Microscope for biological research in space

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Understanding how space environment affects on biological systems has become especially important now, when manned space exploration missions beyond the low Earth orbit are under planning. Mini Fluorescence Microscope is European Space Agency's project with an objective to develop a breadboard model of a microscope that could advance radiation and gravity related biological research in space. A flight model of the microscope would be developed in the subsequent project.

The purpose of this thesis is to investigate the potential of a miniaturized fluorescence microscope in space research. The thesis consists of three main topics. First, the purpose is to specify the fundamental questions in space biology that could be studied with microscopy approaches. Secondly, the state of the art in miniaturized microscopes and space microscopy are reviewed. Lastly, it is determined what are the potential platforms for this kind of instrument.

Based on the review, applications for miniaturized fluorescence microscope in space research are diverse. The health threats for humans in space are more or less characterized, but the underlying cellular mechanisms are poorly understood and require more research. Studying the survivability of microorganisms would benefit space exploration in many ways, such as by supporting the further development of planetary protection policies. The smallest of the reviewed microscopes were not standalone instruments and the microscopes previously used in space were relatively large. There is a need for more independently functioning and compact space microscope. The potential platforms are facilities on-board the International Space Station, CubeSats and rovers.

Keywords: space environment, radiation, microgravity, astrobiology, fluorescence microscopy, International Space Station, CubeSat, Mars rover

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Suunniteltaessa miehitettyjä avaruuslentoja Maan matalan kiertoradan ulkopuolelle, on yhä tärkeämpää ymmärtää miten avaruusympäristö vaikuttaa biologisiin systeemeihin. Mini Fluorescence Microscope on Euroopan avaruusjärjestön projekti, jonka tavoitteena on kehittää prototyyppi mikroskoopista, joka edistäisi säteilyyn ja painovoimaan liittyvää biologista tutkimusta avaruudessa. Lentomalli mikroskoopista kehitettäisiin seuraavassa projektissa.

Tämän tutkielman tarkoituksena on selvittää mitä pienikokoisen fluoresenssimikroskoopin käyttö mahdollistaisi avaruustutkimuksessa. Tutkielma koostuu kolmesta pääkohdasta. Ensimmäiseksi määritellään ne avaruusbiologian peruskysymykset, joita voitaisiin tutkia mikroskopiamenetelmillä. Seuraavaksi tarkastellaan pienikokoisten mikroskooppien ja avaruusmikroskooppien teknisiä ratkaisuja. Lopuksi määritellään mitkä olisivat mahdollisia käyttöalustoja tällaiselle mittalaitteelle.

Tutkielman johtopäätös on, että pienikokoisella fluoresenssimikroskoopilla on monipuolisia sovelluskohteita avaruustutkimuksessa. Avaruusympäristön ihmiselle aiheuttamat terveysuhat ovat jokseenkin määritelty, mutta niiden taustalla olevia solutason mekanismeja ymmärretään huonosti. Mikrobien selviytymisen tutkiminen avaruusympäristössä tukisi esimerkiksi planeettojen suojelupolitiikan kehittämistä. Pienimmät tarkastelluista olemassa olevista mikroskoopeista eivät olleet itsenäisesti toimivia laitteita, ja avaruudessa käytetyt mikroskoopit olivat puolestaan suhteellisen suurikokoisia. Itsenäisemmin toimivalle kompaktille avaruusmikroskoopille on siis tarvetta. Mahdollisia käyttöalustoja ovat kansainvälisen avaruusaseman laitteistot, CubeSatit ja mönkijät.

Asiasanat: avaruusympäristö, säteily, mikrogravitaatio, astrobiologia, fluoresenssimikroskopia, kansainvälinen avaruusasema, CubeSat, Mars mönkijä

Contents

Introduction

As of today, the Apollo missions remain the only manned missions beyond the low Earth orbit (LEO). The longest of them was Apollo 17, which lasted for 12.5 days [1]. Currently, more extensive manned missions beyond the LEO are under planning (e.g., NASA's Moon to Mars [2]), which is why it is particularly important to understand how the space environment affects on biological systems. It is important to establish a good overall picture what is the impact on humans, but also to understand how well bioregenerative life support systems function in space, what kind of risk pathogens pose during space travel and what is the possibility of microorganisms transferring interplanetary distances naturally or on-board spacecraft. In order to gain better understanding, more data is needed. A fluorescence microscope would be an advantageous instrument for research in this domain. Development of fluorescence microscope for biological research in space is recommended in the Roadmaps for Future Research (2016) by European Space Agency (ESA) [3]. The Independent Evaluation of ESA's Programme for Life and Physical Sciences in Space (ELIPS) also recognizes fluorescence microscope as a required technology for in-flight in-situ analysis [4].

Mini Fluorescence Microscope (MFM) is an ESA project with an objective to develop a breadboard model of a miniaturized fluorescence microscope that could perform live cell imaging in space [5]. A flight model of the microscope would be developed in the subsequent project. In order to plan the roadmap from the breadboard to a flight model, the specific application of the microscope needs to be determined. The purpose of this thesis is to investigate the potential of a miniaturized fluorescence microscope in space research. The thesis consists of three main topics. In Chapter 1, the purpose is to examine what are the fundamental questions in space biology that could be studied with a microscope. In Chapter 2, the fluorescence microscopy as an imaging technique is explained and the state of the art in miniaturized microscopes and space microscopy are reviewed. In Chapter 3, the purpose is to present in which platforms the microscope could be used in space and to specify what kind of different research possibilities they offer.

1 Space environment

Space environment differs from the environment on Earth in many aspects, such as radiation, temperature, pressure and gravity. The MFM is dedicated to radiation and gravity related biological research [5], hence these two components of the space environment, and their impact on humans and microorganisms, are presented.

1.1 Radiation

1.1.1 Solar ultraviolet radiation

Electromagnetic radiation is categorized based on wavelength as gamma-rays, x-rays, ultraviolet (UV), visible light, infrared, microwaves and radio waves. The spectral ranges with respective wavelengths and frequencies are presented in Figure 1. The Sun emits electromagnetic radiation from radio wavelengths to soft x-rays and occasionally hard x-rays and gamma rays during solar flares [8]. The Sun's electromagnetic spectrum corresponds roughly to the spectrum of a 5778 K blackbody [8], when about 90 % of its energy is emitted on visible and infrared wavelengths and 8 % on UV wavelengths [9].

The solar UV can be divided into the following subtypes: UVA (315-400 nm), UVB (280-315 nm), UVC (100-280 nm) and extreme UV (10-100 nm) [9]. The Earth's atmosphere is mainly composed of nitrogen and oxygen, from which the nitrogen absorbs effectively wavelengths shorter than 120 nm, attenuating the extreme UV completely [10]. Oxygen and ozone are the main absorbers in the atmosphere [10] and responsible for blocking rest of the UV with wavelengths below 290 nm [9]. This means that all the UVC and part of the UVB are absorbed, while the UVA radiation can penetrate the Earth's atmosphere. In comparison, the Moon does not have an atmosphere or a magnetic field and an unfiltered spectrum of radiation reaches its surface. In the case of Mars, there is some filtering, but due to the different atmospheric composition, UV radiation on the surface of Mars is more severe than on Earth. Wavelengths above 200 nm reach Mars' surface [9].

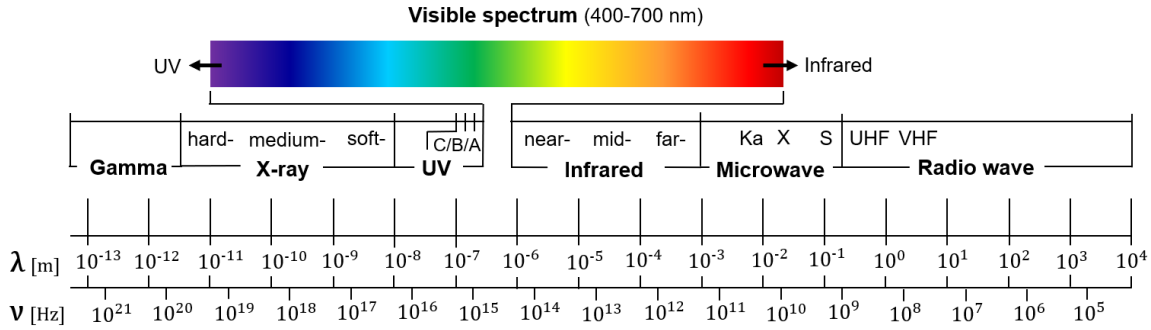


Figure 1: The electromagnetic spectrum with the respective wavelengths (λ) and frequencies (ν). Ka, X, S, UHV and VHF microwave and radio bands are presented according to the IEEE standard [6]. The figure is adapted from [7].

Outside the Earth's atmosphere, the average total solar irradiance is expressed with the solar constant, which is 1366±1.3 W/m^2 at 1 AU (the mean Sun-Earth distance). There are some temporal changes in the solar constant due to the magnetic activity of the Sun [11]. The solar irradiance is inversely proportional to the square of the distance. For example, at 1.524 AU, which is the mean orbital distance between Mars and the Sun, the solar constant is around 590 W/m^2 [9].

1.1.2 Trapped radiation

In addition to the photons originating from the Sun, the space radiation environment consists of energetic charged particles. A planet's magnetic field modulates the flux of energetic particles in vicinity of the planet and in some cases, radiation belts may form. The Earth's magnetic field is a dipole field, which means that the magnetic field converges at the poles. The particle movement in the dipole field consists of gyromotion around the magnetic field lines and drift motion due to the gradient and curvature of the magnetic field. The curvature drift causes electrons and ions to drift perpendicular to the magnetic field and the radius of the curvature, but in opposite directions. In the Earth's dipole field, electrons drift eastward and protons westward. There are mirror points in the northern and southern hemispheres, which form a magnetic bottle, a configuration that confines high energy particles. These trapped particles bounce between the mirror points,

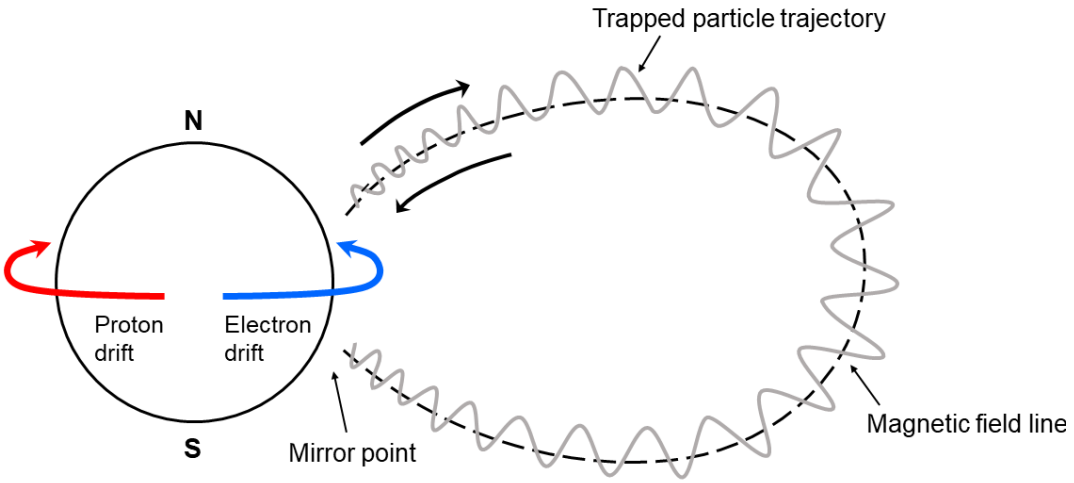


Figure 2: Particle movement in the Earth's dipole field. The particles are in gyromotion around the magnetic field lines and they drift due to the gradient and curvature of the magnetic field. The magnetic bottle traps the particles in bounce motion between the two hemispheres. Adapted from [12].

forming the Van Allen radiation belts [12]. The particle movement in the Earth's dipole field is summarized in Figure 2.

The Van Allen belts consist of two zones, of which the inner zone is more stable and the outer zone has higher variability due to solar activity. Protons with energies above 100 MeV are confined to the inner belt, specifically to altitudes below $3R_E$, where R_E is the Earth radius. Protons with energies below 1 MeV have been observed as far as $7R_E$ [13]. Electrons with energies below 1 MeV have been observed in two zones, which have a slot region that lacks particles in the middle of them. Relativistic and ultrarelativistic (energies above 5 MeV) electrons populate the outer zone [14].

One possible source of the energetic particles in the radiation belts is Cosmic Ray Albedo Neutron Decay (CRAND), where neutrons escaping the atmosphere decay into an electron, a proton and an anti-neutrino. This mechanism can explain the inner belt protons. The protons in the outer belt are from solar origin. The CRAND cannot explain electron energies above 800 keV and it can therefore contribute to the inner belt electrons, but does not explain all of them. Based on current knowledge, there are acceleration mechanisms that are responsible for the high energies [13].

Earth is not the only place in the Solar System to have radiation belts. Mercury has an intrinsic magnetic field like Earth and quasi-trapped particles, but with relatively low energies (typically between 1 and 10 keV) [15]. There is no evidence that Venus and Mars have significant magnetic fields and consequently, they do not have radiation belts either [16, 17]. The outer planets on the other hand are highly magnetized and have radiation belts with high energy particles [18]. Just to give examples, ultrarelativistic electrons with energies up to 50 MeV have been observed in the Jupiter's radiation belts [19]. Saturn has two radiation belts: an outer belt that extends outside the planet's rings and an inner belt, located inside the rings, containing high energy protons with energies from 25 MeV up to few GeV [20]. Measurements of Voyager 2 spacecraft revealed that there are significant fluxes of electrons and protons with energies above 1 MeV trapped in the magnetospheres of Uranus [21] and Neptune [22].

Solar activity plays a role in the radiation belt dynamics and in the case of the outer planets, moons and rings function as absorbers of trapped radiation and add complexity to the systems. All in all, radiation belts are very dynamic and not yet completely understood environments.

1.1.3 Solar energetic particles

The solar energetic particle (SEP) events and can be observed as distinct increases in energetic electron, proton and heavy ion fluxes [23]. These particles originating from the Sun contribute to the radiation belt dynamics, but also pose a major hazard during space travel because of their yet unpredictable nature.

SEP events can be divided into impulsive and gradual events, from which impulsive events are associated with solar flares and gradual events with coronal mass ejections (CMEs) [24]. Impulsive SEP events are more common and less intense than gradual events. Typically impulsive events last less than one day, when in comparison, gradual events can last for several days. Typical energies of SEPs range from hundreds of keV up to few GeV. The GeV protons are from gradual events, where CME-driven shocks accelerate particles [23]. Impulsive and gradual events usually

differ in particle abundances as well. Impulsive events are dominated by electrons, also being rich in heavier ions and ^{3}He, while gradual events mainly consist of protons. Because the SEPs are produced by solar flares and CMEs, the number of events is correlated with solar activity [24].

SEPs are not detected directly at the surface of Earth because the Earth's magnetic field limits the access of majority of SEPs to the atmosphere. If the particle's magnetic rigidity is above the geomagnetic cut-off value, it can penetrate the magnetic field, and in general, the higher the particle's energy is, the deeper it can penetrate the atmosphere. Sometimes particles also gain access due to disturbances in the magnetosphere. The high energy particles that reach the atmosphere, either get absorbed or collide with nuclei of the atmospheric gases and start a cascade of secondary particles [25].

1.1.4 Galactic cosmic rays

Galactic cosmic rays (GCRs) are particles that originate from outside of our Solar System. Around 90% of GCRs are protons, the rest are helium nuclei and smaller abundances of heavier ions [26]. The cosmic ray spectrum is roughly a power law and covers a wide range of particle energies, as can be seen in Figure 3. The intensity maximum of the cosmic ray spectrum is around 0.3 GeV, meaning that the largest percentage of GCRs have energies close to that [28]. According to the current knowledge, the bulk of GCRs are accelerated in supernova remnants by diffusive shock acceleration [26, 29]. These are the particles on the left side of the "knee". After the knee, the spectrum steepens a little bit and the relative elemental abundances change, which indicates to a different source mechanism. At the "ankle" the spectrum shape and particle compositions change again. The cosmic ray spectrum reaches up to ultra-high energies of 10^{20} eV [27]. In comparison, the magnitude of the highest proton energies achieved in the most energetic particle collider on Earth, the Large Hadron Collider in CERN, is 10^{12} eV [30]. It is hard to identify the source of the highest energy particles, because the flux is so weak that the particles are hard to detect [27].

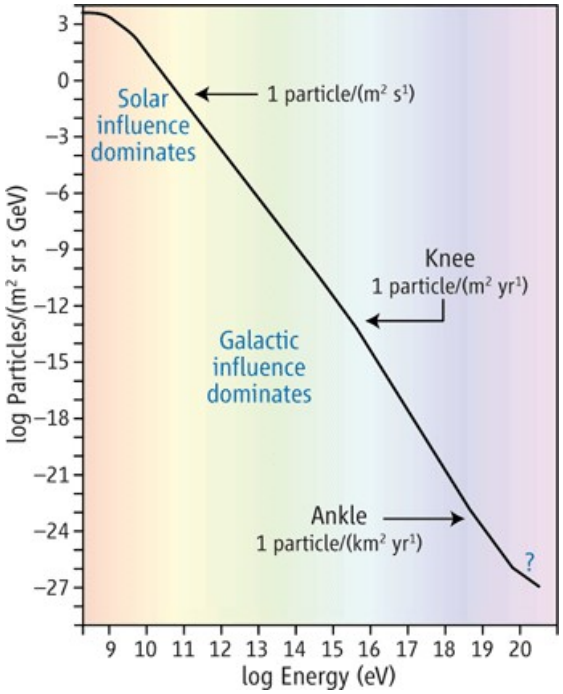


Figure 3: Cosmic ray flux as a function of particle energy. Reprinted with permission from [27].

In order to be observed in the near-Earth space, the GCRs need to penetrate the heliosphere's magnetic field. The magnetized solar wind modulates the GCR flux, which means that particles below certain energies are deflected at the heliopause and termination shock. This is why low energy particles are not shown in the GCR spectrum. Of the particles that enter the heliosphere, the low-energy end of the spectrum is still strongly affected by the solar magnetic field [27]. The GCR flux modulation follows the 11-year solar cycle and is anti-correlated with the solar activity. This means that the GCR flux is the largest during solar minimum and the smallest during solar maximum [31]. When compared to SEP, the GCR flux is much more stable and predictable, but the fact that GCRs are protons and heavier ions, travelling at almost the speed of light, makes it really difficult to shield against them.

1.2 Gravity

The Earth's gravity is a combination of two forces: the gravitational force and the centrifugal force acting on a unit mass. The gravitational force is one of the four fundamental interactions (the other three are electromagnetic, strong and weak interactions) and the centrifugal force arises from the Earth's rotation. Gravity is described with the gravity vector **g** and its magnitude g, and it has the same unit as acceleration, which is in SI units m/s^2 or N/kg, equivalently. Numerical value of g depends on the location, but near the Earth's surface, it is around 9.83 m/s^2 at the poles and 9.78 m/s^2 at the equator [32].

The MFM is designed for partial and microgravity related biological research [5]. In practice, a zero gravity environment, and thus, real weightlessness, cannot be achieved. Consequently, the term "microgravity" is used to describe nearly zero gravity environments, which can be achieved by counterbalancing the gravitational and inertial forces [33]. Microgravity environment can be created for short periods of time for experiments. Up to ten seconds of microgravity can be achieved by using drop towers, which are vertical structures allowing free fall of the payload. Around 25 s of microgravity can be achieved in parabolic flights when the aircraft executes maneuvers at 45 degree angle. Some microgravity experiments have also been conducted by dropping the payload from a stratospheric balloon. With a sounding rocket, from five to six minutes of microgravity can be achieved [34]. In a laboratory, a microgravity experiment can be conducted by placing the payload into a random positioning machine (RPM). The RPM is an instrument, typically used in biological experiments, that rotates along two independent axes, causing the gravity vector of the payload to average to zero over time. The microgravity created with RPM is comparable to true microgravity if the object responses to gravity slower than the direction changes [35].

The only way to establish a long-term microgravity environment is to go to space. A spacecraft in an orbit around Earth is in constant free fall, when this equilibrium of gravitational and inertial forces occur [33]. An example of a facility where microgravity related research can be conducted in space is the International

Space Station (ISS). The ISS is the largest and most complex vehicle in the low Earth orbit, an achievement of the international collaboration between NASA, Russian space agency Roscosmos, ESA, Japan Aerospace Exploration Agency (JAXA) and Canadian Space Agency. The construction of the ISS began in 1998 with the Russian Zarya module, and the assembly of the station remained the primary objective of its first 11 years of operations. The main laboratories that were added are the U.S. Destiny laboratory, integrated in 2001, and the European Columbus and Japanese Kibo scientific modules, integrated in 2008 [36].

The microgravity level achieved on the ISS is between 10^{-3}g and 10^{-6}g. It is a complicated environment, since many factors contribute to the accelerations. These factors are aerodynamic drag, rotational effects, gravity gradient and operation of all the systems and equipment on the station. The first three of these factors are the main sources of quasi-steady accelerations. Typical definition for quasi-steady accelerations is that at least 95 % of the power of accelerations is below 0.01 Hz, when measured over the approximate duration of one orbit. Based on the ISS requirements, the resupply, reboost and maintenance of the space station are planned so that there are at least six 30 day periods per year, when the quasi-steady accelerations in the main research facilities are below 10^{-6}g [37].

The term "partial gravity" is used to describe the gravity between microgravity and Earth's gravity. Partial gravity environments are, for example, surfaces of the Moon and Mars, which have average surface accelerations of 1.62 m/s^2 ($\sim$0.166g) and 3.72 m/s^2 ($\sim$0.379g), respectively [38,39]. Partial gravity can also be simulated in a laboratory with a RPM.

1.3 Impact on humans

In terms of radiation and gravity, the space environment is very different from the environment on the surface of Earth. The Earth's atmosphere absorbs the ionizing part of the electromagnetic radiation. The energetic charged particles that are able to penetrate the Earth's magnetic field either collide in the atmosphere producing secondary particles or get absorbed. Even the LEO is not yet that severe environ-

ment in terms of radiation, because the Earth's magnetic field blocks most of the SEPs and GCRs. There is one region in LEO where the radiation is more intense, called the South Atlantic Anomaly (SAA). In this region the radiation belt reaches altitude of approximately 200 km. Major part of the radiation doses that astronauts on the LEO receive comes from passages through the SAA [40]. Near Earth the planet itself acts as a solid shielding as well.

Exploration missions beyond the protection of Earth are exposed to all the types of radiation described previously, in addition to different gravity levels. Considering astronaut safety and the different radiation sources, the solar UV is the easiest to cope with. The shorter wavelengths of solar UV are biologically harmful, because DNA can effectively absorb them and this can lead to mutations, cancer or death of cells. However, humans can easily protect themselves from the solar UV with proper shielding, as long as the material's response to UV radiation is known [41]. The relatively low energy protons and electrons of the solar wind are also easy to shield against and the risk they pose is considered negligible [42].

More problematic are the energetic charged particles, which contain high charge (Z) and energy component (HZE ions). From these particles, SEPs are typically less energetic and therefore more manageable than GCRs. Bulk of SEPs can be stopped with well designed shielding, but the main hazard related to them is their unpredictable nature. There is a risk that SEP event occurs during extravehicular activity, when astronauts are less protected [43]. This problem can be tackled with storm shelters and alert systems. GCRs are harder to shield against, since they penetrate greater distances in materials. In general, the radiation doses absorbed by the astronauts can be reduced with shielding, but not eliminated completely. Adding shielding means adding mass to the spacecraft, and the spacecraft's mass cannot be increased arbitrarily due to launch system limitations [40].

Linear Energy Transfer (LET) is a quantity used to describe the energy that a charged particle transfers to material. Mathematically,

$$\text{LET} = \frac{dE_L}{dl},$$

which means that a charged particle with specific energy traverses a distance dl and locally transmits an average energy dE_L to the medium [44]. When HZE ions traverse shielding material or tissue they can also produce secondary particles, such as protons, neutrons, alpha-particles, mesons and gamma-rays [45]. The ionizing radiation is typically described as low-LET or high-LET radiation. Impact of radiation on humans can be studied from the data gathered from radiation workers, patients exposed to radiation treatment and the Hiroshima and Nagasaki atomic bomb survivors. However, they have been mostly exposed to low-LET radiation, which is mainly gamma- or x-rays. Astronauts would be exposed to high-LET radiation, such as protons and HZE ions. There is not a lot of data on humans exposed to high-LET radiation, since only 24 astronauts have travelled beyond the LEO during Apollo era, with maximum mission duration of 12.5 days [1]. It is clear that this is not enough data to build statistical models.

From biological point of view, the low-LET is not directly comparable to the high-LET environment. High-LET radiation causes different reactions and damage in cells and tissue than low-LET radiation, such as more complex DNA damage and lower repair rate [46]. Cells also seem to react to high-LET radiation differently if they have first been exposed to low-LET radiation [47]. Typically only one type of radiation is studied in laboratories, which does not correspond to the space environment, where simultaneous exposure to multiple types of radiation is possible [42]. The synergistic effect of space radiation and microgravity at cellular level has also been studied, but due to conflicting results the effects are not well characterized [48, 49].

Radiation exposure and reduced gravity are the main factors endangering the astronaut's health, alongside with isolation, which can cause psychological problems [40]. Radiation effects can be divided into acute and late effects. Radiation

sickness with symptoms like fatigue, nausea and vomiting, or in extreme cases death, is an example of an acute effect. Exposure to a large SEP event is an example of a situation that can cause radiation sickness in space. Late effects include cancer and degenerative tissue effects. Microgravity causes reduced immune response, muscle atrophy and bone mass loss [46, 50, 51]. There are countermeasures to battle some of these effects, for example, the bone mass loss can be diminished by doing regular workouts. Still, even with daily workouts with specially designed equipment, the astronauts on the ISS lose on average 1-2 % of their bone mass during every month in space (in normal gravity conditions, the bone formation and resorption are balanced in healthy people) [52].

Many of the health effects are not due to one factor, but the combination of radiation and microgravity. For example, radiation also induces bone mass loss, but the synergistic effect of radiation and weightlessness is not very well known. Few studies have been conducted in laboratories using simulated microgravity, but more research about the cellular mechanisms is required in order to establish a good overall picture [52]. Both radiation and microgravity can cause problems in the cardiovascular system [50, 51]. Radiation and microgravity have impact on the central nervous system as well, and acutely this combination can affect on short-term memory, motor function or behavior. Possible late risks include premature aging and dementia [45].

The possible health effects are more or less characterized, but it is widely emphasized that the biological processes that cause these health effects are poorly understood (e.g., [40, 41, 45, 47, 50, 52–54]). Terrestrial analogs can be used for this kind of research, but they have their limitations. Due to the complexity and the dynamic nature of the space environment, it cannot be fully simulated on the ground. Animal models have also been utilized in aim to gain better understanding of the effects of space radiation on humans. However, animal models are challenging due to many reasons. Animals typically used in the experiments, such as rodents, are much smaller than humans and scaling the particle energies down also changes the LET-spectrum. The usage of larger species that would probably have better corre-

lation with humans involves ethical problems. The animals used in the experiments typically have shorter lifespan than humans and there are differences in the response to radiation between species, which complicates the extrapolation to humans [42].

In order to build better and more realistic models for assessing the health threats, more reseach in space environment is needed. Understanding the functionality of cellular mechanisms in space also has relevance for Earth applications, such as cancer research [55]. Space based pharmaceutical research is also a growing field [56]. Summary of major topics in space biology and common experimental endpoints that could be studied with microscopy approaches are presented in Table 1.

1.4 Impact on microorganisms

Another application for a microscope in space research would be to study microorganisms. Generally, the solar UV is one of the most immediately lethal components of the solar radiation for microorganisms, because biological macromolecules, such as proteins, nucleic acids and lipids can efficiently absorb it [57]. Similarly to humans, HZE ions can cause severe damage, such as DNA double-strand breaks, mutations and even cell death. Microgravity seems to cause different biological responses on microorganisms than normal gravity conditions, but it is still under debate whether these effects are due to microgravity itself or due to the changes in chemical environment that the microgravity causes. When exposed to microgravity, the physical forces acting on a body and its environment change, which alters the transfer of nutrients and metabolic by-products. This modifies the chemical environment, which leads to a different biological response [56].

However, there are microorganisms that can survive and even thrive in conditions, that are extreme or deadly from anthropocentric point of view. These conditions include, for instance, high and low temperatures, pressure, pH, and radiation, vacuum and chemical extremes. To give examples, there are microorganisms with maximum growth in temperatures above 80°C and others with maximum growth below 15°C. There are also many microorganisms that can be successfully preserved in -196°C [58]. Of the radiation tolerant bacteria, one of the most famous

Table 1: Summary of major topics in space biology with experimental endpoints that can be studied with microscopy approaches. Adapted from [55].

Theme	**Major topic**	**Experimental endpoint**
Cell physiology	Cell viability and growth kinetics	Cell number, live/dead ratio
	Cell morphology and architecture	Cell shape, granularity, cytoskeletal features (e.g., size and number of focal adhesion points or stress fibers)
	Cellular metabolism	Oxygen or glucose consumption rates, ATP levels, intra- and extracellular pH
	DNA damage and repair kinetics	Repair foci number or occupancy, repair protein immobilization and residence time, repair complex composition, non-linear responses (e.g., bystander effects)
	Cellular stress and redox balance	Reactive oxygen species and antioxidant levels, mitochondrial potential, transcription factor translocation
Spatiotemporal behaviour	Gene expression patterns and regulation	Promoter activity, transcription factor translocation, protein turnover rate
	Cell migration	Wound healing speed, cell invasion potential, chemotaxis
Cellular micro-environment	3D cell and tissue architecture	Morphology, cytoskeletal features, cell type distribution
	Cell-cell and cell-ECM interactions	Abundance and distribution of cell adhesion molecules, receptor interactions, enzyme activation, mechanotransduction pathway activation
	Cell-cell communication	Gap junction abundance, synchronized cell activity (e.g., calcium signaling), extracellular cytokine levels, exo-/endocytosis rates
Developmental biology	Cell plasticity and differentiation	Morphological features, cell-line specific biomarkers
	Developmental aberrations	Defects in proliferation, migration, morphology, gene expression
Diagnostic and therapeutic applications	Tissue specific and individual radiosensitivity	DNA damage repair and kinetics, cytotoxicity
	Tissue specific and individual gravisensitivity	Cytoskeletal reorganization, activation mechanotransduction pathways
	Hadron therapy	DNA damage repair and kinetics, cytotoxicity, bystander effects
	Tissue regeneration	3D cell organization, morphology, differentiation
	Countermeasures	Any of the above in the presence of acute or chronic administration of small molecules or bioactive peptides

is *Deinococcus Radiodurans*, which can survive radiation doses up to 5000 Gy (in SI units J/kg) without measurable decrease in viability [59]. In comparison, a whole body dose of over 10 Gy, or even lower without medical treatment, typically leads to death in humans (the value can vary due to many factors, but it gives an estimate of the magnitude) [60].

In addition to microorganisms specialized in certain extreme, there are microbial systems that can cope with the combination of radiation, microgravity, vacuum and temperature of the space environment, such as spores of *Bacillus Subtilis* [61]. Direct exposure to the solar UV radiation kills the spores within seconds, but with some shielding against the UV, such as dust, rock or clay, survival is possible. It is also possible for another layer of microorganisms to work as shielding. After six years in space during the NASA's Long Duration Exposure Facility mission, a significant amount of *Bacillus subtilis* spores survived, when exposed to space environment in multilayers. The uppermost layer had inactivated and formed a crust that protected the inner layers from the UV radiation [62]. More recent SPORES experiment of the EXPOSE-R mission, conducted outside the ISS, showed again that the extraterrestrial UV radiation is lethal to *Bacillus Subtilis* spores. All the spores exposed directly to the UV in monolayers were inactivated, when analyzed after two years of exposure. However, some of the samples were stacked, when the bottom layers had higher survival rate similarly to the previous experiment [63].

If microorganisms can survive in the space environment, then a fundamental question is that can life be distributed in the Solar System naturally, for example, by meteorites? Based on a hypothesis called panspermia, microorganisms could be transported interplanetary distances by natural processes. The theory has gotten both strong support and criticism. Mileikowsky *et al.* [64] concluded that if there existed or exists procaryote microbes on Mars, the transfer to Earth by natural processes is highly probable. Contradictory conclusions, for example by Clark *et al.* [65], state that the interplanetary transfer of microorganisms is unlikely. A question related to the transfer of microorganisms in space is that how well does the space environment sterilize spacecraft? Planetary protection policy has been formulated

by Committee on Space Research (COSPAR) to avoid biological contamination of planets and other celestial bodies. There are certain limits to the allowed bioburden and procedures to reduce it. Celestial bodies have also been categorized based on their potential to sustain life and the policies are more strict in the case of potentially habitable places. For example, the policies are more strict in the case of Mars, Europa and Enceladus than in the case of the Moon [66].

In the PROTECT experiment of the EXPOSE-E mission, a trip to Mars was simulated by exposing *Bacillus subtilis* and *Bacillus pumilus* spores to the space environment and to Martian surface conditions ($\geq$ 200 nm UV radiation and Martian atmospheric concentration inside the experiment container) for 1.5 years. The experiment was conducted outside the ISS. The *Bacillus pumilus* spores were isolated from the airlock between the clean room and entrance of the spacecraft assembly facility in Jet Propulsion Laboratory. Similar results were obtained than in the previous experiments: the solar UV was the most lethal component of the space environment and the samples in multilayers had higher survival rate than the ones in monolayers. When covered from the UV radiation, but exposed to all the other factors of the space environment, over 50 % of the *Bacillus subtilis* and 15 % of the *Bacillus pumilus* spores survived [67].

The results indicate that spores could survive the journey to Mars if shielded by the spacecraft, or if placed in multilayers in cracks of the spacecraft surface. This kind of research is important for the further development of planetary protection policies. Especially now it has become particularly important to know what is the possibility to spacecraft biologically contaminate its destination, when rover missions with aim to study biosignatures on Mars are being prepared. ESA is sending its ExoMars rover to Mars with a sole purpose to hunt down traces of past or present life [68]. NASA's Mars 2020 Perseverance rover has similar science objectives [69]. It is important to understand what is the possibility of terrestrial life surviving the journey to Mars and on the planet, to avoid false positive results.

There have been multiple exposure studies concerning microorganism survival in space in addition to the ones presented in here [70, 71]. However, the current

exposure facilities have limitations, which is why Cottin *et al.* [70] summarized recommendations for future facilities and instruments. Commonly the samples are only analyzed on the ground before and after the experiment. This means that the adaptation processes or the response to the microgravity cannot be studied. Therefore, new instruments that allow analysis throughout the experiment are recommended. One of the limitations of current facilities is that the results are typically extrapolated from few replicates of the test sample. Multiplication of the samples is recommended to attain statistically better results. Most of the exposure experiments have been conducted on the LEO (apart from few CubeSat missions presented in Chapter 3.2.2). Suggestions include instruments that could be implemented in platforms, such as CubeSat, which could take the samples on orbits with greater exposure to energetic charged particles [70]. One of the suggested analysis methods is to quantify the viable cells by using fluorescent dyes [71]. A miniaturized fluorescence microscope enabling in-situ analysis of the samples would answer to these needs.

Another topic is the risk microorganisms pose and the benefits they offer for humans in space. It has been observed that some bacteria become more pathogenic and more drug resistant during space flight, but the reason for this is not yet fully understood. This can be a detrimental combination with the possibly reduced functionality of the astronaut's immune system [56]. On the other hand, microorganisms could be useful during space flight. They could be used in bioregenerative life-support systems, performing waste degradation, water recycling and food and oxygen production. In order to build reliable systems and to create mathematical models that can be used to improve and maintain control of the life-support, a comprehensive database including, for example, growth parameters and metabolic responses of the candidate microorganisms is needed [72]. The use of microscope could advance the research in these domains.

2 Microscope

The MFM project is about miniaturizing a fluorescence microscope for space applications [5]. This chapter presents the fluorescence microscopy as an imaging technique and reviews the state of the art in miniaturized microscopes and space microscopy. Based on the review, some useful features for a miniaturized space microscope are presented.

2.1 Fluorescence imaging

Fluorescence microscopy comprehends a wide range of optical microscopes that make use of the fluorescence phenomenon. A simple epifluorescence microscope, confocal and multiphoton microscopes and novel super-resolution achieving stimulated emission depletion (STED) and saturated structured illumination (SSIM) microscopes are all examples of fluorescence microscopes [73]. Fluorescence microscopy is widely used and powerful imaging technique among cell and molecular biologists, since it allows to acquire images that have a high contrast between the sample and the background.

The physics behind the fluorescence phenomenon is related to the transitions between different energy levels due to excitation and emission. The molecular energy levels, or states, can be divided into three different types: electronic states, vibrational states and rotational states. Figure 4 represents the so-called Jablonski diagram, which describes the energy states in a molecule, the possible transitions between them and the typical time scales of different transitions.

Excitation is a process when an electron in a molecule absorbs a photon and uses this energy to transition into a higher energy state. The singlet states are electronic states that can be occupied by two electrons with opposite spins according to Pauli's exclusion principle. If one of the electrons in a singlet state is excited to an upper singlet state, the spin stays coupled to its original pair, meaning that the spins of the two electrons remain opposite to each other. The singlet states are denoted as S_0, S_1, S_2,..., from which the S_0 is the ground state of the molecule. It is also

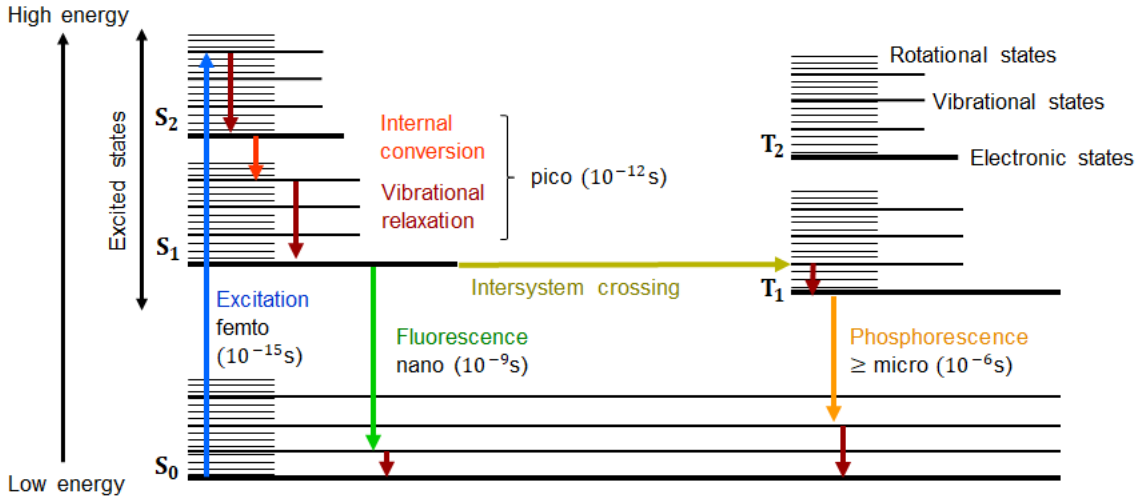
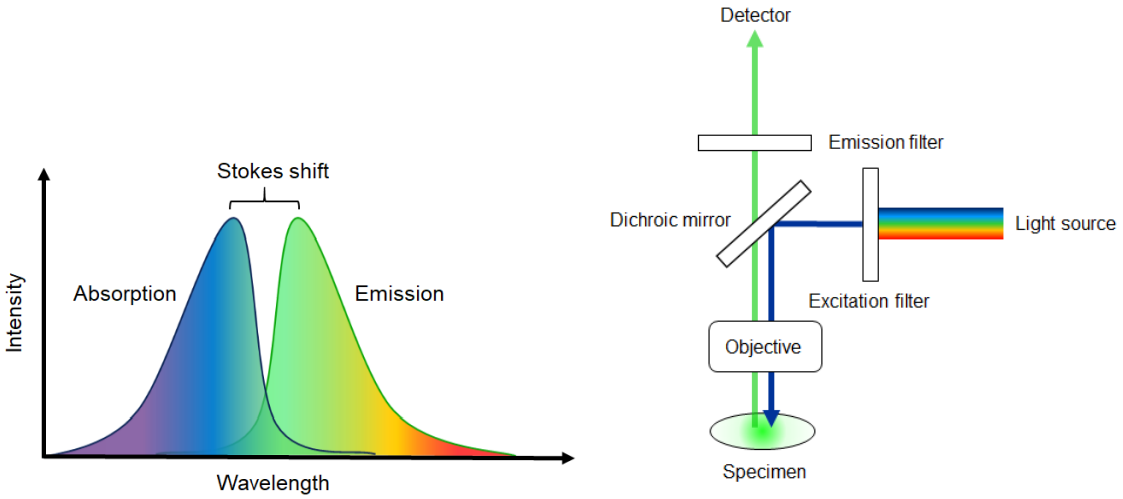


Figure 4: The Jablonski diagram describing different energy states in a molecule, the possible transitions between them and the typical time scales of different transitions. Adapted from [74].

possible that the excited electron in the upper state has a spin parallel to its original pair. These states are called triplet states and are denoted as T_1, T_2,... [74, 75].

Probable de-excitation path is via internal conversion, vibrational relaxation and fluorescence emission. Internal conversion is a non-radiative transition between electronic states. An example of internal conversion could be a transition from S_2 to S_1. The vibrational level of S_1 can be higher than the vibrational level of the original electronic state S_2. Transitions between vibrational levels within an electronic state happen via vibrational relaxation, which is a non-radiative process that transfers energy to the surrounding molecules. Internal conversion and vibrational relaxation can return the molecule back to its ground state, but typically for fluorophores the energy difference between S_1 and the ground state S_0 is so large that this path is not likely. Instead, fluorescence emission occurs. Fluorescence emission is a radiative process, meaning that a photon is emitted, and it is typically a relaxation from S_1 to S_0. For few exceptional substances the dominant fluorescence emission is from S_2 to S_0 [75]. Because some of the absorbed energy has been lost due to vibrational processes, the wavelength of the fluorescence emission is higher than the absorption wavelength. The energy difference between the absorption and emission maxima is



(a) Stokes shift

(b) Principle of epifluorescence microscope. Adapted from [76].

Figure 5: The energy difference between the absorption and the fluorescence emission is called the Stokes shift. This feature is utilized in epifluorescence microscopes, where the same light path is used for both excitation and emission wavelengths.

called the Stokes shift. It is also possible, albeit relatively unlikely, that the electron goes through a so-called forbidden transition, when its spin gets reversed. This transition from singlet to triplet state is called intersystem crossing. From a triplet state the system can return to its ground state via phosphorescence, which is a photoluminescent event like fluorescence, but slower. Fluorescence imaging typically depends on fast signals, which is why this de-excitation path may result in weaker overall signal [74, 75].

At room temperature, the absorption and emission related to fluorescence phenomenon have spectral shapes instead of sharp lines, and usually there is some overlapping between the spectra. This is because at room temperature a small number of molecules is in higher vibrational levels. The absorption and emission spectra typically have skewed shape and are mirror symmetric. A simplistic representation of the spectra and the Stokes shift is presented in Figure 5a. The Stokes shift is a useful feature, because it enables the use of only one light path for both excitation and emission light. Epifluorescence microscopes utilize this feature and the basic principle of it is presented in Figure 5b.

In epifluorescence configuration, a specific excitation wavelength is filtered from the light source with an excitation filter. The path of the excitation light is bent with a dichroic mirror, which is a beam splitter that reflects the excitation wavelength, but transmits the emission wavelength. The excitation light goes through an objective and illuminates the sample, when absorption and consequently fluorescence emission occurs. Now the emission light has a different wavelength and it can pass the dichroic mirror. With an emission filter it can be made sure that only the emission light reaches the detector and all other light is filtered. In real applications, the light path may include more filters and mirrors and have more complicated configuration. Selection of light source and objective also have an impact on the resulting image [74, 75]. Using the same light path for both excitation and emission light saves space, which makes epifluorescence configuration a good solution for space applications.

The fluorescence microscopy as a technique is based on samples that fluoresce. Many organic substances are autofluorescent, meaning that the ability to fluoresce is an intrinsic feature in them. Sample can also be stained with synthesized fluorescent compounds. In addition, proteins of living systems can be tagged with fluorescent proteins, such as green or yellow fluorescent protein. Tagging with fluorescent proteins means that the fluorescence feature is kind of genetically encoded to the sample. The absorption and emission wavelengths and also the efficiency depends on the used fluorophore. Fluorophore's ability to fluoresce weakens when excitation and emission cycles are repeated and at some point, it permanently fades. This is called photobleaching. To avoid bleaching, typically no more than necessary amount of light is used for excitation. With some samples however, the bleaching is only related to the number of cycles, so reducing the amount of excitation light will not help. Typically with good fluorophores there is a limit of 10000-40000 excitation emission cycles [74].

2.2 State of the art in miniaturized microscopes

Miniaturized fluorescence microscopes have been developed for several different areas of research. The ideal application and the main driver for miniaturized microscope development seems to be in vivo rodent experiments, in which a compact lightweight microscope offers new kinds of neural imaging possibilities. Typically these microscopes are head-mounted, allowing the animals to move naturally while the brains are being imaged. There are two main types of miniaturized microscopes for animal brain research: integrated microscopes and microscopes that are based on the use of fiber bundles. In the fiber bundle microscopes the optical signals are carried away from the microscope via fibers, which makes it possible to separate the objective lens from the sensor in a flexible way. Examples of fiber bundle microscopes are presented in e.g., [77, 78]. However, this solution increases the total volume and mass, and the optical resolution is limited by the fibers, which is why it is not considered as an ideal basis for space applications.

In 2011, an integrated miniature microscope was developed by Gosh *et al.* [79]. The microscope is presented in Figure 6. It is head-mounted, made of mass-producible components and has a mass of only 1.9 g. The optical path is designed in epifluorescence configuration. A light-emitting diode (LED) is used as a light source and the light path includes a collector lens, an excitation filter, a dichroic mirror, an objective, an emission filter, an achromat lens, and a complementary metal-oxide-semiconductor (CMOS) sensor as a detector. The optical system also includes a focusing mechanism. This microscope offered a larger field of view and better fluorescence transmission efficiency than the previous fiber bundle microscopes. The microscope body included all the optical parts, but the control signals, power and data were carried to and from the microscope by electrical wires. Therefore, the microscope was not a standalone instrument and it required a continuous computer connection [79].

Many open-source integrated microscopes followed. Liberti *et al.* [80] developed an open-source head-mounted miniature microscope, that was capable of wireless communication. Design of this microscope was partially based on the design of

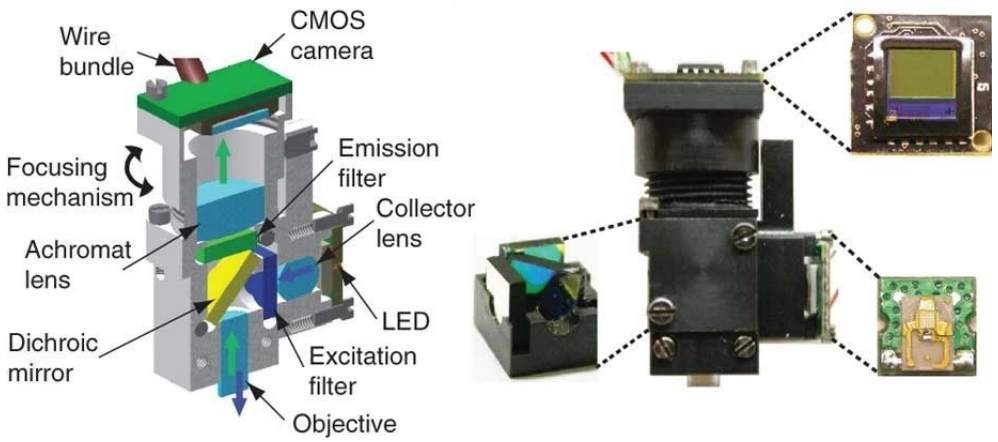


Figure 6: Miniaturized integrated epifluorescence microscope developed by Gosh *et al.* [79]. The microscope included all the optical parts, but the control signals, power and data were carried out via electrical wires. Reprinted with permission from [79].

Gosh *et al.* [79]. The microscope was made from commercial off-the-shelf (COTS) components and 3D printed parts and its mass was less than 1.8 g. The battery and wireless transmitter added approximately 2 g to the total mass [80]. Another example of an open-source microscopy project is the Miniscope [81], which offers a platform for sharing source-code and design files, among other useful information. Integrated miniaturized microscopes based on the Miniscope were developed for example, by Cai *et al.* [82] and Barbera *et al.* [83].

Experimental fluorescence microscopes were modified from existing low-cost cameras. Zhang *et al.* [84] successfully modified a COTS USB webcam into a portable miniature microscope for biomedical applications. The system is presented in Figure 7. The webcam modification included disassembling the webcam to obtain the CMOS sensor chip and detaching, inverting and re-attaching the lens to gain magnification. Different magnifications were achieved by using a spacer that varied the distance between the sensor and the lens. The possible magnifications were 8x, 20x, 40x and 60x, with distances between the sensor and the lens being 5 mm, 12 mm, 24 mm and 48 mm, respectively. The microscope was suitable for imaging live cells, tracking cellular processes and fluorescence analysis [84].

Sung *et al.* [85] modified a mobile phone into a fluorescence microscope. In this microscope, a lens system together with fluorescence illumination and sample housing was attached to a modern mobile phone, which was used as a camera system.

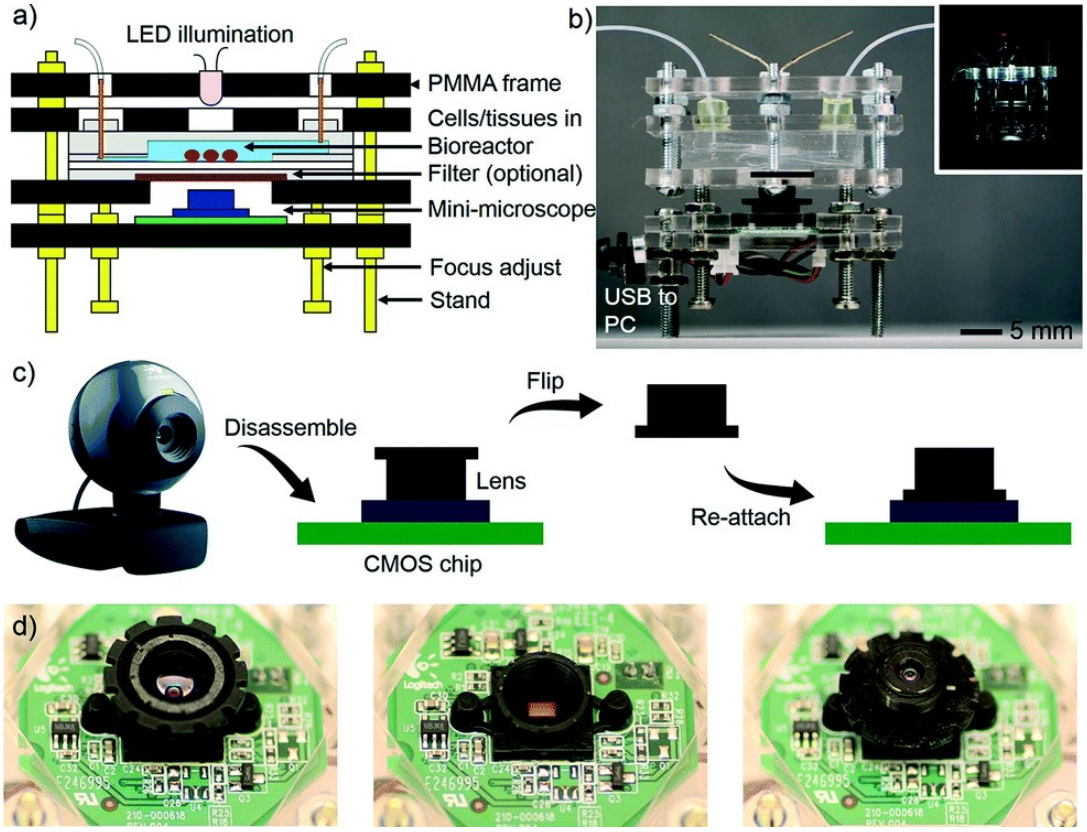


Figure 7: A miniature microscope modified from a webcam by Zhang *et al.* [84]. (a) Schematic showing of the microscope design. (b) Photograph of the final device. (c, d) The webcam modification. Reprinted with permission from [84].

The idea of using mobile phone resources, such as processing power, data storage, communication and camera, is an intriguing approach for experimental microscope development. However, the unknown components and materials, together with potential electromagnetic compliance issues, are problematic for space applications.

Forcucci *et al.* [86] designed a prototype of all-plastic, miniature fluorescence microscope for white blood cell measurements. The purpose of this microscope was to be an inexpensive tool for diagnosing and monitoring diseases at the point-of-care. The microscope used a custom objective made of plastic, which was integrated into a plastic housing made by 3D-printing. The optical parameters were especially designed to be suitable for white blood cell imaging. For instance, the field of view of the microscope was designed to be larger than 1 mm that statistically significant amount of cells could be captured in each frame. A similar design was later developed by Wong *et al.* [87].

Table 2: Comparison of different miniaturized microscopes. (N/A = information not available.)

Microscope	**Integrated miniature microscope**	**Microscope modified from a webcam**	**All-plastic microscope for point-of-care**
Reference	[79]	[84]	[86]
Purpose	Animal brain research	Biomedical applications	Diagnosing and monitoring diseases at the point-of-care
Sensor	640 x 480 / 0.3 MP	1280 x 1024 / 1.31 MP	4096 x 2160 / 8.8 MP
Pixel size	5.60 µm	N/A	1.55 µm
Resolution	∼2.5 µm	2.19 µm	362.0 - 456.1 line pairs/mm
Optical magnification	5x	8-60x	4.5x
Field of View	600 x 800 µm	1060 x 850 µm - 130 x 105 µm (depending on the magnification)	1.2 mm (diameter)
Frame rate	36 fps	30 fps	21 fps
Size	8.4 x 13 x 22 mm^3	42 x 55 mm^2 (microscope form factor)	78.95 mm (optical system length)

Optical capabilities of different types of miniaturized microscopes are compared in Table 2. The smallest microscopes were the ones designed for animal brain research. For a space application, the size this small is not required, but rather it is better to have all the components inside the microscope envelope and to be able to function as a standalone instrument. Most of the reviewed microscopes were inexpensive, utilized COTS components and were manufactured by 3D printing. The usage of a color filter on the sensor was also common for all low-cost and compact fluorescence microscopes. Typically, this is a Bayer filter which has two green pixels for every red and blue pixel. The downside of the pixel specific filter is the loss of resolution, since all the pixels cannot be used to image all wavelengths. Therefore, a filter free monochrome sensor is considered better for high resolution applications.

2.3 Space microscopy

There has already been microscope based biological space research decades ago. An example from the early stages of space microscopy is the slow rotating centrifuge microscope NIZEMI, which was flown in the Spacelab mission in 1994. Its purpose was to study the effects of gravity on small biological systems, such as small plants, fungi, spores and cell cultures. It consisted of control module, experiment control unit and experiment module. The system was relatively large, since the combined mass of the modules was 98 kg. The experiment module had a centrifuge, which was capable of accelerations between 10^{-3}g and 1.5g. The microscope was accommodated on top of the centrifuge and it rotated together with the sample. The possible imaging modes were bright field, dark field and phase contrast [88].

The laboratory modules on the ISS are also equipped with microscopes, which are integrated parts of the facilities. Light Microscopy Module (LMM) is a multifunctional sub-rack of the Fluids Integrated Rack on-board the Destiny module. LMM is originally modified from commercial light microscope and it includes multiple imaging techniques, such as bright field, dark field, phase contrast, differential interference contrast and confocal microscopy. The microscope is operated remotely from the ground [89]. Despite the LMM being primarily designed for fluid physics experiments, it has also been used for biological research (e.g., [90]). Another rack including a microscope is the Biolab on-board the Columbus module [91]. Biolab is covered more thoroughly in Chapter 3.1.2. In the Kibo module there are two microscopes. One microscope is located in a sterilized glovebox in the Saibo multi-purpose rack. This microscope includes bright-field, phase contrast and fluorescence imaging options. The other microscope is JAXA Microscope Observation System, which is a fluorescence microscope located either in the Multi-purpose Small Payload Rack or in the cabin area. The microscope is operated remotely from the ground and dedicated for biological studies [92]. In addition to the large microscopes that are integrated parts of the facilities, there is a need for smaller and more independently functioning space microscopes. The state of the art in these kind of microscopes is reviewed in the following.

Autonomous Microscope for Examination of Radiation Effects (AMERE) was a conceptual design of a microscopy system, developed for the ESA Lunar Lander mission. The AMERE project ended in 2012 and it was stated in the project's executive summary report [93] that finishing the instrument for the Lunar Lander (2018) would not be realistic. Furthermore, the Lunar Lander mission was put on hold in 2012. However, the project addressed many interesting challenges and generated ideas that could be adapted for future space microscopy projects. The project was about developing a conceptual design of an instrument, which could visualize how DNA damage, resulted from HZE particle traversal, is repaired in live cells, and assessing technological and biological requirements for such instrument [94].

The AMERE concept consists of three main parts: an automated fluorescence microscope, a radiation detector and a cell life support system. The whole system is designed to fit inside a 476 x 476 x 260 mm^3 box. The main working principle is the following. The cell culture chamber is underneath a position sensitive radiation detector, which is able to detect particles with LET $>$ 50 keV/µm. When a particle hits the detector, the trajectory is derived from the detector data and extrapolated to the biological sample. The impact location is passed on to the XY-stage, which is then moved so that the affected cells can be imaged. The concept is illustrated in Figure 8. The microscope includes two fluorescence excitation channels in addition to dark field imaging possibility and an autofocus [94]. Including the dark field imaging possibility to a space microscope would make the system more versatile. The dark field imaging mode can be used for experiments in which fluorescence is not needed, for example, to find the area of interest or to track cell movement.

One of the main challenges distinguished during the project was the preservation of the samples during the relatively long pre-launch period and the travel to the Moon. In the study it was estimated that the samples must be handed over to the launcher three months before arrival to the Moon. It was concluded that an optimal solution would be to preserve the live human cells by freezing them to -80°C and then heat them up to human body temperature when reaching the destination. Three candidate cell lines for the mission were also selected: HBEC3, U2OS and

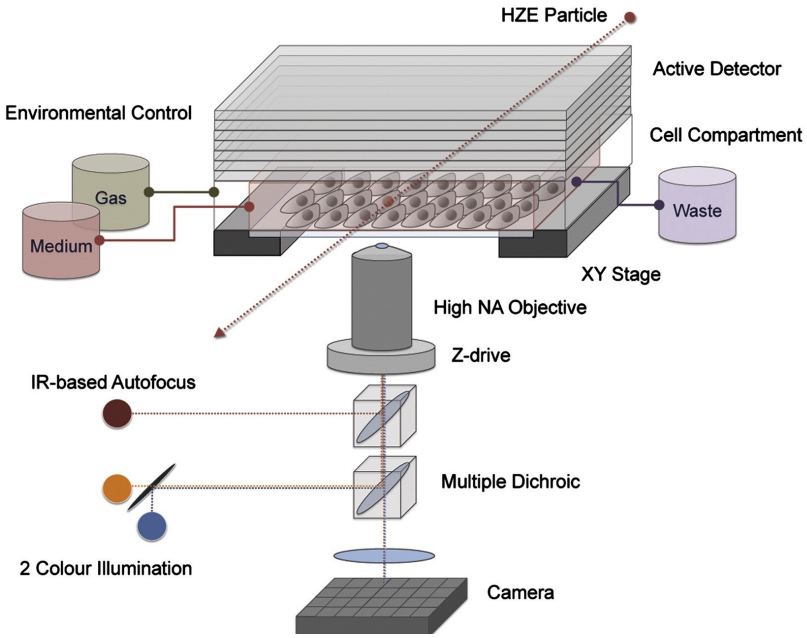


Figure 8: The AMERE concept. The position sensitive radiation detector detects HZE particles with LET>50 kev/µm. The particle trajectories are derived from the detector data and extrapolated to the biological sample, which is located underneath the detector. Information about the impact location is passed on to the XY-stage, which is moved so that the affected cells can be imaged. The sample environment is controlled with the cell life support system. Reprinted with permission from [94].

HTI1080, from which the U2OS was considered the most promising. The cells were selected based on their ability to resume metabolism after the freezing and heating cycle, preliminary vibration test results and compatibility with a specific fluorescent marker (53BP1) [93]. Combining a radiation detector with the microscope would be a powerful approach for radiation related biological space research.

Fluorescence Microscopy Analysis System (FLUMIAS) is a project that focuses on human cell imaging in microgravity. The first version of the FLUMIAS microscopes was FLUMIAS-TEXUS, which was a confocal laser spinning disc fluorescence microscope. It was designed for parabolic flights and suborbital rockets and it was first used in 2015 [95]. FLUMIAS-TEXUS weighted about 120 kg and was not suitable for the use on the ISS [96]. The second FLUMIAS microscope, FLUMIAS-DEA, was a technology demonstrator and a simplified version of the future microscope FLUMIAS-ISS. FLUMIAS-DEA was a project of the German Aerospace Center (DLR), implemented by TILL I. D. and Airbus DS with cooperation of University of Zurich and NASA. FLUMIAS-DEA successfully operated on the ISS in 2018.

It was used as an integrated part of Space Tango facility on-board the Destiny module, where it obtained high-resolution 3D images during 21 days. The microscope based on structured illumination microscope technology and had dimensions of 400 x 200 x 90 mm^3. It used four LEDs as light source and was capable of stage displacement in x and y directions and the objective could be moved for acquiring z-stacks. The XY-stage is necessary for finding the area of interest from the cell culture. Since human interaction is minimal (e.g., on the ISS) or non-existent (e.g., in CubeSats and rovers), automated stages, that can be operated remotely or via PC connection, are the best solution for a space microscope.

The FLUMIAS research on the ISS was done with two different samples, one with fixed cells and one with living human macrophages. The cells were chosen so that they could survive in temperature range from approximately 25°C to 35°C. The experiment was packed in a phase shift material during the transportation to ISS to diminish temperature variations. The cell culture chamber volume was 120 µl and no medium exchange was possible, since no pumps were included in the system. In the AMERE study it was stated that the samples must be handed over to the launcher already few months before the launch. Depending on the launcher, the pre-launch period is not necessarily that long anymore. The FLUMIAS experiment was launched by SpaceX, when the handover was only 24 hours before the launch [96]. An alternative for fluidic culture systems could be to use immobilization techniques (e.g., method to produce *Synechocystis* PCC6803 biofilms in [97]). Immobilized cell systems could be easier to implement for in-situ space research than fluidic culture systems. It has also been observed that immobilized cells preserve viable for longer periods of time [98].

In addition to the optical microscopes, a small portable electron microscope was developed for the ISS. The microscope, named as Mochii, is a commercial product of Voxa. The microscope is 250 mm tall and has a mass of around 12 kg [99]. Generally, electron microscopes have high resolution, but to use one in space, additional shielding against the magnetic field is required to minimize the interactions between the electron beam and the magnetic field [100].

3 Potential platforms

The potential platforms for miniaturized fluorescence microscope in space range from the facilities on-board the ISS to satellites and rovers. The ISS is especially suitable for gravity related biological research and technology demonstrations. The radiation levels on the ISS are higher than on ground, but still lower than in the interplanetary space. Especially the high-LET particles are relatively less abundant due to the Earth's magnetic field coverage [101]. To study the impact of space radiation on cells, a potential platform is a small satellite, such as a CubeSat. The possibility to use a microscope in a rover is also discussed.

3.1 International Space Station

The ISS is an unique and ideal site to study the effects of gravity on biological systems, not only because of the microgravity environment, but because it also has research facilities where a 1g reference environment can be created. With the reference environment it can be ensured that the possible effects in the biological system are due to the weightlessness and not due to some other environmental factors. The reference conditions can be created with an on-board centrifuge. European facilities for cellular research that have a centrifuge are KUBIK and Biolab. There is also a facility called European Modular Cultivation System (EMCS), which has a centrifuge, but it is dedicated for plant research [102].

3.1.1 KUBIK

KUBIK is a cubic shaped incubator with removable insert. It has a long history on-board the ISS, since it was already used in the Russian segment before the Columbus module integration. The removable insert can be either a centrifuge or a KUBIK Interface Plate, which is for accommodating custom made experiment hardware. KUBIK with the centrifuge insert and experiment containers in static positions is presented in Figure 9. The temperature of the incubator can be set between 6°C and 38°C with 0.1°C increments, and the acceleration of the centrifuge can be set

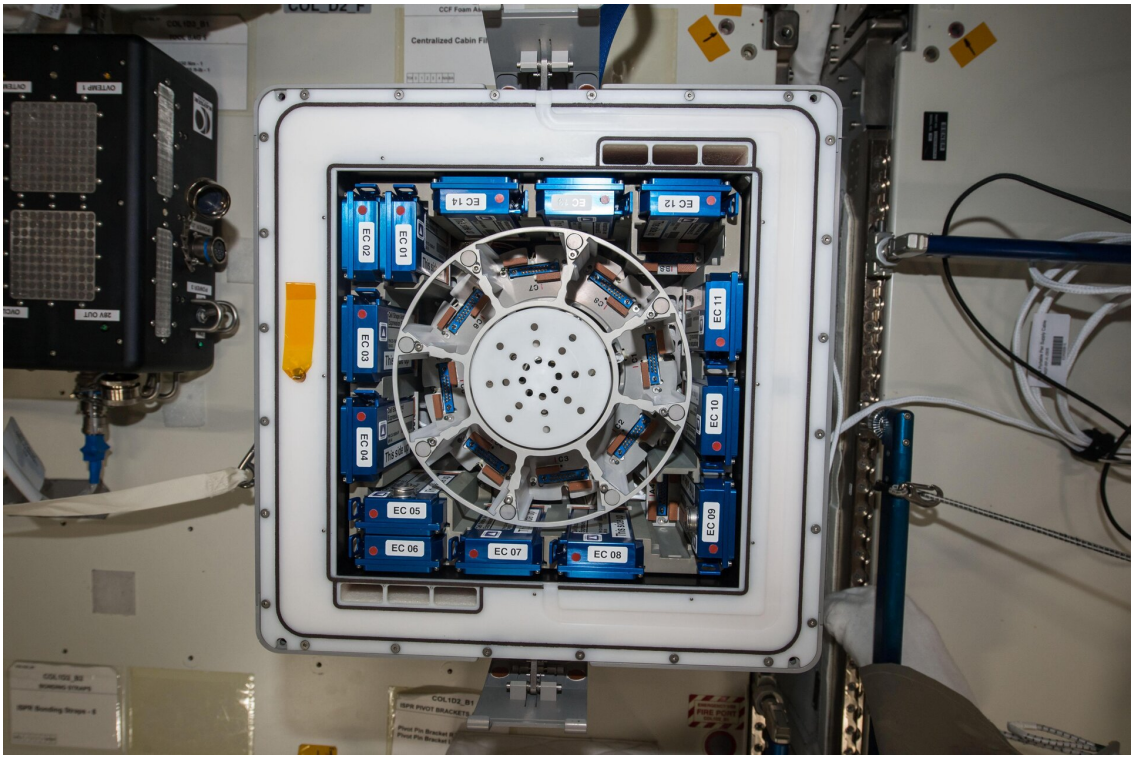


Figure 9: KUBIK incubator with the centrifuge insert and experiment containers in static positions. Credit: NASA.

between 0.2g and 2.0g with 0.1g increments [103]. The centrifuge has a diameter of 175 mm [102] and there are two types of experiment containers (ECs) it can host: a smaller standard size EC and a larger extended EC. In static position the insert can accommodate either 16 standard or 8 standard and 4 extended type containers. The centrifuge itself can accommodate 8 ECs, not depending on the type. The dimensions of the containers are approximately 20 x 40 x 80 mm^3 for the standard EC and 30 x 40 x 80 mm^3 for the extended EC. KUBIK requires the experiments to be self-contained and operations to be automatic, since the data and communication possibilities with the ECs are very limited [103].

3.1.2 Biolab

Biolab is a single-rack payload on-board the Columbus module. It was launched in 2008 and it is mainly dedicated to study microorganisms, tissues and cells [91]. It is a larger facility than KUBIK with more functions. A description of Biolab is presented in Figure 10. The facility is divided into two sections, which are the automated

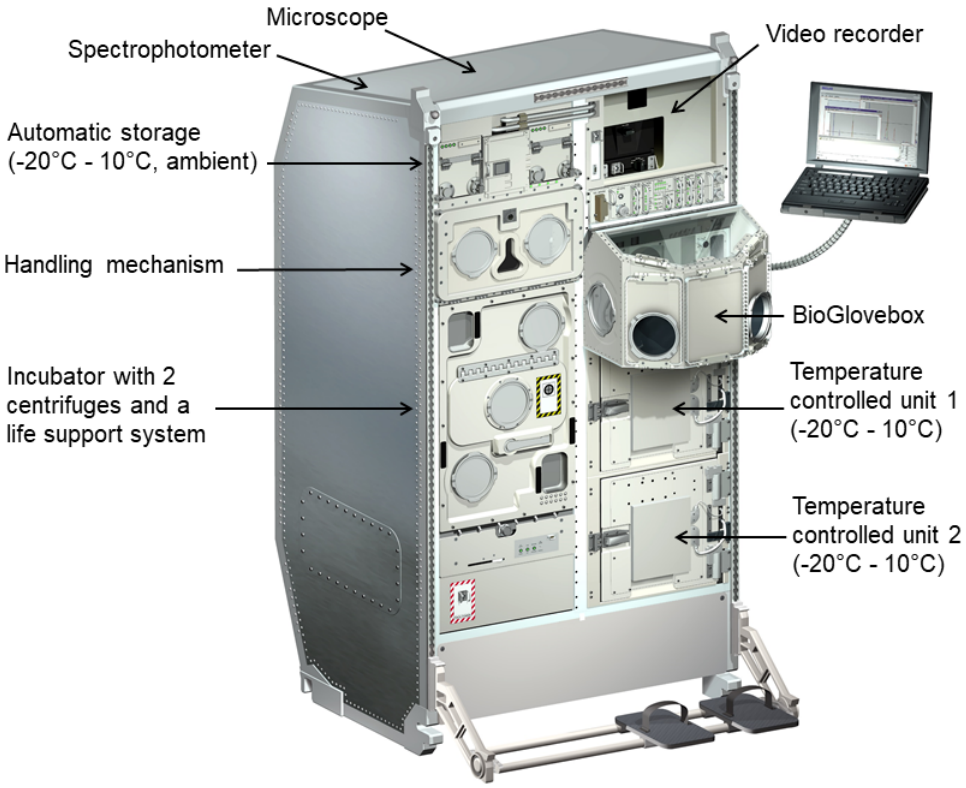


Figure 10: Biolab is divided into an automated section on the left and a manual section on the right. Credit: ESA/ D. Ducros (explanations have been added to the figure).

section on the left and the manual section on the right. The automated section includes an incubator, which has two 600 mm diameter centrifuges that are able to simulate gravity between 0.001g and 2.0g [91]. The centrifuges can accommodate standard and advanced ECs, which have internal volumes of 60 x 60 x 100 mm^3 and 108 x 150 x 137 mm^3, respectively [104]. Each of the centrifuge rotors have positions for two advanced ECs and can accommodate up to six ECs in total, meaning that either six standard ECs or a combination of four standard ECs and two advanced ECs can be used. Temperature in the incubator is selectable from 18°C to 40°C with 0.5°C increments and humidity and atmospheric concentrations are adjustable with a life support system [91].

In addition to the incubator, the automated section includes a handling mechanism that reduces the astronaut interactions by allowing some automated operations and communication to the ground. It is possible to control the functions of the incubator, such as the life support system and centrifuge rotor speed, from ground. That also applies for automated hardware inside the ECs, if any. On the ground, the controlling and data retrieval are handled by the Biolab user support and operations centre at DLR in Germany or possibly by the user home base. Available analysis instruments in Biolab are a microscope and a spectrophotometer. The microscope and spectrophotometer are located above the handling mechanism and behind the automatic storage compartments. It is possible to inject the sample from the EC to the microscope, where an in-flight analysis can be made at the end of the experiment. After the analysis, the sample is flushed into a waste reservoir. Since the microscope is an integral part of the Biolab facility, it is not possible to place it inside the experiment containers and thus, image the samples during the experiment [91].

The manual section has two temperature-controlled units and a BioGlovebox-section, which is an enclosed area for manual operations, also including an ozone generator. The ozone generator is used for the sterilization of the working space. In the manual section the astronauts perform sample preparations and experiment completion activities. The experiments are transported to the ISS in the experiment containers or in some cases the samples can be transported in small vials. The samples can be stored before use and then installed into the instrument in the BioGlovebox [104].

3.1.3 ICE Cubes

The International Commercial Experiment Cubes (ICE Cubes) service is a commercial facility on-board the Columbus module. The ICE Cubes platform is quite new, as it was launched on May 2018 [105]. ICE Cubes facility can host experiment cubes of size 1U (10 x 10 x 10 cm^3) or modular combinations of that size scaled along two axes, with maximum size of 4U x 3U. The facility is presented in Figure 11. It is composed of a container and a framework that has 20 locations for accommodation

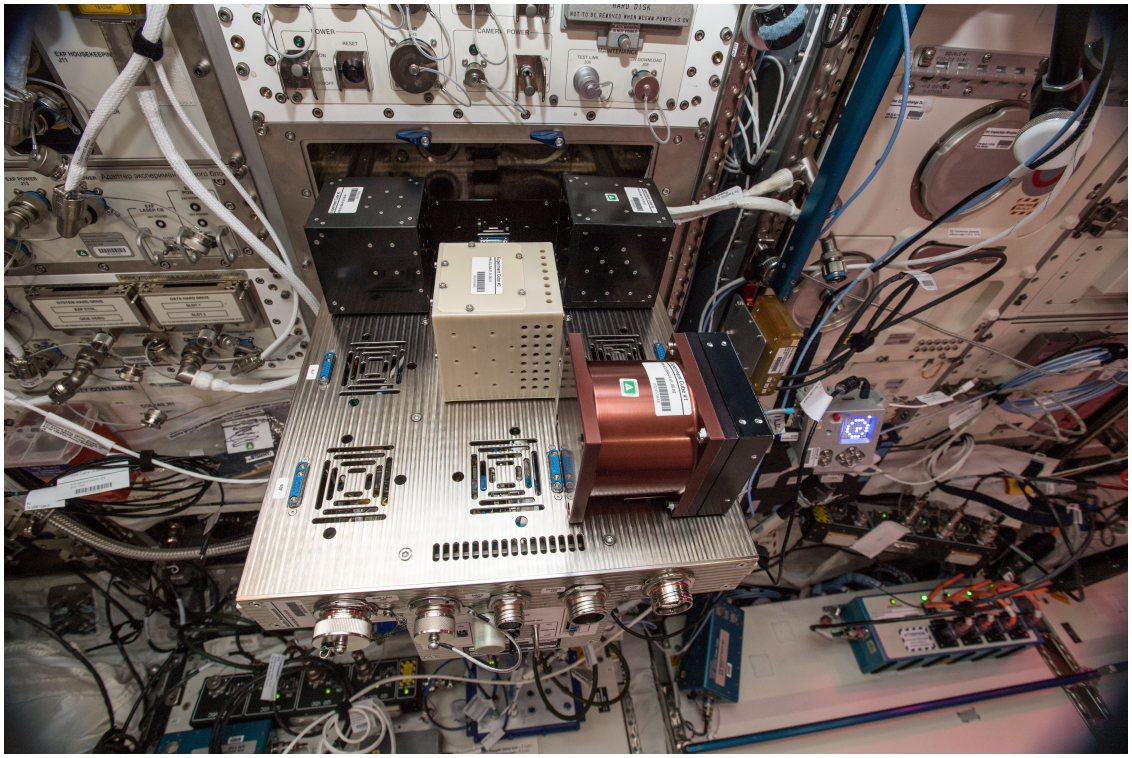


Figure 11: ICE Cubes facility with experiment cubes. Credit: NASA.

and management of the experiment cubes, providing power and gigabit Ethernet. The whole system is operated from the ground, but the framework has hosting bay for two SSDs and ports for two USB 3.0 flash drives, which can be used for data storage before downlinking. There is a possibility to operate the system from user home base, when a private IP address would be assigned by the ICE Cubes mission control centre for communication. It is expected that the astronauts only need to activate the power switch, exchange the experiment cubes and possibly install or remove SSD or USB flash drives. Temperature range inside the ICE Cubes facility is between 17°C and 30°C, but the exact temperature of the air surrounding the experiment cube depends on the location of the cube inside the facility [106].

3.1.4 Comparison

All the platforms presented above offer different experiment possibilities. KUBIK and Biolab are especially dedicated for biological research. From these two, Biolab has larger experiment containers than KUBIK and can therefore accommodate more

sophisticated experiment hardware. Due to the larger volume, more features, such as a fluidic system for media exchange, could be implemented in the EC together with the microscope. If the smaller EC is used in KUBIK, it can host more experiments than Biolab (16 on static position and 8 on the centrifuge), but developing a fully functional and automatically operating microscope in that volume is extremely difficult task. If the larger EC is used in KUBIK, the advantage is lost since the number of ECs in a static position reduces to four.

Both KUBIK and Biolab can be used for studying the impact of microgravity on biological systems with a 1g reference environment. In Biolab this can be done by keeping one of the centrifuges in a static position and rotating the other. With two centrifuges, it is also possible to conduct partial gravity research with a 1g reference environment. For example, it is possible to investigate how biological systems react to the gravity of the Moon or Mars compared to the Earth's gravity.

The communication and data transfer possibilities in Biolab are also better than in KUBIK, since it is basically not possible to transfer images from the KUBIK during the experiment. ICE Cubes is not as flexible from the biological point of view, because the temperature is not as controlled and it does not have a centrifuge. However, it is more flexible in terms of volume and has excellent communication and data transfer capabilities. It is a suitable platform for technology demonstrations, meaning that it could be used for in-orbit demonstrations or validations to raise the technology readiness level (TRL) of an instrument.

Aspects that are not considered in this comparison are the availability and cost of the facilities.

3.2 CubeSats

The CubeSat project started in 1999 as a collaboration between Jordi Puig-Suari from California Polytechnic State University (Cal Poly) and Bod Twiggs from Standford University's Space Systems Development Laboratory. The main purpose of the project was to develop a standard for a small satellite design that would reduce the cost and development time and would increase the accessibility to space [107].

The originally envisioned purpose of CubeSats was to be low cost platforms for educational tools and technology demonstrations. However, the purpose of CubeSat missions has started to shift towards more advanced, real science missions and commercial applications. As a result, CubeSats are nowadays also potential platforms for spaceborne science laboratories [108].

CubeSats consists of one or more 10 cm x 10 cm x 10 cm units (1U). Common sizes are 1U, 1.5U, 2U and 3U, from which the 3U is the most used size as to date [109]. Based on the CubeSat design specification [107], the maximum mass is 1.33 kg for 1U and 4.00 kg for 3U CubeSat. Recently, standards for larger 6U, 12U and 27U CubeSats were also developed [110, 111]. The number of launched CubeSats has grown rapidly in the past couple of years. The hundredth CubeSat was launched in 2012 [112], and in 2020, the number of launched CubeSats already exceeds 1200 [109]. One of the reasons of CubeSat's success is the advances in the miniaturization of COTS components that enable to build a small satellite from compact, low cost and low power components that are already available in the market. Another reason is that the CubeSat standard makes it possible to use standardized deployment systems in the launch vehicles [108]. CubeSats have also made it possible to smaller countries like Finland to launch their first satellites (e.g., Aalto-1 [113]).

3.2.1 Subsystems

In addition to the payload, a spacecraft typically includes the following subsystems: propulsion, attitude and orbit control system (AOCS), electrical power supply, communications, command and data handling and thermal control [114]. The subsystems are embedded in the spacecraft structure, which can also work as shielding against radiation or as thermal control [108].

The need for a propulsion system in a CubeSat depends on the mission. Most of the launched CubeSats do not have propulsion systems, but with more advanced mission goals it might be needed. The propulsion system can be used for changing the orbit, attitude control or deorbiting at the end of the mission to avoid creating space

debris. There are some requirements presented in the CubeSat specification [107] that set restrictions for the propulsion system. For example, the use of pyrotechnics is not permitted on a CubeSat, which excludes the use of solid propellants without a waiver. The chemical energy stored in a CubeSat is also limited to 100 W-hours. At least cold gas propulsion, electrospray and vacuum arc thrusters and solar sail propulsion systems have been used in CubeSats [115].

The AOCS is responsible for determining and adjusting the orientation of the spacecraft. The attitude determination can be done with star trackers, Sun sensors, Earth sensors, magnetometers, radio transponders or on Earth orbit with global navigation satellite system (GNNS) such as Global Positioning System (GPS) or Galileo. The spacecraft can be stabilized and oriented with thrusters, reaction wheels or magnetorquers [108, 114].

The electrical power supply system includes power source, energy storage and control. Primary power source for CubeSats is photovoltaic solar cells, which can be either deployable or mounted in the CubeSat structure, depending on the power requirements of the mission. Energy storage is needed to supply power during eclipses. Typically batteries, such as lithium-ion or lithium-polymer batteries, are used in CubeSats. Control of the power distribution and regulation is typically done with custom built systems [108, 116].

The communication system receives the operational commands that are transmitted from the ground station and transmits the collected payload and housekeeping data from the CubeSat. Almost all of the CubeSats have transceiver and antennas and use frequencies from the radio spectrum. Most of the launched CubeSats use either very high frequency (VHF), ultrahigh frequency (UHF) or S-band for communication [109, 116]. The frequencies of those bands are 0.03-0.3 GHz, 0.3-1 GHz and 2-4 GHz, respectively, and the data rates achieved vary from kbps to some Mbps. Data rate of tens of Mbps can be achieved by using the 8-12 GHz X-band or the 27-40 GHz Ka-band [116]. The locations of the communication bands in the electromagnetic spectrum are presented Figure 1. Based on statistics of April 2020, 450 nanosatellites (the term nanosatellite includes other same size class satellites

in addition to CubeSats) use X-band for downlinking [109]. In advanced science missions, the communication subsystem is typically one of the limiting factors. If more data is generated than can be transmitted to ground, the communication subsystem becomes a bottle neck. Significant improvement in the data rates would be attained by using optical communication, which has potential to achieve data rates from hundreds of Mbps up to several Gbps [108]. Optical communication has already been demonstrated in a CubeSat (e.g., NASA's Optical Communications and Sensors Demonstration program [117]).

The command and data handling system is responsible for collecting and storing the data before transmission and distributing the received commands to the subsystems. Commonly used systems for on-board data handling in CubeSats are field-programmable gate arrays (FPGAs) or microcontrollers, such as mixed signal processors (MSPs), peripheral interface controllers (PICs) and advanced RISC machines (ARMs). Low cost open-source hardware and software microcontrollers (e.g., Arduino) and single-board computers (e.g., Raspberry Pi), are also promising for CubeSats [108, 116].

Thermal control is needed to protect the payload and all critical components from the extreme temperature variations between the times when the spacecraft is in the Sun and when it is in eclipse. In addition to the direct sunlight, external heat inputs are the sunlight that is reflected from planets and moons and the infrared energy from the central body of the CubeSat's orbit. The subsystems of the spacecraft also generate heat inside the spacecraft. Thermal control means balancing the heat inputs and outputs and it can be passive or active. Passive thermal control includes techniques that do not use any power input, such as, multi-layer insulation blankets, surface coating, Sun shields, heat pipes and radiators. Passive thermal control techniques are often reliable and have low cost, mass and volume. More accurate thermal control can be achieved with active control, such as heaters or cryocoolers [108]. Biological payloads typically have strict temperature requirements, which is why efficient thermal regulation is needed. To achieve precise control, a combination of passive and active approaches could be used. Examples are given in the next chapter.

3.2.2 Biological missions

Among other purposes, CubeSats are also a great opportunity for biological research in space. NASA's first biological CubeSat was launched in 2006. GeneSat-1 was a 3U CubeSat with an autonomous microorganism life support system. It studied the *E. coli* bacteria with an optical sensor system. In the experiment the green fluorescent protein was used to tag the gene associated with metabolism of the bacteria. This way the fluorescence phenomenon could be utilized to observe metabolism of the sample by using a LED for excitation and an intensity-to-frequency detector for emission light detection. The density of the bacteria population could be observed by using a green LED and the same detector. The payload was inside a pressurized and sealed cylinder. The subsystems included a passive magnet/hysteresis rod (magnetorquer) for orientation, body-mounted solar panels and a battery. Communications were handled by a S-band (2.4 GHz) transceiver with a data rate budget of 86 kbps. An amateur radio beacon was also included. A PIC-based board was used for command and data handling [118].

Following to GeneSat-1, PharmaSat was developed by the same group. PharmaSat was launched in 2009 and it was also a 3U CubeSat. One of the challenges of human spaceflight was that the pathogens may become more drug resistant in the microgravity environment. PharmaSat studied this phenomenon by using yeast and antifungal agent. The payload consisted of a microfluidic system that provided nutrients to the samples and dosed the antifungal agent, and a similar optical system than the GeneSat-1 to determine the size and health of the sample populations. Instead of the green fluorescent protein, a colorimetric reagent Alamar Blue was used [119]. The subsystem design was inherited from GeneSat-1. PharmaSat used hybrid thermal control, which consisted of low thermal conductance paths, aluminum gold plating and multi-layer insulation, in addition to active heaters that increased the temperature of the payload [120].

Both GeneSat-1 and PharmaSat were launched to approximately 450 km LEO and the main experiment duration was four days. GeneSat-1 was mainly focused on technology demonstrations, but biological results, showing slower growth rate in

flight samples, were also obtained [121]. The PharmaSat mission reported interesting results: at high concentrations of antifungal agent, the growth was almost completely inhibited in ground control, but significant activity was still observed in flight samples [122].

Organism/Organic Exposure to Orbital Stresses (O/OREOS) was launched in 2010 to approximately 650 km LEO with 72° inclination. Due to the high inclination, the CubeSat frequently passed over the SAA or the Earth's magnetic poles, where the magnetic field does not provide shielding against cosmic radiation. O/OREOS worked as a science demonstration mission in the NASA's Astrobiology Small-Payloads program and it studied how microorganisms and organic molecules respond to microgravity and ionizing radiation [123]. Description of the O/OREOS spacecraft is presented in Figure 12.

O/OREOS was a 3U CubeSat with a control unit and two payload modules, each of the size of 1U. The control unit was derived from PharmaSat. The payloads were the Space Environment Survivability of Living Organisms (SESLO) experiment and the Space Environment Viability of Organics (SEVO) experiment. The purpose of the SESLO experiment was to measure survival, metabolism and growth of two different types of microorganisms by using similar method than the NASA's previous biology CubeSats. The SEVO experiment measured changes in organic molecules by using UV and visible spectroscopy. In addition to the spacecraft bus and the payload modules, O/OREOS included a self deploying de-orbit mechanism "NanoKite", which increases the spacecraft surface area and results to faster de-orbiting (approximately 22 years) [123]. It was observed during the O/OREOS mission that the microorganims had slower growth rate in microgravity (similar results than GeneSat-1 obtained), and that there was no significant decrease in viability in the space environment [124].

After O/OREOS, NASA developed one more 3U biological CubeSat. SporeSat was launched in 2014 to 325 km LEO. The spacecraft bus was derived from PharmaSat and O/OREOS. The purpose of the SporeSat was to study the effects of microgravity on spores. The payload consisted of three lab-on-a-chip devices and

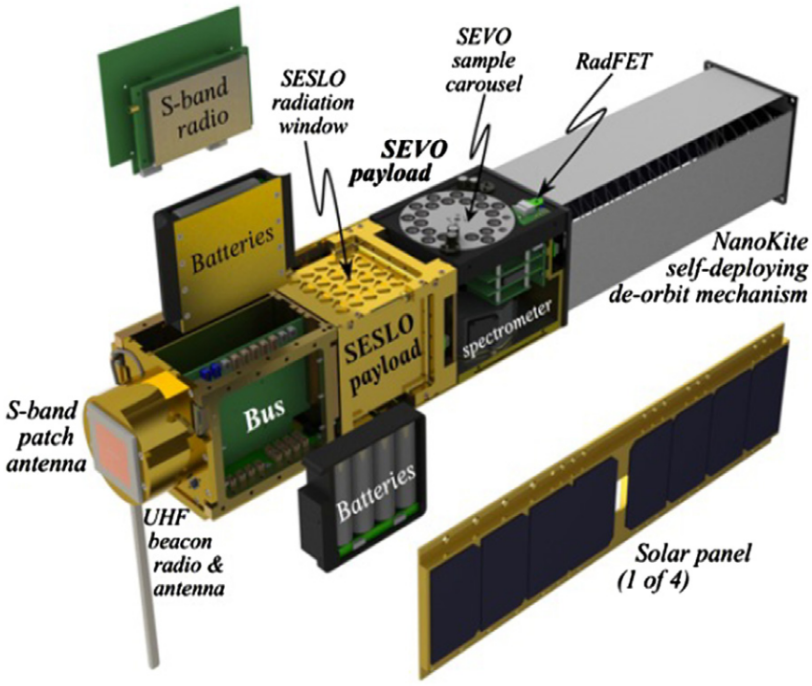


Figure 12: The O/OREOS spacecraft. Reprinted with permission from [123].

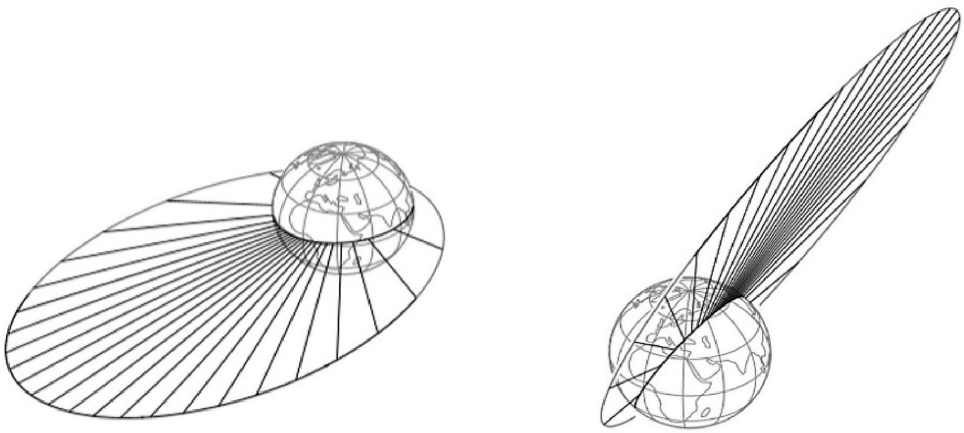
two 50 mm miniature centrifuges, which were for creating a reference environment. The research focused on germinating spores and especially on the changes in the calcium ion channel activity. The mission successfully demonstrated the functionality of the miniature centrifuges, but no findings concerning the spores were made due to a LED failure [121, 125].

Recently, biological payloads have been accommodated in larger 6U CubeSats. NASA's Escherichia coli Antimicrobial Satellite (EcAMSat) was a 6U CubeSat launched in 2017. The EcAMSat bus was a copy of the PharmaSat bus and the scientific instrument had 90 % commonality with the PharmaSat's. It studied the antibiotic resistance of uropathogenic *E. coli* in microgravity environment. EcAMSat was deployed from the ISS and its main experiment lasted for 6 days [121]. Result of the study was that microgravity did not enhance the antibiotic resistance of this specific bacteria. It was also concluded that targeting the rpoS gene led to slower metabolims of the uropathogenic *E. Coli* and that the method could be an effective way to combat bacterial infections in space [126].

BioSentinel is also a 6U CubeSat developed by NASA, with planned launch in 2020. BioSentinel will be the first biological CubeSat going to the interplanetary space. It will go to a heliocentric orbit and its purpose is to study cell metabolism and growth, and to monitor how cells respond to DNA damage induced by the deep space radiation. It has similar optical sensor system than the previous NASA biology CubeSats, with some improvements. It will, for example, accommodate more samples than the previous missions. *S. cerevisiae* yeast was selected for the BioSentinel mission, because it shares hundreds of homologous genes with humans, including the genes governing the DNA damage repair, and because it has already been used in a spaceflight [121]. In addition to the optical sensor system, the payload includes a radiation spectrometer that measures linear energy transfer within 0.2-300 keV/µm range and the total ionizing dose [127].

The previous biological CubeSats used magnetorquers that utilize the Earth's magnetic field for orientation. This method is not applicable in deep space, and therefore, BioSentinel has three orthogonal reaction wheels and a cold gas propulsion system, which are responsible for the orientation of the spacecraft. Star tracker is used for attitude determination. Electrical power supply system includes deployable solar panels and lithium-ion batteries. X-band transponder and the deep space network are used for communication. A hybrid thermal control, consisting of passive interface materials and coatings and active heaters is used. In addition to the BioSentinel travelling to the interplanetary space, a copy of the payload will be used on the ISS and in ground control, to compare the three different radiation environments [127].

Europeans are also developing a biological 6U CubeSat. SpectroCube's goal is to study the photochemical changes in organic molecules in the space environment. The payload includes a COTS Fourier transform spectrometer, five UV sensors measuring different wavelengths and radiation dose sensors, which are capable of measuring 35 keV-6 MeV electrons and 600 kev-500 MeV protons. The sample holders can accommodate up to 60 samples, of which part of are shielded from the UV radiation but not from the energetic charged particles. The purpose is to study what changes



(a) Geostationary transfer orbit. (b) Molniya orbit.

Figure 13: Different high Earth orbits. Reprinted with permission from [128].

in the samples are due to the UV and what are due to the cosmic radiation. The subsystems include cold gas thrusters, deployable solar panels and a S-band transceiver. The AOCS consists of four reaction wheels, three magnetorquers and a Sun sensor. The launch of SpectroCube is expected to be somewhere between 2020 and 2022 into a high Earth orbit. The baseline orbit is the highly elliptical geostationary transfer orbit (GTO) [128]. The GTO has an apogee at 35786 km, perigee between 200 and 650 km, inclination of 7° and an orbital period of around 10.5 hours. The orbit is presented in Figure 13a. There is significant radiation exposure in GTO, since the spacecraft traverses the radiation belts and encounters GCRs and possibly SEPs [114].

3.2.3 Microscope as a payload

Microscope as a CubeSat payload would be a suitable instrument to study the synergistic impact of space radiation and microgravity on cells and microorganisms. If the objective was to study only the effects of microgravity, the platforms on-board the ISS would be better due to the centrifuges and lower radiation doses. If the objective was to study only the effects of UV radiation, LEO would be an adequate destination.

The advantage of a CubeSat with respect to the ISS is that it can be launched into an orbit with greater exposure to energetic charged particles. These kinds of orbits are, for example, high Earth orbits such as GTO or Molniya orbit, or high inclination LEO. Molniya orbit, presented in Figure 13b, is a highly elliptical orbit with inclination of 63.4° and orbital period of 11 h 58 min. The height of the apogee is around 39000 km and due to the highly elliptic shape of the orbit, it has good communication coverage. A spacecraft in Molniya orbit passes through the Van Allen belts and altitudes where there are GCRs and possibly SEPs during each orbit [114]. In addition, there is also the possibility to go to interplanetary space.

Significant radiation exposure is damaging for biological systems, but also for spacecraft components, especially for the electronics, which needs to be considered in the subsystem design. In the high Earth orbits, propulsion system might be needed to re-orbit the spacecraft into a graveyard orbit at the end of mission lifetime. Depending on the height of the perigee, re-orbiting from a high orbit to graveyard orbit can be more energy efficient than de-orbiting. Critical subsystems from the perspective of a microscope and biological samples are communication and thermal control. If the goal is to take high resolution fluorescence images, communication system providing sufficient data rate is essential. For example, if the microscope had a 5 megapixel camera sensor and it acquired images with a bit depth of 12, one full resolution image would be 60 Mbit. Metadata, housekeeping data and data from other possible payload systems need be taken into account as well. The duration of the acquisition of signal period depends on the orbit and the ground station location. The shorter the acquisition of signal period is, the higher data rate is required. Thermal control needs to be effective and precise in order to make sure that the samples do not die of the temperature variations (if it is not the purpose of the mission to study e.g., how microorganisms survive in the space environment, including the temperature variations). Hybrid thermal control, as was done in the previous biological CubeSats, is viable option. In the end, the requirements for the subsystems are designated by the mission objectives.

CubeSat mission concept ideas including a miniaturized microscope are presented in the following. The concept ideas are derived from the science questions related to humans and microorganisms in space and the biological missions concepts presented in the previous chapters.

A compelling mission concept would be to study the effects of HZE ions on biological systems with similar configuration than was proposed in the AMERE-project. In this concept, a position sensitive radiation detector would be placed on top of the microscope system. The working principle would be similar to AMERE: particles with high enough energy are detected and the detector information is used to extrapolate the impact location on the sample. The impact location is passed on to the XY-stage, which is moved so that the affected cells can be imaged. This concept requires that the microscope system includes a XY-stage and that the cell culture chamber is underneath the radiation detector. The system would have to operate autonomously, meaning that the calculation of the impact location and moving of the stage is handled by software, because continuous connection to the ground is not possible. In addition to the microscope and the radiation detector, some kind of fluidic system would be needed for cell life support.

Another mission concept would be to compare the effects of different types of radiation with similar approach than in the SpectroCube mission. In this concept, two miniaturized fluorescence microscopes would be on-board the CubeSat. One of the microscopes would be shielded better and the other would be more exposed to radiation. This concept could also be used to simulate different shielding materials and shielding material thicknesses and to determine how the different shielding techniques reduce the damage in biological systems.

Third mission concept would be to study the interplanetary transfer of microorganisms. The idea of adding a microscope payload into a satellite is not restricted to only CubeSats. A miniaturized microscope with, for example, microorganism samples could be a "hitch-hiker" in larger satellite missions as well. This way, realistic information about the survival of microorganism in the interplanetary space could be gained.

3.3 Rovers

The platforms on-board the ISS as well as CubeSats are suitable for studying how terrestrial life is affected by the space environment. Rovers on the other hand, are the platforms for searching extraterrestrial life. The evidence suggests that liquid water was once present in Mars' surface, which is one of the reasons that make Mars a prime candidate in the search of extraterrestrial life [129].

Four NASA rovers have been landed and operated successfully on Mars in the past. The first wheeled vehicle to move on Mars was the Sojourner rover that arrived to Mars on-board the Mars Pathfinder lander on July 4, 1997 [130]. The Sojourner was a microrover with dimensions of 65 cm x 48 cm x 30 cm and a mass of 10.5 kg. The payload included front and rear cameras for detecting hazards and imaging the terrain, and an alpha proton x-ray spectrometer to identify the elemental composition of the surface materials [131]. Sojourner used an UHF link to communicate with the lander. The rover operated for three months and roved 104 m on the Martian surface doing investigations. It returned 564 images, chemical analyses of the soil and rocks, and performed 10 technological experiments that provided information for the upcoming Mars rover designs [130].

Mars Exploration mission was the next mission to Mars that included rovers. The two twin rovers, Spirit and Opportunity, landed on Mars around three weeks apart in January 2004. The science objectives of the Mars Exploration rovers focused on the habitability of the planet, including finding evidence that Mars had liquid water in the past and studying the geological processes that have shaped the Martian surface. In addition, the mission had technology related objectives [132]. The rovers were considerably larger than the Sojourner. Measured at wheelbase, the length of the rovers was 141 cm and the width was 122 cm. The solar panels mounted on top of the rover had larger dimensions.

The Mars Exploration rovers had a remote sensing package, including a panoramic camera and a miniature thermal emission spectrometer assembled in a mast. Each of the rovers had a mass of 180 kg [132, 133]. The in-situ science package was embedded in a robotic arm, which included four tools: a microscopic imager, an alpha

particle x-ray spectrometer, a Mössbauer spectrometer and a rock abrasion tool. The microscopic imager was for taking close-up images of the rocks and soils and it was capable of taking in-focus images with spatial resolution of 30 µm [133]. In addition, the rovers had a magnetic properties experiment. For communication, there were two options: direct communication to Earth via X-band or a relay communication via Mars orbiters and an UHF band. The highest supported data rate was 28.8 kbps [132]. The expected lifetime of the rovers was 90 sols, (one sol being one Martian day, which is approximately 40 minutes longer than one day on Earth) [132]. Spirit operated over six years (2210 sols) [134] and Opportunity operated over 14 years (5111 sols) and travelled roughly 45 km [135].

The Mars Science Laboratory rover, Curiosity, landed on Mars on August 2012. The objective of the mission is to investigate the habitability of Mars by studying the geology of Gale Crater site, the Martian environenment and how the atmosphere moderates the radiation environment. The rover's payload consists of 10 scientific instruments, including five different kinds of spectrometers, gas chromatograph, x-ray diffractometer, a meteorology package, radiation monitor and multiple cameras, including a high resolution micro imager [136]. The Curiosity rover is larger than the Mars Exploration rovers with 2.8 m width, 3.0 m length and a mass of nearly 890 kg. The communications are via X-band or UHF band either directly to Earth or via relay system using the two orbiters: Mars Odyssey and Mars Reconnaissance Orbiter. The primary data return is via UHF band and the relay system, with designed minimum data rate of 250 Mb per sol [137]. As of August 2020, the Curiosity rover is still operational [138].

The previous rover missions focused more on the technological demonstrations and the study of Mars' habitability. The first three generations of Mars rovers are presented in Figure 14. The upcoming Mars missions are aiming to find biosignatures on Mars and to prepare for future human exploration missions. Biosignatures can be divided into three categories. Cellular fossils are preserved microbial remains and their extracellular matrices. Studying cellular fossils requires sophisticated sample preparation process and a high-resolution instrument. Morphological biosigna-

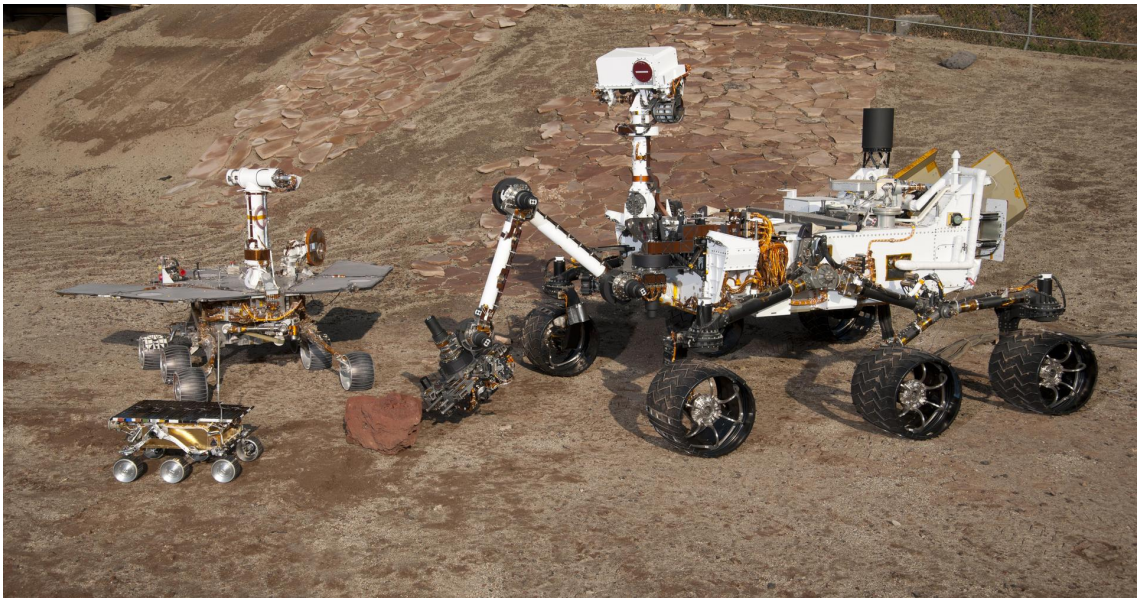


Figure 14: Two test rovers and one flight spare represent the three generations of Mars rovers. From the left: Sojourner, Spirit/Opportunity and Curiosity. Credit: NASA/JPL-Caltech

tures are traces from microbial colonies preserved as textural information on rocks. These biosignatures can be identified and studied with cameras and close-up imagers. Chemical biosignatures are primary biomolecules, such as nucleic acids, proteins and amino acids, the degradation products of biomolecules and biomarkers [68]. NASA 2020 Mars rover, named Perseverance, was launched in July 2020 [139]. ESA's first Mars rover is planned to launch in 2022 [140].

The Perseverance mission objectives are to explore ancient environments that are astrobiologically relevant. The rover will characterize geology and climate, search biosignatures, collect and store samples for potential later return to Earth and demonstrate key technologies for future human exploration missions [69]. The Perseverance rover is approximately the same size than the Curiosity and it has seven scientific instruments. The instruments include a weather station that is able to measure temperature, radiation, dust, humidity, wind and pressure. Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals (SHERLOC) is an instrument for detection of organic molecules, minerals and biosignatures. The SHERLOC imager has a spatial resolution of 30 µm [139]. In the

rover's mast are assembled a panoramic camera system and a SuperCam, which is capable of laser-induced breakdown spectroscopy, Raman spectroscopy and visible and infrared passive reflectance spectroscopy. It is able to determine the elemental composition with 10 % accuracy and detecting minerals and organics, including aromatics, amino acids and carotenoids. SuperCam also has a microphone for recording sounds and a camera for high-resolution imaging [141]. There are also an x-ray spectrometer, an ultraviolet spectrometer and a subsurface radar. Mars Oxygen In-Situ Resource Utilization Experiment (MOXIE) is a technology demonstrator for an instrument that generates oxygen from carbon dioxide [139].

The ESA's ExoMars rover is smaller than the most recent NASA rovers, with 1.3 m width at wheelbase and an approximate mass of 310 kg. The ExoMars rover has a panoramic camera system for imaging the rover's environment and a close-up imager that can take images with sub-mm resolution. With a drill, the ExoMars rover is able to acquire samples with less radiation damage from depth down to 2 m. The samples will then be crushed and analyzed in the analytical laboratory drawer, which includes three scientific instruments: a visible near-infrared imaging spectrometer MicrOmega, a Raman laser spectrometer and a Mars Organic Molecule Analyzer (MOMA). The MicrOmega instrument will identify and study the composition and structure of the sample and pass information to the Raman spectrometer and MOMA. Followingly, the Raman spectrometer and MOMA investigate whether the sample is organic. ExoMars rover is therefore capable of identifying morphological and biochemical biosignatures, but it is not capable of visually recognize cellular fossils. The payload also includes infrared spectrometers, neutron detector and subsystems to support the drilling and sample preparation [68].

Epifluorescence microscopy is one of the most useful techniques for in-situ life detection [142, 143]. Including a fluorescence microscope in a rover would make it possible to find conclusive evidence of extinct or extant life on Mars. Rover is also the most demanding from the presented platforms for a microscope, since the instrument would have to operate autonomously. The autonomous operations include robotic sample preparation, which is one of the main challenges. Typically in labo-

ratories the sample preparation includes sample extraction, centrifugation, washing and labeling. This kind of procedure might be difficult to implement robotically and it could even destroy delicate biosignatures found from the Martian soil and rocks.

Sophisticated sample preparation procedures could be replaced by using the kind of fluorescent dyes that have little fluorescent binding with rock or soil and do not require washing. Organic fluorescent probes are not ideal, because they could be destroyed in the sterilization procedures specified in the planetary protection policies. Long-term storage, possible freezing and heating cycles, as well as the radiation and oxidants on Mars are also challenging. Fluorescent semiconductor nanocrystals, called quantum dots, have been suggested as an alternative for organic probes. Quantum dots have many promising features, such as a broad absorption spectrum, which means that a single LED could be used for excitation. They can also be made in a way that they only fluoresce in the presence of a certain target compound [143]. Nadeau *et al.* [143] tested a variety of different probes and summarized the potential ones for in-situ life detection.

A sophisticated science laboratory including a microscope could also support the sample return mission [144], which is under planning. The microscope could characterize and identify interesting samples that would then be sent to Earth.

4 Discussion and Conclusions

The objective of this thesis was to investigate the potential of a miniaturized fluorescence microscope in space research. It was examined that in which topics within space research a microscope could significantly advance the current knowledge. The space radiation consisting of solar ultraviolet radiation, trapped radiation, solar energetic particles and galactic cosmic rays, together with microgravity or partial gravity, form a complex and dynamic environment. This combination of space radiation and reduced gravity has an impact on humans and poses a threat for astronauts' health. Specifically, the gap in current knowledge is in the underlying biological mechanisms that cause the health problems. To be able to realistically estimate the

health risks of future manned missions beyond the LEO, and to develop effective countermeasures, more research in space environment is needed. A miniaturized microscope would enable in-situ research and generation of data that is crucial for future space travel.

Other topic that could be studied with a microscope in space is the survivability of microorganisms. There is a possibility that resilient microorganisms survive the journey to another planet or celestial body naturally or on-board a spacecraft. The current exposure facilities on the ISS lack instruments that can do observations throughout the experiment, leaving the adaptation processes or the response to microgravity undetected. Microscope could provide real-time information on these processes. Studying the survivability of microorganisms in space would help the further development of planetary protection policies and would also prevent obtaining false positive results when searching for extraterrestrial life on Mars. Other questions that could be further studied with microscope based research are that do bacteria become more pathogenic or drug resistant in the space environment and if so, why does this happen? In addition, the usage of microorganisms as part of life support systems could also be examined.

Fluorescence microscopy as an imaging technique, the state of the art in miniaturized microscopes and space microscopy were reviewed. The smallest of the reviewed microscopes were not standalone instruments and the microscopes previously used in space were relatively large. There is a need for more independently functioning and compact space microscope that could be used in the platforms that enable real-time in-situ research. In epifluorescence microscopes, the same light path is used for both excitation and emission light. This configuration saves space, which is why it is a good solution. Some useful features for a space microscope were presented. These included XY-stage, dark field imaging mode and fluidic system for cell life support. The usage of immobilization techniques instead of fluidic cell systems was also suggested.

Potential platforms for a miniaturized fluorescence microscope and the different research possibilities they offer were studied. From the facilities on-board the ISS,

KUBIK and Biolab are particularly suitable for studying the effects of microgravity or partial gravity due to the centrifuges that can be used to create a reference environment. The ICE Cubes platform is especially suitable for technology demonstrations. The advantage of the facilities on-board the ISS is that human interaction is possible, which enables more sophisticated biological research. However, the ISS is shielded against the majority of the cosmic radiation by the Earth's magnetic field. To study the synergistic effect of space radiation and microgravity, CubeSats are promising platforms, because they can be taken into orbits with significant exposure to energetic charged particles. Other advantages are relatively low cost and short development time, which make CubeSats suitable for technology demonstrations.

While the facilities on-board the ISS along with CubeSats are suitable for studying how terrestrial life copes with the space environment, rovers are the platforms for searching extraterrestrial life. Fluorescence microscope could be the instrument to find conclusive evidence of extinct or extant life on Mars. The robotic sample preparation would be challenging, but fluorophores that do not require washing and have little binding with background materials could be a solution. However, these techniques are not yet mature enough to be reliably used in Mars missions. Moreover, including a microscope in a rover would be more relevant after Perseverance and ExoMars deliver results from Mars. The biosignature findings, or lack of them, could then formulate the requirements for the microscope. From the reviewed platforms, rovers are also the most expensive and the largest projects, hence it is advisable to demonstrate the microscope technology in other platform first.

As a result, the recommended platform for a miniaturized fluorescence microscope is a CubeSat. The biggest open questions are related to the synergistic impact of space radiation (especially HZE particles) and microgravity on humans and microorganisms. An advantageous mission concept would be to include a radiation detector as a second payload in addition to the microscope. With a position sensitive radiation detector on top of the microscope and automatized determination of the area of interest, the cells affected by the HZE particle traversal could be imaged. Demonstrating the functionality of the microscope in space environment with

CubeSat would also raise the TRL of the instrument.

All in all, the applications for a miniaturized fluorescence microscope in space research are diverse and it can be concluded that there is a need for this kind of instrument. Next step would be to specify the application and continue the development of the breadboard towards a space qualified instrument.

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