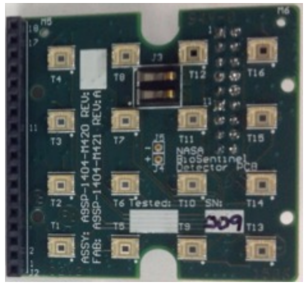


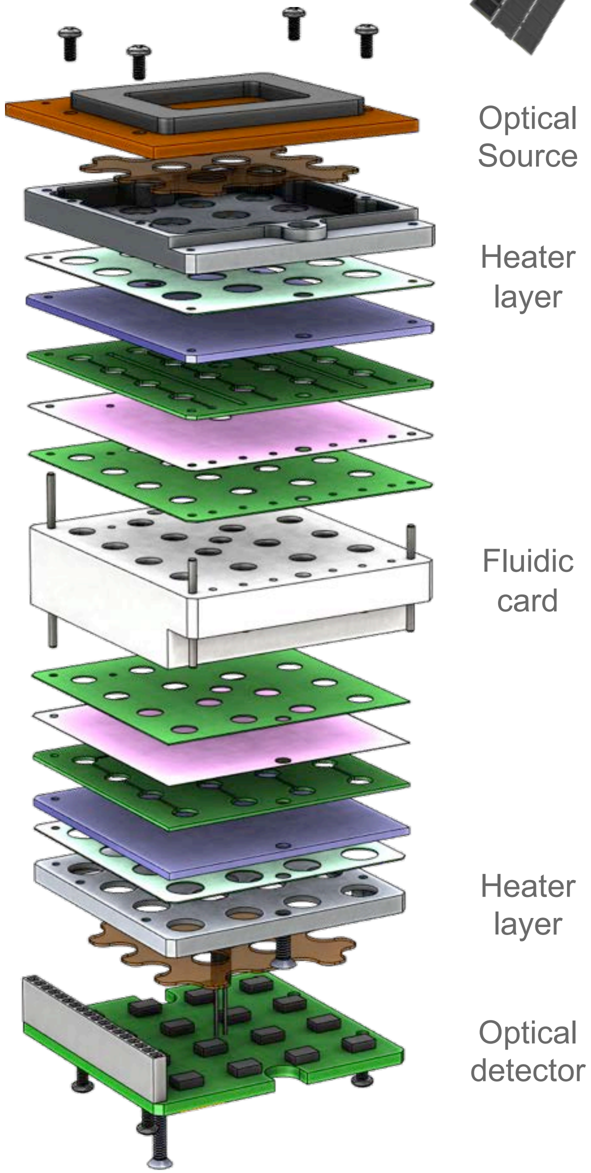


3-LED emitter

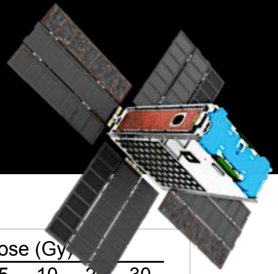


Photodiode detector array





BioSentinel: optical detection system

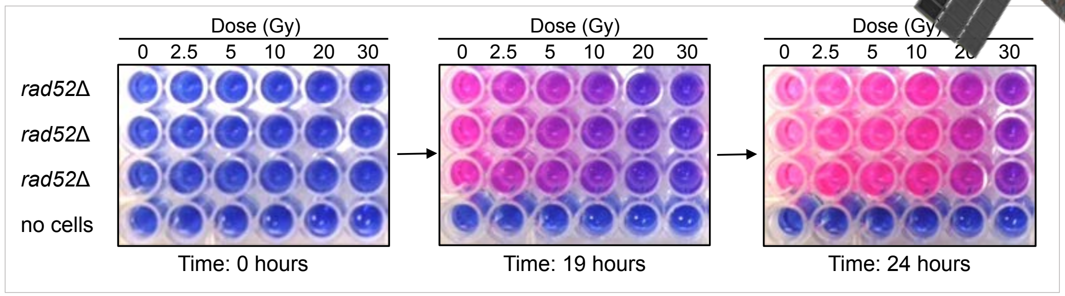


Dedicated 3-color optical system at each well to track growth *via* optical density and cell metabolic activity *via* dye color changes

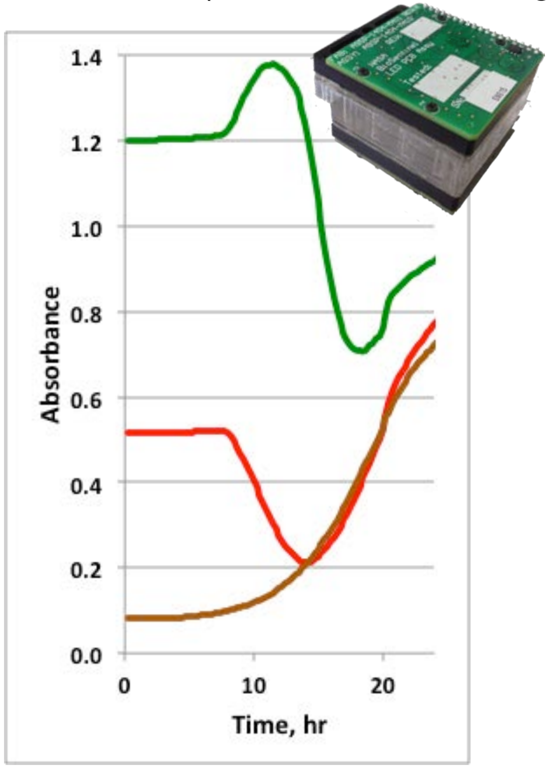
<u>LEDs</u>: 570 nm (green, measures pink)

630 nm (red, measures blue)

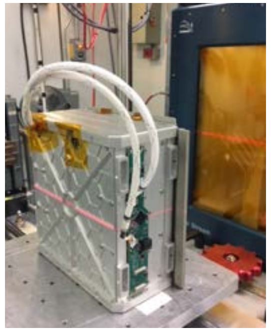
850 nm (infrared, measures growth)

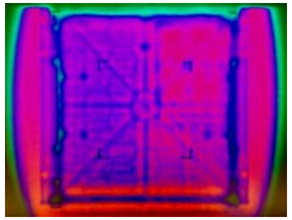


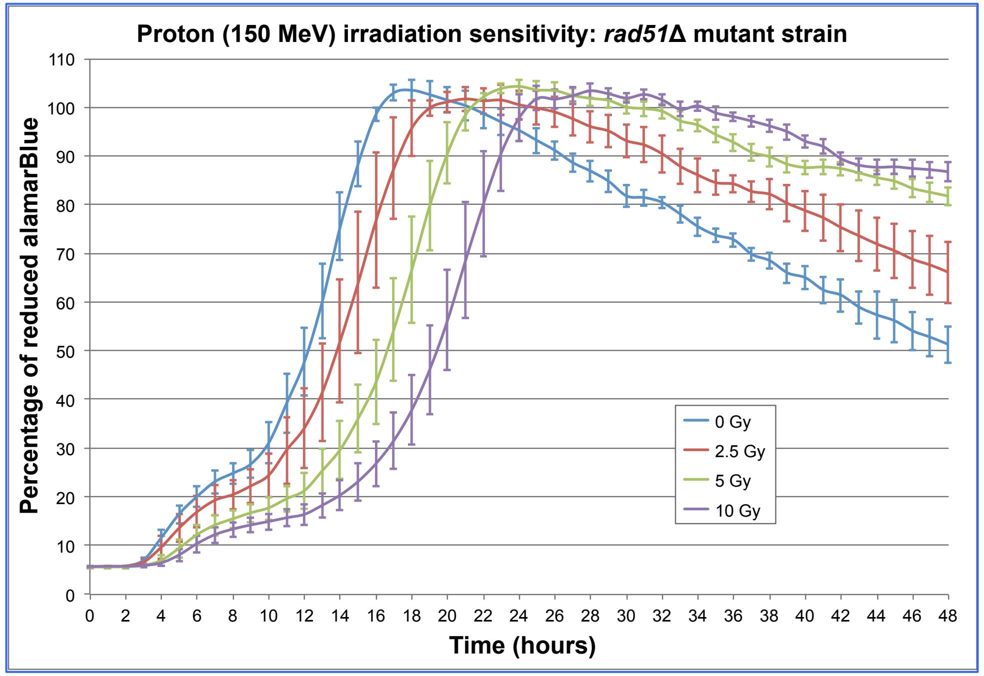
alamarBlue turns pink when cells are metabolically active



Yeast growth with flight-like optical unit



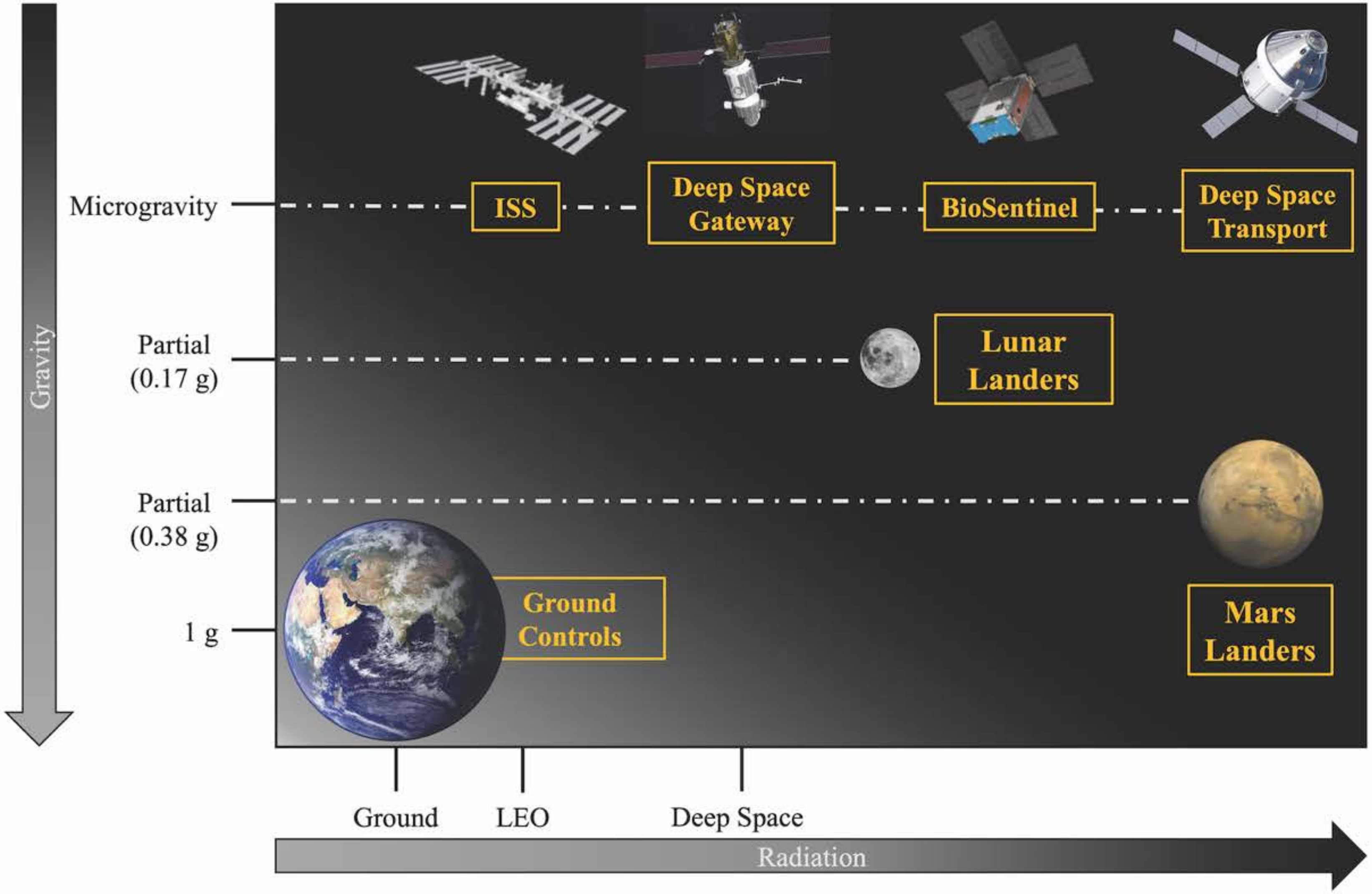




HR repair defective cells show sensitivity to ionizing radiation

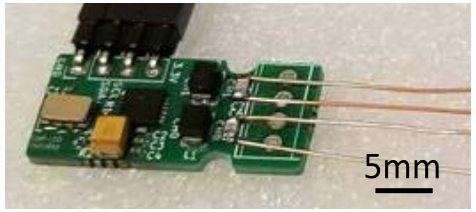
BioSentinel: future & ongoing objectives

A flexible design that can (and will be) used on different space platforms

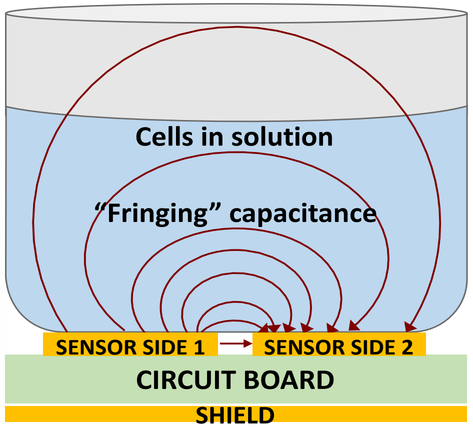


Examples of future biosensor technologies

Dielectric spectroscopy biosensor



Fluidic well

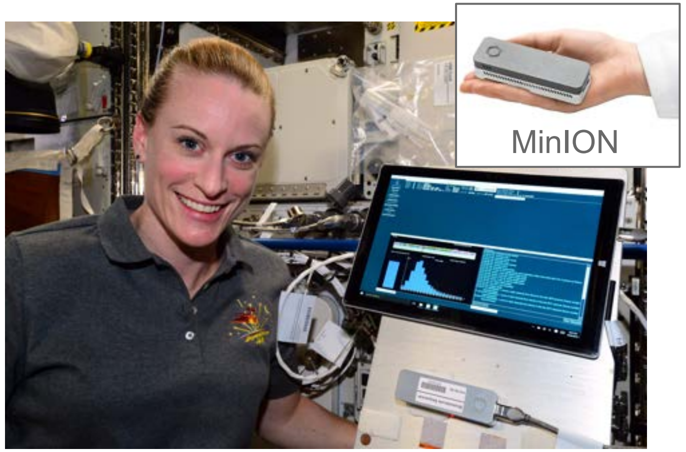


Goal: Develop miniaturized instrument for continuous non-invasive monitoring of the biological response to deep space radiation via bio electrical signatures.

TOP: Dielectric sensing prototype with electrodes connecting to microwell containing bio organisms.

BOTTOM: Rendering of prototype and cross-sectional illustration of contactless sensing mechanism.

Miniaturized bio sequencing devices



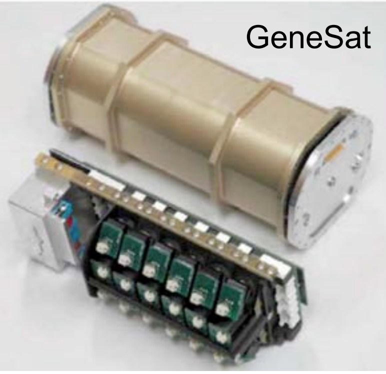
Credit: Oxford Nanopore Technologies

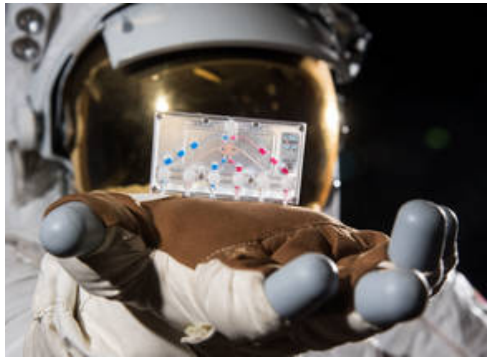
Goals: Design and develop instruments with integrated bio growth, sample extraction, and sequencing capabilities to study effects of space environment on microorganisms (*e.g.*, DNA mutations, gene expression)

Repurpose proven LEO technologies for deep space

Microcentrifuges to generate artificial gravity

Fluorescence microscopy





Organ / tissue on chip microfluidics

Conclusions

- **Nanosatellites like CubeSats can do real science in low Earth orbit (LEO) and in interplanetary deep space**
  - Instrument miniaturization & new micro/nano technologies
  - Fully automated instruments
  - Adaptable technologies for different platforms (ISS, free-flyers, Lunar landers & gateway)
  - Real-time, *in-situ* experiments provide insights on dynamics not available from expose-and-return strategies

- **Heritage of astrobiology and fundamental space biology experiments in LEO is a major enabler for interplanetary biological missions**
  - Flying biology in desiccated form, filling microfluidic cards/wells in microgravity
  - Long-term material & reagent biocompatibility (long-duration pre-launch preparation)
  - Radiation-tolerant design
  - High-heritage components: microfluidics, optical measurements, environmental sensors