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**International Space Station  
Research Results Accomplishments:  
An Analysis of Results from 2012-2014  
Addendum – June 2017**



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International Space Station Research Results Accomplishments: 2012-2014

# FOREWARD

In 2016, the International Space Station (ISS) partnership published the first-ever compilation of international ISS research publications resulting from research performed on the ISS through 2011. The *International Space Station Research Accomplishments: An Analysis of Results From 2000-2011* is a collection of summaries of over 1,200 journal publications that describe ISS research in the areas of biology and biotechnology; Earth and space science; educational activities and outreach; human research; physical sciences; technology development and demonstration; and, results from ISS operations.

In October 2016 the office of the ISS Program Scientist released the addendum to the original titled *International Space Station Research Accomplishments: An Analysis of Results From 2012-2014*.

This addendum includes Roscosmos results summaries including results from early ISS utilization through expedition 40.

## IDENTIFYING THE GENETIC FEATURES DETERMINING INDIVIDUAL DIFFERENCES IN THE RESILIENCE OF BIOLOGICAL OBJECTS TO LONG-TERM SPACEFLIGHT FACTORS STUDIES WITH THE FRUIT FLY *DROSOPHILA MELANOGASTER* (POLIGEN)



**Research Area:** Biology and Biotechnology: Animal Biology – Invertebrates  
**Expedition(s):** 19-21, 23, 24, 27-30, 34, 35 - Ongoing  
**Principal Investigator(s):** Olga N. Larina, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow Russia

### RESEARCH OBJECTIVES

The Identifying the Genetic Features Determining Individual differences in the Resilience of Biological Objects to Long-term Spaceflight Factors Studies with the Fruit Fly *Drosophila*



View of the Drozofila Kit - used in an experiment to define genetic criteria for revealing living organisms with a maximum possible resistance to extreme conditions of a prolonged space flight - mounted to panel 406 in the Service Module (ISS020E050695)

*melanogaster* (Poligen) experiment studies the links between parameters of the capability of *D. melanogaster* populations to adapt to spaceflight and their genetic structure. At the present time, the causes of the individual sensitivity to genetic mutations and inducing factors during spaceflight have not been fully studied. The ability of an organism to withstand mutagenic factors depends on the features of its genotype, in particular the systems of genes controlling processes of DNA repair.

### EARTH BENEFITS

A possible promising use of the space experiment results on Earth is analyzing the individual genotypes in order to identify genetic indications of resilience to mutational disruptions caused by stress factors and unfavorable environmental conditions.

### SPACE BENEFITS

As a result of the experiment, data are expected to be obtained on genes and gene systems, the functional activity of protein products whose expression influences the resilience of eukaryotic organisms to mutational changes when exposed to a range of spaceflight factors. Determining the individual genotypes of these genes could be used to predict the risk of mutations occurring in biological organisms, including humans, in near-Earth spaceflight. The results of the studies could potentially be used to assess gene mutation vulnerability of spaceflight candidates.

## RESULTS

For the Poligen experiment on the ISS, the Drozofila-2 kit (figure-1) is used, with 2 containers for holding flies and feed. From the experiments, it was found that in some stages of the individual development of the insects, specifically in the stages of middle and late chrysalis, exposure to sub-optimal environmental conditions (environmental stress) during spaceflight and/or ground transportation from the landing site could induce an increase in the frequency of dominant lethal mutations in drosophila. The data obtained confirm the possibility of mutagenesis activation when exposed to unfavorable factors corresponding to actual spaceflight. In this context, in addition to ionizing radiation, exposure to microgravity, dynamic impacts, changes in the atmospheric composition, changes in temperature could affect mutation frequency. The results of the experiment also make it possible to hypothesize that one of the causes for contradicting data on the effect of spaceflight factors on the mutation process found in some literature could be a change in drosophila sensitivity to genotoxic factors occurring during individual development.

## PUBLICATION(S)

Larina ON, Lazebnyy OY, Kulikov AM. Population/genetic studies of *Drosophila melanogaster* on the ISS (Poligen experiment). *Space Biology and Medicine*. 2011;2:384-388.

Sychyov VN, Levinskikh MA, Guryeva TS, Podolskiy IG, Gorgiladze GI, Samarin GI, Larina ON. Biological experiments in manned spaceflight. *Space Medicine and Biology*. 2013:170-192.

Larina ON, Bekker AM. Dominant lethals in *Drosophila melanogaster* natural populations flown on board ISS. *COSPAR 40th Scientific Assembly*, Russia, Moscow. 2-10 August 2014.

***Investigation is ongoing and more results are pending publication.***

## STUDYING THE IMPACT OF VARIOUS SPACEFLIGHT FACTORS ON THE PROCESS OF REGENERATION IN BIOLOGICAL OBJECTS IN TERMS OF MORPHOLOGICAL AND ELECTRO-PHYSIOLOGICAL INDICATORS (REGENERATSIYA-1)

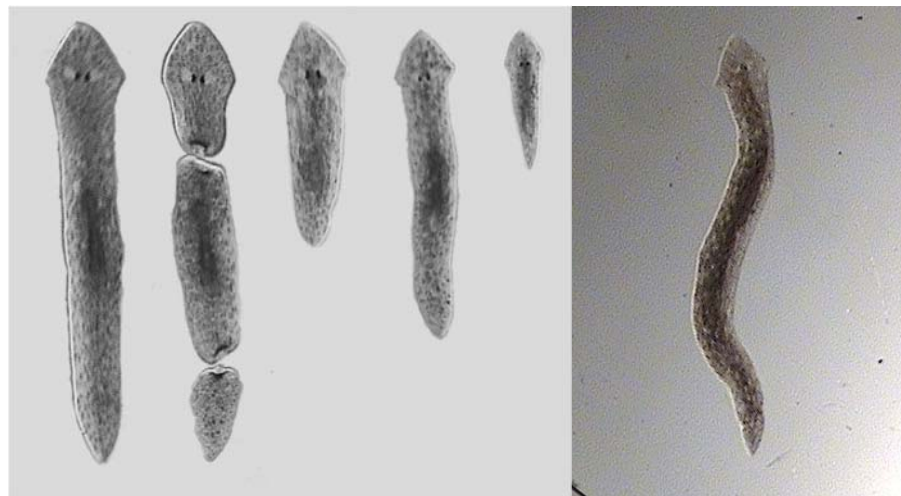


**Research Area:** Biology and Biotechnology: Animal Biology – Invertebrates  
**Expedition(s):** 37 – 40, Ongoing  
**Principal Investigator(s):** Givi I. Gorgiladze, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow Russia

### RESEARCH OBJECTIVES

The Studying the Impact of Various Spaceflight Factors on the Process of Regeneration in Biological Objects in Terms of Morphological and Electro-Physiological Indicators (Regeneratsiya-1) investigation uses the fresh water planarian as a model organism to study the affects of microgravity on regeneration.

Understanding the importance of the force of gravity in different life processes in terrestrial organisms is a paradigm of space biology.



From left to right: planarian in its normal state, planarian cut across in three sections, three regenerated specimens (1). Planarian regenerated from one half of its body (planarian's body is curved) (2).

### EARTH BENEFITS

In the experiment, new knowledge was obtained on the possibility for organism development in a sensory field in the absence of gravitation. The results obtained could be used by experts working in the field of developmental biology, and to develop and conduct lecture series in the relevant disciplines for students at university biology faculties.

### SPACE BENEFITS

An increase in manned spaceflight duration, increase in the volume of work, and complication of operator tasks increase the risk of accidents and various types of injuries requiring surgical intervention in crewmembers. Because of this, information on the regeneration of damaged organs and tissues in animals and the anomalies occurring in a number of cases in the regenerated formations could be useful to refine the strategies of medical support during long-term manned spaceflight.



## **RESULTS**

In flights lasting 11-14 days (ISS-13, ISS-14, and ISS-17), it was shown that planarians retain a high regenerative capacity. In specimens in flight, like in the control on Earth, the restoration of missing body parts was noted.

From this, it was concluded that the absence of gravity is not a limiting factor for regeneration mechanisms. In addition, in a number of cases anomalies were identified in the flight regenerated specimens: planarians that regenerated from the longitudinal half of the body were noticeably curved compared to controls. The need to identify the possible causes of this phenomenon led to studies with longer exposure to spaceflight factors.

In the post-flight examination of capsule contents, in all the planarian specimens the appearance of missing body parts was established: in the right halves the missing left part regenerated; in the left halves, the right part. This same picture was observed in the cut halves of the torso during planarian regeneration. In each half of the torso, the anterior part of the body also regenerated. In the majority of cases, the regenerated planarians' bodies curved to the right or the left by 30-90°. They would swim in circles, typically in the direction of the curve of the body.

In the first two runs of the experiment on station, data were obtained that were similar to previous data on the ISS with exposure three times shorter than exposure in the indicated flights. The background radiation recorded in the area of the planarian containers was approximately 120 higher than that in Moscow. This could be one of the causes of the anomalous development of the regenerated specimens, which requires further study.

***Investigation is ongoing and more results are pending publication.***

## STUDY OF THE MICROGRAVITY EFFECT ON INTRACELLULAR CHARACTERISTICS DETERMINING THE CELL'S FUNCTIONAL STATE (BIOSIGNAL)



**Research Area:** Biology and Biotechnology: Cellular Biology  
**Expedition(s):** 40 – 42, Ongoing  
**Principal Investigator(s):** Lyudmila B. Buravkova, M.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow Russia

### RESEARCH OBJECTIVES

The Study of the Microgravity Effect on Intracellular Characteristics Determining the Cell's Functional State (Biosignal) investigation studies the effects of microgravity and other space flight factors on the intracellular pH regulation system as an integral indicator of the overall functional state of the cell. Using a suspension culture of human lymphocytes isolated from peripheral blood the effects on the intracellular pH-regulation system are evaluated using fluorescent probes.



Fluor-C Device containing human lymphocyte cell suspension.

### EARTH BENEFITS

The Fluor-C device is a compact standalone multi-channel fluorometer with a programmable operating algorithm. It is used for recording the changes in the differential fluorescence signal from organic or inorganic objects (cellular organelles in suspension, human and animal cells, unicellular algae, bacteria, and fluorescent colloid solutions of various origin) in small volumes with synchronous temperature measurements. Experiment results are used to assess the gravitation contribution when evaluating the effects of temperature on cellular processes, as well as when comparing effects on various cellular systems.

### SPACE BENEFITS

Results facilitate advances in methodologies for studying the state of intracellular systems in microgravity and also to develop a screening system of express tests for assessing the functional cell state during various phases of space flight. This system aims to expand the testing capabilities of space biology and biotechnology. Experiment data allow researchers, for the first time, to assess the cell function changes during each phase of space flight and contribute to our understanding of the fundamental processes of cell adaptation to changes in gravitational stimulus and facilitate the development of countermeasures.

### RESULTS

Data analysis is in progress and results are pending future publication.

*Investigation is ongoing and more results are pending publication.*

## STUDYING THE IMPACT OF MICROGRAVITY ON THE SOLUBILITY OF CALCIUM PHOSPHATES IN WATER (KALTSIY)



**Research Area:** Biology and Biotechnology: Cellular Biology  
**Expedition(s):** 27 – 39, Ongoing  
**Principal Investigator(s):** Anatoliy D. Ukraintsev, Ph.D., Biokhimmash, Moscow, Russia  
K. Krashennnikova, Ph.D., Biokhimmash, Moscow, Russia Alexey N. Sinitsyn, Ph.D., Biokhimmash, Moscow, Russia  
Olesya A. Semelyova, Biokhimmash, Moscow, Russia  
Yekaterina V. Sinchurina, Ph.D., Biokhimmash, Moscow, Russia

### RESEARCH OBJECTIVES

The Studying the Impact of Microgravity on the Solubility of Calcium Phosphates in Water (Kaltsiy) investigation determines the of solubility of calcium phosphates and human bone tissue samples in water in microgravity to reveal the possible causes of calcium homeostasis destruction and demineralization in human bone tissue.

### EARTH BENEFITS

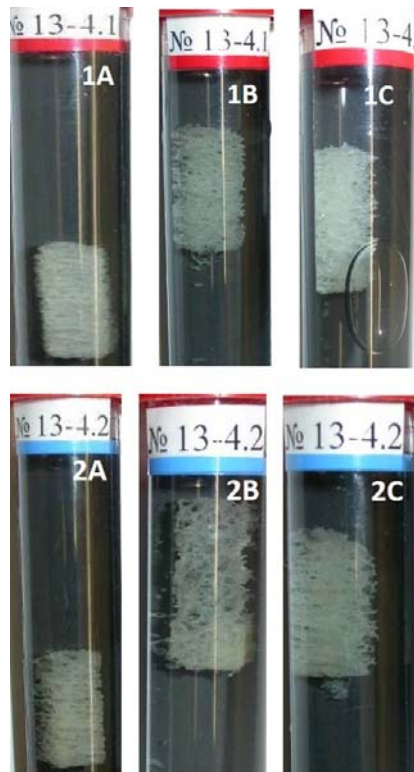
No earth application has been identified for this experiment.

### SPACE BENEFITS

The systematic study of calcium elimination from bone tissue in long-duration spaceflight is necessary to understand the primary causes of the rapid development of symptoms of osteoporosis in crewmembers and to develop means of prevention.

### RESULTS

During the long-term space experiment (291 days), the sterility of flight and ground control test tubes was not disturbed. In 2 test tubes (one flight and one Earth control) out of the 32 used in the experiment session, air bubbles were detected, indicating a failure of test tube integrity. In both cases the cause of the integrity failure was a defect in the test tube cap seal. Visually, no noticeable changes were discovered in the external appearance of the cortical bone tissue specimens



Photographs of flight test tubes containing specimens of cortical bone tissue in physiological solution and distilled water before and after the third session of the experiment. Series 1 images show samples in a physiological solution solvent. Image 1A shows when the science equipment is filled. Image 1B shows after exposure on ISS and 1C shows after storage on Earth. Series 2 images show samples in a distilled water solvent. Image 2A shows when the science equipment is filled. Image 2B shows after exposure on ISS and 2C shows after storage on Earth.



either in water or physiological solution and either on the ISS or on Earth. At high magnification, extremely small particles of bone tissue were visible in all test tubes. The external appearance of trabecular bone tissue specimens did not change noticeably in either solvent. The mechanical strength of cortical tissue was no worse.

The results of determining four concentrations of calcium and phosphorus in solvents during exposure on the ISS and on Earth confirm the previously established trend: the elimination of calcium in both types of bone tissue in physiological solution was greater than in distilled water. Another impacting factor in static experiment conditions (immobile, test tube sealed closed) was the correlation between the mass of the bone tissue specimen and the volume of solvent: at a constant test tube volume (12 ml) the lower the specimen mass, the greater the mineral elimination. In the majority of the experiments, calcium and phosphorus elimination in physiological solution and distilled water on the ISS was noticeably higher than on Earth. The elimination of phosphorus and calcium was not at a steady rate. In the initial period (50-100 days), the transfer of elements into the solvent increased, then slowed down and began to decrease. This makes it possible to hypothesize that in the absence of thermal movement of the solvent, the main process of element elimination becomes the diffusion of liquid to the micro-pores of the bone tissue. On Earth, this phenomenon was manifested to a lesser degree than on the ISS.

*Investigation is ongoing and more results are pending publication.*

## STUDYING THE IMPACT OF SPACEFLIGHT FACTORS ON ENZYME ACTIVITY (KONSTANTA)



**Research Area:** Biology and Biotechnology: Cellular Biology  
**Expedition(s):** 21-22, 25, 27, 29, 31, 33, 35, 37, 39  
**Principal Investigator(s):** Valentin I. Evstigneev, Ph.D., Biopreparat, Moscow, Russia  
Alexey N. Sinitsyn, Professor, Ph.D., Biokhimmash, Moscow, Russia

### RESEARCH OBJECTIVES

The Studying the Impact of Spaceflight Factors on Enzyme Activity (Konstanta) investigation identifies the existence and nature of the impact of spaceflight factors on the activity of a model enzyme preparation relative to a given substrate. An increase in enzyme activity in orbital flight can impact the condition and function of the human body. Enzyme studies could clarify the impact of spaceflight on organs and tissues of the human body living long-term aboard the International Space Station (ISS).



Photographs of Konstanta trays on exposure day 6 during ISS-31 and in the parallel experiment on Earth. Image provided by Roscosmos.

### EARTH BENEFITS

Experiment data on changes in the characteristics of biocatalysis reactions in space provide the opportunity to come closer to identifying the causes of changes in the molecular mechanisms of reactions of living matter in space. Such data are necessary to create reliable means of preventing and protecting against the corrosive factors of the surrounding environment.

### SPACE BENEFITS

Experimentally determining the total impact of spaceflight factors on the stability and biocatalysis activity of crucial enzymes in mammals provides an opportunity to determine the feasibility, both individually

and systematically, to monitor biochemical indicators in crewmembers during spaceflight using enzyme test systems, which is necessary to realize long-term space flights and colonize space objects; determine the possible directions for using enzyme systems to protect against the undesired effects of spaceflight factors.

### RESULTS

The first experiment session was carried out by the ISS-22 crew. A total of three sessions were conducted with the Rekomb-K equipment. The capability to determine the activity of an isolated enzyme preparation in microgravity was demonstrated experimentally using simple

equipment, and to visually record the expected effect in real time. The information obtained using the Rekomb-K hybridizers on the optimal composition of receptors (specific substances, their concentrations and ratios) and on the nature of changes in the properties of their components during ascent and orbital flight was directly used to develop enzyme-substrate-indicator receptors for the special Konstanta equipment. As of 2011, Konstanta equipment has been used for the space experiment on the ISS RS and on Earth. Parallel experiments demonstrated that on Earth enzyme activity quickly and steadily decreased, and on exposure day 25 it was detected in only one of the four compartments. On the ISS, enzyme activity initially increased, then rapidly dropped, but was detected after 39 days of exposure in individual compartments.

It is clear that on the ISS (white timer), the reaction has already stopped in all indicator compartments, while on Earth (black timer) the reaction continues. The growth in enzyme activity on the ISS complicates comparison with the experiment on Earth. It was established that the  $K_M$  values calculated increased consecutively as exposure duration increased on the ISS Russian Segment (RS) and on Earth, while  $K_M$  on Earth increased faster. Analysis of the patterns of the decrease in enzyme activity established that the rate of enzyme denaturation was lower on the ISS RS than on Earth.

On a qualitative level, the fourth session fully confirms the previously identified pattern: in orbital flight conditions the enzyme activity of butyrylcholinesterase is significantly higher than on Earth, beginning with the first run. A methodological approach to calculating the Michaelis constant was developed based on photo and video imagery of the course of the enzyme reaction, and specific  $K_M$  values were obtained. On the ISS, the enzyme reaction was virtually complete within 3 minutes; on Earth, in 6 minutes. The increase in the reaction rate on the ISS can be assessed at no less than 2 fold compared to on Earth.

The results obtained make it possible to assert that in the sessions conducted, the main task of the Konstanta experiment science program was successful. Through experiments, the typical correlation between the rate of an enzyme reaction and specific substrate concentration was studied, and the Michaelis constants were calculated. It was established that in space (orbital) flight, the activity of butyrylcholinesterase relative to butyrylcholine was significantly higher than in a parallel experiment on Earth. Microgravity is the most likely spaceflight factor capable of having such an effect. The increase in enzyme activity in orbital flight that was identified can impact the condition and function of organs and tissues of the human body.

***This investigation is complete; however, additional results are pending publication.***

## OPTIMIZING THE PROCESS OF GENETIC MATERIAL TRANSMISSION USING BACTERIAL CONJUGATION (KONYUGATSIYA)



**Research Area:** Biology and Biotechnology: Cellular Biology  
**Expedition(s):** 7, 9, 12-19, 21, 24, 27, 33, 35, 36, 39 – Ongoing  
**Principal Investigator(s):** Yu. P. Zerov, OOO Proteinovyi Kontur, St. Petersburg, Russia  
Nikolay A. Staritsyn, Biopreparat, Moscow, Russia

### RESEARCH OBJECTIVES

The Optimizing the process of genetic material transmission using bacterial conjugation (Konyugatsiya) is devoted to the development of methods for constructing new recombinant strains producing biologically active substances (BAS) via the transmission of plasmid and chromosome DNA using bacterial conjugation.

### EARTH BENEFITS

Gene-engineering technologies for obtaining new highly effective therapeutic and preventive drugs for human and veterinary medicine are currently a high priority in the pharmaceutical industry. The developed method of obtaining hybrid strains producing BAS through the transfer of chromosome or plasmid DNA using bacterial conjugation under space flight conditions and subsequent ground selection may be used to produce proteins that are valuable for health care. The introduction of recombinant producer strains obtained during this work into manufacturing and the subsequent use of the developed methodology to obtain new strains producing BAS and their use at other sector companies may have a significant economic effect.



ISS035E030074 - Roscosmos cosmonaut Alexander Misurkin holds a Recomb-K Apparatus for the Konyugatsiya (Conjugation) experiment.

### SPACE BENEFITS

Data obtained on the influence of orbital flight conditions on the effectiveness of transferring chromosome and plasmid DNA using bacterial conjunction may be used to create process lines to produce recombinant proteins under microgravity conditions.

## RESULTS

Recombinant donor and recipient strains are engineered on the ground for each experiment session. As a result of implementing two Konyugatsiya experiments during ISS Expeditions 23/24 and 27/28, two recombinant plasmids that encode the end human Cu, Zn superoxide dismutase (SOD) protein were successfully transferred. From the biomaterial obtained during conjugative hybridization in the experiment, four potential variant hybrid clones of SOD-producing *E. coli* were identified: two groups depending on the recipient strain and plasmid and two hybrid alternatives each in each group: type I hybrids, which received only the recombinant plasmid as a result of mobilization, and type II hybrids, which also received the F' conjugative plasmid. The mean productivity of the obtained clones of the two variants of strains producing SOD was 170–185 µg/ml of culture, which is sufficiently high for primary clones. In both cases the productivity of obtained lines of hybrid strains is sufficiently high, as to allow them to be used as original material for the selection of high-productivity producer strain variants.

Another object of study that is of great practical interest is the human epidermal growth factor (EGF). EGF is a unique growth factor that ensures monolayer skin growth, i.e., wound healing without the formation of scar tissue. This factor has ensured its wide use in treating burns, for healing post-surgical sutured incisions, for treating ischemic ulcers, bed sores, and freezing injuries, for preventing and treating radiation dermatitis, as well as for cosmetic surgery. Obtaining hybrid producers of EGF in the Konyugatsiya experiment met with serious difficulties. Owing to the high toxicity of the end product for bacteria cells, the obtained hybrids gradually lost the ability to synthesize EGF. Finally, as a result of the Konyugatsiya experiment implemented during ISS Expedition 39/40, two stable and pure lines of a strain producing EGF—*E. coli* BL21(DE3)/F';pEThEGForiT—were obtained by combining the preliminary selection in the experiment with the subsequent ground-based selection. Both obtained lines of strains producing EGF exhibited high genetic stability and reproducibility with respect to end product output.

Obtained variants of the strain *E. coli* BL21(DE3)/F';pEThEGForiT are of interest as promising sources for the production of the EGF-producing strain. Results confirmed that the method of mobilizing recombinant plasmids in bacterial conjugation in the experiment allows bacterial producer strains with a levels of end product biosynthesis high enough for practical use to be obtained.

## PUBLICATION(S)

Zerov YP, Murashev BV, Smirnova GV. Plasmid transfer using bacterial conjugation under on-orbit space flight conditions. *Cosmonautics and Rocket Engineering*. 2007;4(49):95.

***Investigation is ongoing and more results are pending publication.***



## THE EFFECTS OF SPACE FLIGHT FACTORS ON A LACTOLEN PRODUCER STRAIN (LACTOLEN)



**Research Area:** Biology and Biotechnology: Cellular Biology  
**Expeditions:** 16-19, 21, 23, 24, 27, 29, 32  
**Investigator(s):** Alexey I. Kobatov, State Scientific and Research Institute of High-Purity Medicinal Products, Moscow, Russia  
Valentin I. Evstigneev, Biopreparat, Moscow, Russia

### RESEARCH OBJECTIVES

The probiotic based on strains of *Lactobacillus acidophilus* is a representative of a new generation of immunobiological preparations, created with due regard for modern ideas in medicine and biotechnology. It exhibits high therapeutic effectiveness and safety, and does not cause allergic reactions. The Effects of Space Flight Factors on a Lactolen Producer Strain (Lactolen) investigation studies the effects of exposure to space flight on the biomedical properties of investigated strains of *Lactobacillus acidophilus*. Microgravity is believed to be the principal space flight exposure factor acting on living organisms. At the same time, other on-orbit factors cannot be ignored—in particular, cell motion in a geomagnetic field and cosmic radiation.

### EARTH BENEFITS

Selected high-productivity lines (clones) of *Lactobacillus acidophilus* strains with improved biomedical characteristics will be used to produce the Vitaflor probiotic preparation at the facility of the State Scientific and Research Institute of High-Purity Medicinal Products (Federal State Unitary Enterprise), Russia.

### SPACE BENEFITS

Data on the impact of on-orbit exposure on the growth, genetic, and probiotic properties of lactic acid bacteria obtained from *Lactobacillus acidophilus* will be used to establish the production of probiotics on the space station.



Porous Vitaflor tablets. Image provided by Roscosmos.

### RESULTS:

The results of the Lactolen experiment during ISS-16, ISS-19, and ISS-21 confirmed data obtained regarding the high sensitivity of liquid *Lactobacillus* cultures to space flight factors. In addition, flight specimens are characterized by exceptionally high sensitivity to storage—after one week, the number of viable cells had fallen sharply in comparison with ground specimens.

During the next phase of the experiment (during missions ISS-23 to ISS-30), a study was conducted of the effects of space flight factors on the probiotic properties of dry formulations

consisting of nutrient medium and seed material and exposed to space flight conditions aboard the ISS for 0.5, 1, and 1.5 years. The experimental specimens comprised a mixture of polydisperse powder (having a particle size of 10-150 µm), nutrient medium, and seed material (the Vitaflor probiotic).

It was determined that, after exposure aboard the ISS for 1.5 yr, the dry parent stock of the Vitaflor lactic acid product retains its probiotic potential and may be used to obtain a probiotically functional Vitaflor product.

During ISS-32, a long-duration session of the Lactolen experiment was begun, to study the influence of space flight factors on the probiotic properties of dry systems consisting of a dried and pulverized nutrient medium (liquid milk) and seed material in the form of a porous tablet of Vitaflor probiotic. The duration of exposure aboard the ISS was 0.5 yr, 1 yr, and 2 yr.

The distinguishing feature of this session was the change in the seed material from a polydisperse powder to a fragment of a porous tablet, obtained using an innovative method of molding and freeze drying (RF Patent No. 2169547 [cq-Trans]). The use of an initial starter in the form of a tablet obtained via the innovative method of molding and freeze drying (RF Patent No. 2169547) simplifies the technology for obtaining a freeze-dried parent stock for the product, as it eliminates the stage of pulverizing a biological preparation, which reduces the starter's biological activity.

It was shown that over a 2 yr period of exposure aboard the ISS at the working compartment temperature, the freeze-dried parent stock of the lactic-acid probiotic Vitaflor product retained a high level of probiotic lactic-acid bacteria. The lactic-acid products obtained from flight specimens are a bioculture of *L. acidophilus* (strains D75 and D76) that is characteristic of the Vitaflor probiotic, with a high level of physiologically active lactic-acid bacteria. Lactic-acid bacteria exhibit typical stability to antibiotics, which are the drugs of choice used to treat infectious diseases of various bacterial nature; they show high antagonism to opportunistic pathogenic bacteria; they are stable to the adverse antagonism of *S. aureus* and *K. pneumoniae*.

Thus, lactic-acid products obtained by fermenting freeze-dried parent stock exhibit high probiotic potential. At the conclusion of the experiment during ISS-32, a ground selection was made of highly stable clones of *L. acidophilus* strain D75, obtained from a flight specimen. The obtained clones will be used to develop a new generation of bacterial probiotics, with their subsequent production by the manufacturing section of the State Scientific and Research Institute of High-Purity Medicinal Products (Federal State Unitary Enterprise), Russian Federal Biomedical Agency.

#### **PUBLICATIONS**

Kobatov AI, Verbitskaya NB, Dobrolezh OV, Petrov LN. The Vitaflor probiotic as a potential way to protect cosmonauts against the adverse consequences of exposure to ionizing radiation. *Meditcina ekstremalnykh situatsiy [Emergency Medicine]*. 2007;2(20):72–79.

Kobotov AI, Verbitskaya NB, Dobrolezh OV, Rybalchenko OV, Petrov LN. A study of the probiotic characteristics of *Lactobacillus acidophilus* grown under space flight conditions. *Medsina ekstremalnykh situatsiy [Emergency Medicine]*. 2008;4(26):66–78.

Kobotov AI, Verbitskaya NB, Dobrolezh OV, Petrov LN. Optimizing the process of cultivating the probiotic *L. acidophilus* bacteria under space flight conditions. *Medsina ekstremalnykh situatsiy [Emergency Medicine]*. 2010;4(34):77-86.

Verbitskaya NB, Dobrolezh OV, Kobotov AI, Petrov LN. Aspects of obtaining and using bacterial probiotics aboard the ISS under conditions of long-duration space flight. *Kosmonavtika i raketostroenie [Cosmonautics and Rocket Engineering]*. 2011;3(64):130-135.

***This investigation is complete; however, additional results are pending publication.***

## CRYSTALLIZING BIOLOGICAL MACROMOLECULES AND OBTAINING BIOCRYSTALLINE FILMS IN MICROGRAVITY CONDITIONS (KRISTALLIZATOR), 50 INVESTIGATIONS



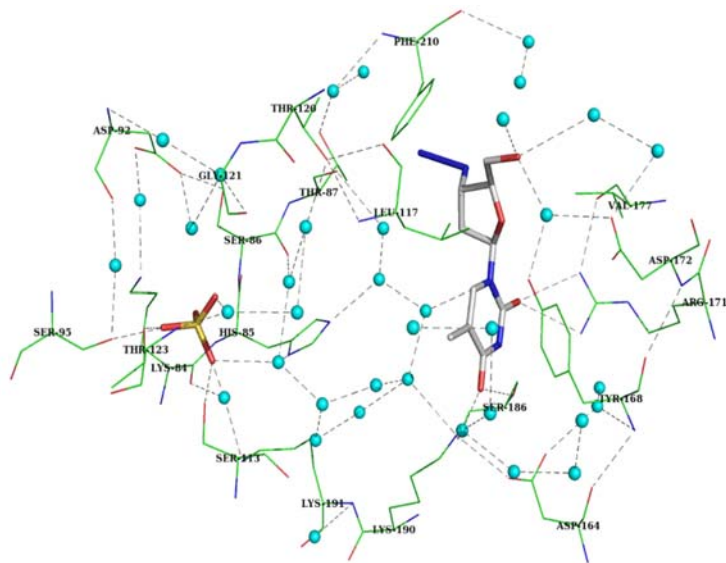
**Research Area:** Biology and Biotechnology: Macromolecular Crystal Growth  
**Investigator(s):** Alexey E. Voloshin, Ph.D., A. V. Shubnikov Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia  
**Expeditions:** 12-14, 16-18, 20, 22-25, 28, 30, 35-36, 39

### RESEARCH OBJECTIVES:

The Crystallizing Biological Macromolecules and Obtaining Biocrystalline Films in Microgravity Conditions (Kristallizator) investigation studies the physical processes of protein crystallization to obtain perfectly structured single protein crystals that are suitable for X-ray structural analysis, and biocrystalline films from a three-dimensional solution formed on substrates using the artificial epitaxy effect. Study of protein crystals is essential for visualizing proteins and developing new drugs and agricultural products.

### EARTH BENEFITS:

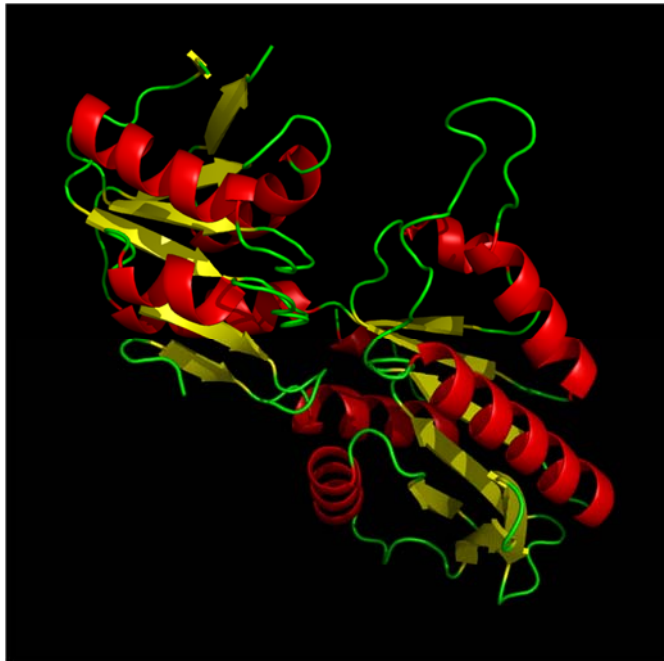
Crystallization of proteins with subsequent determination of their structure via X-ray structural analysis is one of the most promising areas of modern structural biology. In many cases, a knowledge of the structure of a protein and its complexes, for example, with an inhibitor, is key for the creation of pharmaceuticals. X-ray structural analysis is the principal method that allows aspects of the structural organization of macromolecules to be studied. Crystallization in microgravity significantly improves the quality of obtained crystals and allows greater three-dimensional resolution to be achieved when decoding macromolecule structures.



Structure of thymidine phosphorylase in complex with an inhibitor: envelopment of azidothymidine in the active center of thymidine phosphorylase. Image provided by Roscosmos.

### RESULTS:

In the period since 2005, crystals of 69 proteins and their complexes have been obtained. Structural data have been obtained for 59 proteins. Data on 24 protein structures has been deposited in the PDB.



Independent part of a lattice cell in a PRPP crystal. Image provided by Roscosmos.

In recent years, the results of the space experiment have obtained and refined the three-dimensional structures of proteins: carboxypeptidase T (CpT) carboxypeptidase T complexed with sulfamoyl-L-arginine (SPArg), thymidine phosphorylase complexed with an inhibitor, a protein of unknown function from *Salmonella typhimurium* It2 (2Q02), a protein of unknown function from *D. radiodurance* (3E80), lactose from *B. aclada*, purine nucleoside phosphorylase, carboxypeptidase B, 5-keto-4-desoxy uronate isomerase from *E. coli*, O-acetyl-L-homoserine sulfhydrylase from *B. metelensis*, aldehyde dehydrogenase from *Pyrobaculum sp 1147* (apo form), Kdul with inhibitor, FBA with inhibitor, phosphoribosyl pyrophosphate synthetase (PRPP), the DJ-1 protein from *H. sapiens*,

and Xaa-pro aminopeptidase from *T. sibiricus*. Beginning in 2009 this investigation has been carried out jointly with JAXA.

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***Investigation is ongoing and more results are pending publication.***

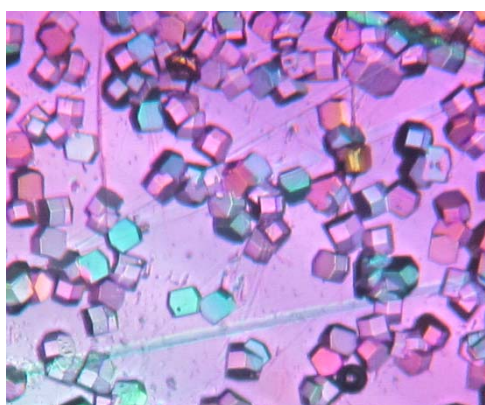
## OBTAINING HIGH QUALITY CRYSTALS OF RECOMBINANT PROTEINS (STRUKTURA), FOUR INVESTIGATIONS



**Research Area:** Biology and Biotechnology: Macromolecular Crystal Growth

**Expedition(s):** 18, 21, 22, 25, 27, 29, 31, 33, 35, 37, 39 - Ongoing

**Principal Investigator(s):** Valentin I. Evstigneev, Ph.D., Biopreparat, Moscow, Russia  
A.I. Miroshnikov, Ph.D., Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia



Results of the control experiment "Lizotsim" on Earth during ISS-33. Contents of the crystallization compartment. polarized light.

### RESEARCH OBJECTIVES

Struktura is a study of protein crystallization processes and growth of single crystals which are suitable for X-ray structural analysis and structural decoding. Proteins are large molecules that are involved in all processes which support the vital activities of cells in an organism. The main advantage for using space to obtain biological crystals is that in space there is virtually no convective disturbances, which negatively impacts crystal growth on Earth.

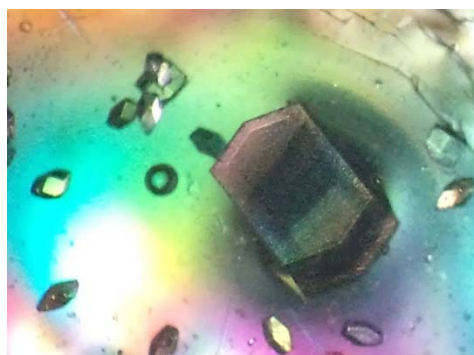
### EARTH BENEFITS

High quality crystals of recombinant proteins grown in space have scientific and commercial value, and data

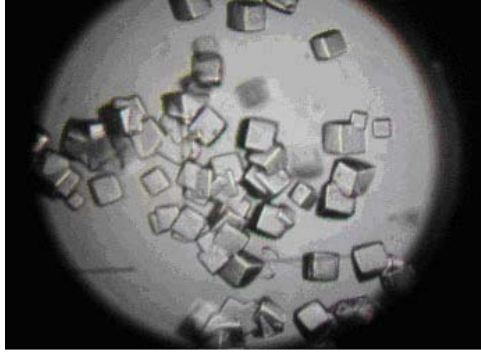
obtained in the experiment are used to understand the structure and function of these molecules. Peptides could be synthesized, using microgravity data, which possess the main properties of proteins but without gravity effects present in the whole molecules. Conducting experiments in microgravity, in which substances are transported to the growing crystal by diffusion, offers an excellent opportunity to develop the understanding of the growth mechanisms of protein crystals, which is also important for improving technologies on Earth.

### SPACE BENEFITS

Since the beginning of spaceflight, growing single crystals of various compounds, including proteins, has become an area of practical application of spacecraft. Space research creates fundamental technological processes for crystallizing valuable proteins based on different methods and specially designed protein solutions.



Results of the Lizotsim experiment during ISS-35. Contents of the on-orbit crystallization compartment, polarized light.



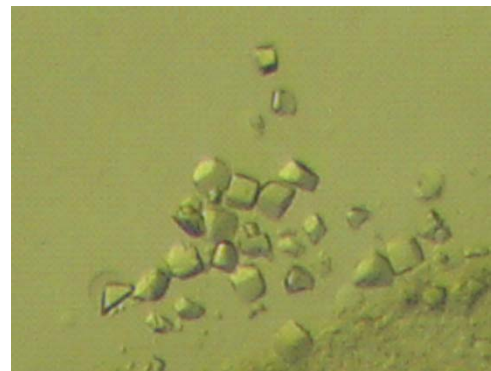
Results of the Sialoinsulin experiment during ISS-29. Contents of the on-orbit crystallization compartment, typical light. (cubic crystals, average size 50x50x50  $\mu\text{m}$ ).

## RESULTS

The Struktura experiment was carried out during multiple increments using Luch-2 equipment. Beginning in increment ISS-27, the greatly upgraded Luch-2M biocrystallizer has been used to support the Struktura experiment. The Luch-2M equipment consists of two kits, each containing six standard biocrystallization trays (Figure-1). The compartments of the crystallization tray are filled on Earth with protein solutions and precipitant, and delivered to the International Space Station (ISS). The duration of each session is 30-90 days.

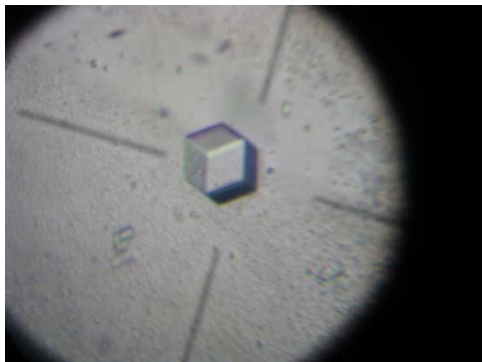
Based on reference data and promising biological and research benefits, the following proteins were selected

for crystallization on the ISS Russian Segment (RS): Recombinant alpha-fetoprotein (AFP), recombinant alpha-fetoprotein in a complex with bilirubin, recombinant gamma-interferon, recombinant human growth hormone (somatotropin), recombinant phage T5 muralytic enzyme (T5mur), recombinant phage phiKZ fibrillar adhesin, recombinant human insulin modified by polysialic acid 23 kDa INCA23 ("Sialoinsulin"). Results show that in standard conditions on Earth, the proteins forms multiple small crystals. Under the same chemical conditions, but in microgravity, the formation of very large straight crystals virtually visible with the naked eye (100 x 100 x 500  $\mu\text{m}$ ) can occur.



Results of the Sialoinsulin experiment during ISS-29. Contents of the Earth control crystallization compartment, typical light. (crystals, average size 20x20x20  $\mu\text{m}$ ).

To clarify whether chemical modification affects the



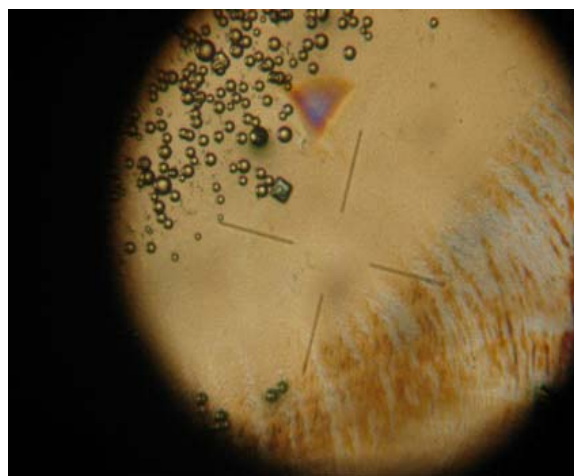
Results of the T5Mur experiment during ISS-25. Contents of the on-orbit crystallization compartment. Typical light.

construction of

the initial protein molecule, recombinant insulin with polysialic acid 23 kDa was crystallized. On Earth, the complex had difficulty crystallizing, producing small and disordered crystals. In microgravity, cubic crystals were obtained. At the DESY synchrotron (Hamburg, Germany), a diffraction set with resolution up to 1.55  $\text{\AA}$  was collected from the flight crystals, and a spatial model was built of the insulin that was part of the complex, with a resolution of 1.60  $\text{\AA}$ . The study showed that during formation of the complex, minimal spatial changes occurred in the insulin molecule that did not impact the biological activity of the substance, but increased the time the drug acted in the body.

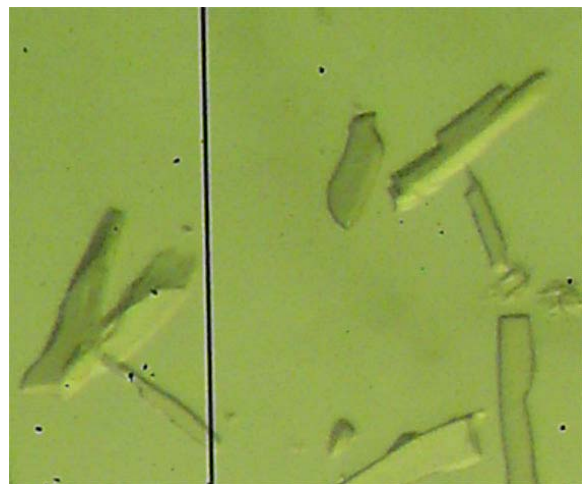
In microgravity, crystals of T5 muralytic enzyme were obtained that exceeded the Earth samples in terms of size. Thus, a straight crystal was obtained with a size of approximately 50x50x50 (Figure-6), while on Earth crystal size did not exceed 10  $\mu\text{m}$  (Figure 7). Unfortunately, the geometric increase in the crystal in the space experiment did not result in obtaining a spatial protein model, because the protein structure in the crystal did not enable a high quality set of diffraction data to be obtained.

A significant increase in crystal size in the space experiment was observed for the recombinant human alpha-fetoprotein. In crystallization on Earth, there was virtually no formation of straight crystals from the nucleation centers. In microgravity, large layered crystals formed with linear sizes of over 100  $\mu\text{m}$ , which turned out to be unstable upon returning to Earth.



Results of the Alpha-fetoprotein experiment during ISS-22. Contents of the Earth crystallization compartments. Typical light.

Results showed recombinant protein  $\gamma$ -interferon, a complex of recombinant human insulin with polysialic acid 23 kDa INCA23 (“Sialoinsulin”), recombinant human alpha-fetoprotein (“AFP”) and in a complex with bilirubin (“AFP-Bilirubin”), recombinant human growth hormone (“Somatotropin”), recombinant adhesin of the bacteriophage phiKZ (“Adhesin”), recombinant



Results of the Alpha-fetoprotein experiment during ISS-21. Contents of the on-orbit crystallization compartment. Typical light.

muralytic enzyme of the bacteriophage T5 (“T5mur”), recombinant human butyrylcholinesterase (“BChE”), and highly purified chicken egg lysozyme (“Lizotsim”) were successfully grown. But for the proteins  $\gamma$ -interferon, somatotropin, and adhesin, crystallization in microgravity did not result in the formation of crystals of a size suitable for establishing an X-ray diffraction picture. The imperfect crystals and amorphous formations obtained were kept, and if the opportunity arises, they will be analyzed in a synchrotron radiation source. For the proteins AFP-bilirubin and BChE, it was found that the crystallization conditions in space differed from the nucleation conditions in the control on Earth, likely due to the significant

difference in diffusion conditions. These proteins require subsequent selection and optimization of crystallization conditions, given the conditions of the Luch-2M equipment.

***Investigation is ongoing and more results are pending publication.***



## INITIAL STAGES OF BIOLOGICAL DETERIORATION AND BIOLOGICAL DAMAGE IN SPACE USING THE BIOPROBY KIT (BIODEGRADATSIYA), 20 INVESTIGATIONS



**Research Area:** Biology and Biotechnology: Microbiology  
**Expedition(s):** 5 – 40  
**Principal Investigator(s):** Tatiana A. Alyokhova, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

### RESEARCH OBJECTIVES

Various microorganisms – bacteria and microscopic fungi – unavoidably colonize all places created by humans, spacecraft and orbiting stations are no exception. Many of these microorganisms are capable of causing biological damage to different structural materials. Because of this, the goal of the Biodegradatsiya experiment is to monitor the composition of microscopic fungi and bacteria within the ISS Russian Segment (RS). Experiment tasks include studying the initial stages of the colonization of surfaces, isolating and determining the composition of microorganisms, identifying destroyer microorganisms, and developing effective ways to suppress their activity.

### EARTH BENEFITS

As a result of this work, a collection was created of the biological microorganisms on the ISS RS, which currently counts more than 300 strains. Representatives of the 10 most typical types of fungi capable of causing biological damage were deposited in the union-wide microorganism collection at the G. K. Skryabin Institute of Microorganism Biochemistry and Physiology, Russian Academy of Sciences for official storage for preparation of the patent process.



Process of collecting samples using the Bioproby kit on board the station.

### SPACE BENEFITS

Based on the research done and culture collection created, ways are being developed to prevent the occurrence of microorganisms, and suppress their growth and activity in microgravity to prevent their potential harmful effects on materials and crew.

## RESULTS

Twenty-six samples at various points on the Russian Segment were collected for analysis (20 with main samplers, and 6 additional). Microorganisms were discovered in 8 of them, at very low quantities. The largest quantities of microorganisms were found at the condensate water processor, pressure hull or onboard cable network, and pressure hull of the structural part of the working compartment. Overall, the number of colony-forming units (CFU) of microorganisms isolated from the ISS RS structural surfaces studied using the Bioproby kit was not great (1-12 CFU/sample). Mycelial fungi were represented in 4 genera by 6 types:



Colony of bacteria and microscopic fungi isolated from the sample collected at Point #4 (condensate water processor behind panel 434)

*Aspergillus niger*, *Aspergillus sydowii*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Penicillium crustosum*, and *Ulocladium botrytis*. Two types of yeast fungi were identified: *Rhodotorula sp.* and *Debaryomyces sp.* Bacteria were found in 2 samples, at low quantities. All the types identified had already been seen on the station. As compared to the previous analysis (ISS-35/36, cycle 23: September 2013), the species diversity and numbers of CFU identified of microscopic fungi decreased slightly. Spore growth on ISS RS structural materials and surfaces in this stage was not high.

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***Investigation is ongoing and more results are pending publication.***

**INVESTIGATING AND DEVELOPMENTAL TESTING A STANDALONE CLOSED REACTOR FOR PRODUCING BIOMASS OF BACTERIA AND BIOLOGICALLY ACTIVE SUBSTANCES WITHOUT INTRODUCING ADDITIONAL INGREDIENTS OR REMOVING METABOLIC PRODUCTS. DEVELOPING HIGH-OUTPUT PHARMACEUTICALS PRODUCTION PROCESSES OF FEW STEPS USING THIS REACTOR TYPE (BIOEMULSIYA)**



**Research Area:** Biology and Biotechnology: Microbiology  
**Expeditions:** 14, 15, 17-19, 21, 24, 27, 29, 30, 33, 36, 39 – Ongoing  
**Principal Investigator(s):** Alexey I. Kobatov, Ph.D., State Institute of Highly Pure Biopreparations of the Russian Federal Biomedical Agency, Moscow, Russia  
Valentin I. Evstigneev, Ph.D., Biopreparat, Moscow, Russia

**RESEARCH OBJECTIVES**

The Investigating and Developmental Testing a Standalone Closed Reactor for Producing Biomass of Bacteria and Biologically Active Substances without Introducing Additional Ingredients or Removing Metabolic Products. Developing High-output Pharmaceuticals Production Processes of Few Steps Using this Reactor Type (Bioemulsiya) investigation creates a standalone closed bioreactor to be used as a backbone for developing a few-stage process for emulsion-based culturing of bacteria. The bioreactor is designed for carrying out the culturing process under severe conditions; no air supply for aerating the culture medium or venting of metabolic gases and no metabolic products removal.

**EARTH BENEFITS**

The production process developed can be used (jointly with interested organizations) to create new emulsion forms of biopharmaceuticals and biologically active substances to benefit both the medicine and biotechnologies on Earth.

**SPACE BENEFITS**

As a result of the research, a production process with few stages will be developed to obtain biomass from aerobic bacteria and biologically active substances using emulsion culture media in a standalone bioreactor in microgravity.

**RESULTS**

The distinction of Bioemulsiya space experiments series performed in 2014 as compared to the earlier experiments is in the using dry powder as the culture material obtained from peroral Vitaflor pills produced on the industrial production line at the State Research Institute of High-Purity Biopharmaceuticals. The experiment used the pills of Series #01-0613 produced in June 2013 and stored at room temperature until the experiment (April 2014). Based on the results obtained, several conclusions can be drawn.

During a full culturing cycle on the ISS of the *Vitaflor*<sup>®</sup> symbiotic complex of acidophilus bacteria in Rekomb-K hybridizers, a fermented milk product was obtained with a high titration of *L.*

*acidophilus* (at least  $3.6 \times 10^8$  CFU/mL). Probiotic lactobacilli in the flight and ground-produced samples maintained a high probiotic potential for the entire observation period (52 days). Lactobacillus cells in the flight samples were in a physiologically active state and were of typical size (length) and morphology. The structures of lactobacillus populations in the flight samples were represented by two strains; the flight samples experienced an increase in the fraction of strain D #75, producing extracellular polysaccharides (mucosal strain). Lactobacilli in the flight samples manifested clear antagonism to the opportunistic pathogenic bacteria *S. aureus* and *K. pneumoniae*, and were resistant to the counter-antagonism of opportunistic pathogenic bacteria and antibiotics (tetracycline, ampicillin, gentamicin). Lactobacilli in the flight and ground fermented milk products had a long-term shelf stability at low temperatures (+6°C).

#### **PUBLICATIONS**

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***Investigation is ongoing and more results are pending publication.***

## DEVELOPING METHODS FOR OBTAINING POLYMER MATERIALS THAT ARE RESISTANT TO BIOLOGICAL CORROSION (BIOPOLIMER)



**Research Area:** Biology and Biotechnology: Microbiology  
**Expeditions:** 40 – Ongoing  
**Investigator(s):** Tatiana A. Alyokhova, Faculty of Biology, Lomonosov Moscow State University, Moscow Russia

### RESEARCH OBJECTIVES

The Developing methods for obtaining polymer materials that are resistant to biological corrosion (Biopolimer) investigation aims to develop approaches to halting microbiological corrosion in the enclosed pressurized volume (ISS) and treating surfaces to decontaminate them. Experiment activities include investigating the feasibility of creating technology enabling biological contamination to be halted in the early stages of the development of corrosion damage to structural materials.

### EARTH BENEFITS

Recent decades have been marked by the appearance on the market of a wide variety of disinfectants and fungicides. Here, a number of strict requirements are applied to



Exposure of the Biopolimer container on the ISS SM.

antimicrobials: high antimicrobial effectiveness, environmentally and hygienically safe, long shelf life, and simple to use the treatment solution. With certainty, such agents can include peroxosolvates, which are crystal products joining hydrogen peroxide to cations of inorganic or organic acids plus a few neutral molecules.

### SPACE BENEFITS

The goal of the research planned within the framework of the Biopolimer space experiment is to develop the means to suppress the

growth of microorganism colonies on structural material surfaces and halt them in the initial stages of biological damage. Technologies for using film-type fungicides in enclosed pressurized volumes of spacecraft are tested.

### RESULTS

Three modifications of the fungicide film material were developed: ПМ-1, ПМ-2, and ПМ-3. The polymer material contains a filming agent, activating substance, and plastifier. The filming agent is a mixture of polyvinyl alcohol and polyvinylpyrrolidone. The first modification is a control without the activating substance (ПМ-1), potassium fluoride peroxosolvate is added to



the second (ПМ-2), and hydrogen peroxide is added to the third (ПМ-3), also containing glycerin to give the films elasticity. The toxicological characteristics of potassium fluoride peroxosolvate were studied. It was demonstrated that peroxosolvate is categorized as a Class IV low hazard substance when in contact with the skin.

Experiments were conducted to determine the fungicidal activity of peroxosolvates relative to the fungal destroyer microorganisms identified from ISS RS structural surfaces: *Aspergillus niger*, *Cladosporium herbarum*, *Aspergillus flavus*, and *Penicillium chrysogenum*.

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***Investigation is ongoing and more results are pending publication.***

## **INFLUENCE OF FACTORS OF THE SPACE ENVIRONMENT ON THE CONDITION OF THE SYSTEM OF MICROORGANISMS-HOSTS RELATING TO THE PROBLEM OF ENVIRONMENTAL SAFETY OF FLIGHT TECHNIQUES AND PLANETARY QUARANTINE (BIORISK), THREE INVESTIGATIONS**



**Research Area:** Biology and Biotechnology: Microbiology  
**Expedition(s):** 5 – Ongoing  
**Principal Investigator(s):** Nataliya D. Novikova, PhD, Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

### **RESEARCH OBJECTIVES**

The Influence of Factors of the Space Environment on the Condition of the System of Microorganisms-Hosts Relating to the Problem of Environmental Safety of Flight Techniques and Planetary Quarantine (Biorisk) investigation aims to obtain new data on physical and genetic changes in bacteria and fungi typically found on spacecraft equipment, and also in various biological test objects (higher plant seeds, dormant forms of lower crustaceans) under exposure in the interior ISS compartments and on the exterior ISS surfaces.

### **EARTH BENEFITS**

The survivability limits determined for microorganisms causing biological destruction in extreme conditions and the sequence of their colonization of structural materials will facilitate the selection of the most environmentally safe materials that are resistant to bacterial contamination and can be used on the objects with artificial space habitat, as well as in gas/oil pipelines.



Russian cosmonaut Alexander Samokutyaev, Expedition 28 flight engineer, works with a Biorisk-MSN experiment container in the Zvezda Service Module of the International Space Station (ISS028E018265).

### **SPACE BENEFITS**

The Biorisk experiment results are intended to be used in the development of advanced technologies to reduce the risk of biological damage to space equipment and hardware. The experiment's value in terms of resolving problems of planetary quarantine is of a particular note, because its results have demonstrated the possibility of long-term (comparable to a round trip between Earth and Mars) viability of bacteria/microscopic fungi spore forms exposed to space environment, which consequently indicates the possibility of transferring Earth organisms on the exterior surfaces of interplanetary stations to other planets.

### **RESULTS**

For outfitting the Biorisk-MSV/Biorisk-MSN containers, bacterial and fungal spores were used and applied to the materials used in space technologies. In particular, industrial aramid cloth and AMГ-6 aluminum alloy were used. The seeds of higher plants were placed in cotton bags.

For the first time in the world, the possibility has been demonstrated of long-term (31-month) survival of various biological agents (spores of bacteria and microscopic fungi) when exposed to space, thus providing the basis for assuming that such agents may travel to other planets on spacecraft surfaces and for taking this capability into consideration when developing and validating planetary quarantine measures. It was also demonstrated that not only spores of bacteria or microscopic fungi are capable of long-term survival in space, but also biological objects at the highest levels of development in the evolutionary chain, i.e., higher plant seeds.

#### **PUBLICATION(S)**

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***Investigation is ongoing and more results are pending publication.***

## STUDYING THE IMPACT OF STREAMS OF HEAVY CHARGED PARTICLES FROM SPACE RADIATION ON THE GENETIC PROPERTIES OF CELLS IN PRODUCERS OF BIOLOGICALLY ACTIVE SUBSTANCES (BIOTREK), TWO INVESTIGATIONS



**Research Area:** Biology and Biotechnology: Microbiology  
**Expedition(s):** 14-20, 22, 24, 26, 28, 30, 32, 34  
**Principal Investigator(s):** Anatoliy D. Ukraintsev, Ph.D., Biokhimmash, Moscow, Russia  
Tatiana K. Krashennnikova, Ph.D., Biokhimmash, Moscow, Russia  
Yekaterina.V. Sinchurina, Ph.D., Biokhimmash, Moscow, Russia  
Irina Yu. Baltina, Biokhimmash, Moscow, Russia

### RESEARCH OBJECTIVES

Studying the Impact of Streams of Heavy Charged Particles from Space Radiation on the Genetic Properties of Cells in Producers of Biologically Active Substances (Biotrek) studies the long-duration effect of space radiation factors on cultures of recombinant strains of bacteria and fungi which produce biologically active substances in spaceflight, as well as their growth characteristics and plasmid segregation stability. Also, the investigation tests a technique for selecting highly productive strains from the cultures exposed in spaceflight, records and



Bioekologiya case for the Biotrek space experiment. Image provided by Biokhimmash.

analyzes heavy nuclear tracks and total radiation dose. Researchers are seeking to establish a correlation between alterations of genetic properties of biological subjects with a different level of organization (microorganisms, plant and animal cells) and exposure to heavy charged space particles. Experiment data collected make it possible to look into the action of molecular mechanisms leading to disordering in genetic cell apparatus caused by an increased level of ionizing

radiation acting in a spaceflight along with the microgravity and other extreme environment conditions, which will find in development of orbital space stations of a new generation, as well as during long-duration spaceflights.

### EARTH BENEFITS

Based on highly active strains of *Arthrobacter sp.* OC-1 and *Arthrobacter sp.* МИА-74 obtained after 2069 days of the experiment and a strain of *Arthrobacter sp.* МИП-89 obtained after 660 days of the experiment in prototype fermentation vessels with a capacity of 250 liters, a

preparation of Rodart, a compound containing highly active bacteria which consume petroleum pollution from soil and bodies of water, was produced.

### SPACE BENEFITS

In order to effectively study and develop strategic technologies in the interests of long-term human space explorations, an important task is creating a bio-laboratory model in which laboratory production can be carried out. Currently, through conducting such space experiments as Biotrek, Kaskad, Aseptic, and Glovebox-C, baseline technologies are being sought and experimentally tested to obtain promising biological products in microgravity, to perfect the counterpart production on Earth, and to obtain fundamental knowledge on the impact of spaceflight factors on biological objects.

### RESULTS

As a result of the Biotrek space experiment, four Bioekolgiya kits (4 cases per kit) were successfully delivered and all 16 were successfully returned. The space experiment was conducted from 93-2069 days, starting in ISS-14 and ending in ISS-34. Preserving the viability of the bacterial cultures on the International Space Station Russian Segment (ISS RS) to a great degree depended on the culture strain: if the museum strain in Earth conditions has a tendency for the cell concentration to decrease during storage, then the trend was maintained as a result of being on the ISS RS.

According to the macro-morphological assessment, the most stable properties were maintained in the museum strains and cultures exposed in vials. For cultures located throughout the entire experiment in solid and liquid nutrient media, a typical manifestation was a large number of different isolates in S (virulent strains) and R (non-virulent colonies) form. The number of dissociated forms within one type increased with experiment time, and also depended on the microorganism itself. Cultures in the space experiment which were in suspended form manifested polymorphism. The formation in a suspended culture of cells of varying lengths and varying stages of growth depended on the combination of the conditions in the culture. Culture productivity when cultured in liquid nutrient medium was at the level of standard laboratory indicators. The decrease in cell culture concentration in vials and in liquid nutrient medium after the end of the experiment did not have an impact on productivity; cultures fully recovered their properties upon multiple passaging to nutrient media in laboratory conditions. While studying the dissociated forms of cultures, it was shown that cultures stably retain the potential for active growth, and high cell concentrations were obtained when it is originally programmed in the genes of the cell by nature. All these parameters did not depend on the time a strain spent on the ISS RS and were comparable to ground controls.

For the first time in Russia, after the longest exposure time in the Biotrek experiment over 2069 days, results were obtained for bacterial culture strains of *Arthrobacter sp.* OC-1, *Arthrobacter sp.* МИА-74, *Arthrobacter sp.* МИП-89, *Bacillus Licheniformis* L-34, and of micromycetes *Cylindrocarpon radicolica* Wollenweber HTH-10, *Mycelium sterilia* ЛХ-1, *Mycelium radialis ginseng* HTH-1, and *Mycelium radialis var. ledum* (Phialocephala fortini) НЖ-13, demonstrating that these lyophilized bacterial and fungal cultures survived in solid nutrient medium and

retained the main performance indicators. Also for the first time, a molecular and genetic comparison was conducted of in-flight, Earth, and collection samples of cultures of bacteria *Arthrobacter sp.* OC-1 and *micromycetes Mycelium radialis var. ledum* (*Phialocephala fortini*) HЖ-13. The comparative analysis of the versions of bacterial culture strains identifies genetic changes in the flight versions of the strains by fingerprinting demonstrated genomic changes in the culture strain *Arthrobacter sp.* OC-1 in the form of single-point replacements of individual nucleotides and slight deletion or inversion occurring both in Earth conditions and in space. New bacterial and fungal strains were obtained with enhanced growth and biosynthesis properties from the Biotrek experiment. All the bacterial cultures in the Biotrek experiment were lyophilized and added to the culture collection bank at the space biotechnology laboratory at OAO Biokhimmash.

***This investigation is complete; however, additional results are pending publication.***



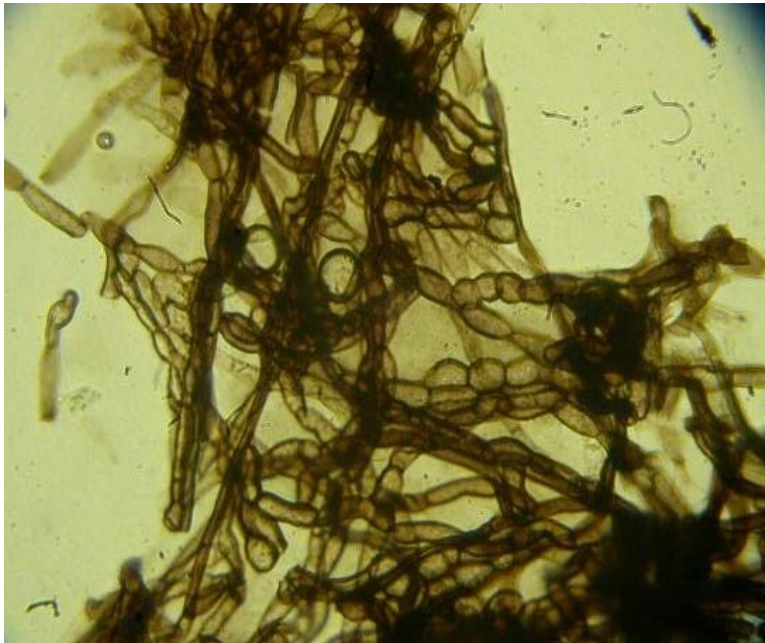
## STUDYING THE PROCESSES OF CULTURING VARIOUS TYPES OF CELLS (KASKAD)



**Research Area:** Biology and Biotechnology: Microbiology  
**Expedition(s):** 19-24, 27-29, 31-33, 35-38, 40 – Ongoing  
**Principal Investigator(s):** Anatoliy D. Ukraintsev, Ph.D., Biokhimmash, Moscow, Russia  
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Natalya G. Nikishenkova, Biokhimmash, Moscow, Russia

### RESEARCH OBJECTIVES

The Studying the processes of culturing various types of cells (Kaskad) investigation studies the processes of culturing cells of microorganisms, animals, and humans in microgravity to obtain concentrated biomass with a high concentration of cells producing a high output of target biologically active substances. The impact of flight factors on the properties of fungal culture cells and products of their biosynthesis was studied in order to achieve the maximum possible output of biomass and target biosynthesis products, i.e. biologically active substances. Work was performed on the fungal culture *Mycelium radicis*, a producer of the biostimulator substance of plant growth.



Fungal culture *Mycelium radicis* var. *ledum* strain HJK-13. Image provided by Biokhimmash.

### EARTH BENEFITS

No earth application has been identified for this experiment.

### SPACE BENEFITS

During Kaskad, the properties and characteristics are studied of a fungal strain grown through culturing in orbital flight in a liquid nutrient medium, and optimizing the process parameters of placing a planting material and culturing cells in an enclosed bioreactor. As a result of the work, proposals and comments are formulated on science equipment operation, time and process parameters of conducting the experiment, which in turn lead to the creation of

standard on-station biotechnology equipment to conduct stages of the biotechnological process of obtaining biologically active substances in orbital space flight.

## RESULTS

A culture liquid of the fungal culture *Mycelium radicis* was obtained from the Kaskad experiment during ISS-36 and ISS-38. Based on the laboratory investigations of the culture liquid, it was concluded that the specimen obtained during these missions did not differ visually from the control sample. The fungal culture obtained in flight during ISS-37 was in a suppressed state while returning to Earth and lost its viability. The cause of this could have been bacterial contamination discovered in the culture liquid.

During laboratory investigations of the biochemical indicators of the culture liquid obtained during expeditions 36 and 38, a decrease was noted in all values in the flight sample compared to the ground counterpart obtained by culture according to the space experiment timeline and a control sample grown in a flask. This effect may be related to the impact of various spaceflight factors on the rate of metabolism of the fungal culture, resulting in a more intensive consumption of substrate components of the nutrient medium. During orbital flight while growing the fungal culture in the bioreactor, culture liquids were obtained with a balanced complex of biologically active substances that, at a specific incubation of working solution, manifest maximum growth stimulating activity for both roots and stems.

The most balanced complex of biologically active substances in the culture liquid obtained during expedition 36 was identified at a working solution dilution of up to 10 ppm. In the space experiment, a culture was obtained for the first time whose growth stimulating activity for stems and roots exceeded by 10 fold compared the indicator in the ground culture, and whose growth stimulating activity was higher than the control culture. In the culture liquid obtained in expedition 38, the optimal ratio of growth stimulating activity for roots and stems was observed at a working solution concentration of up to 1 ppm. For a statistically justified finding on the levels of biotechnological indicators and growth stimulating activity based on substances obtained from flight culture liquids, additional sessions of the Kaskad experiment will be carried out in the future.

***Investigation is ongoing and more results are pending publication.***

## TRANSFER OF PLASMID DNA DURING CONJUGATION IN SPACEFLIGHT (PLAZMIDA)



**Research Area:** Biology and Biotechnology: Microbiology  
**Expedition(s):** 14 – 35  
**Principal Investigator(s):** Vyacheslav K. Ilyin, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

### RESEARCH OBJECTIVES

The transfer of Plasmid DNA During Conjugation in Spaceflight (Plazmida) investigation examines the microgravity effect on the rate of transfer and mobilization of bacteria plasmids. It is well known that the sensitivity of crewmember microflora to antibiotics undergoes significant changes during spaceflight. This manifests in the formation of strains with signs of increased resistance to many antibiotics. By spreading among crewmembers, these strains can decrease the effectiveness of the antibiotics used to provide medical treatment to crewmembers. It is hypothesized that the main mechanism of the formation of dangerous bacterial strains is related to the recombining of plasmids (molecules of DNA encoding different bacterial properties, including pathogenicity and antibiotic resistance, that exist, reproduce, and multiply independently of chromosomes), which are determined both by the frequency of autonomous plasmid transfer and their capability to be mobilized by different genetic factors. It



Roscosmos cosmonaut Alexander Misurkin holds a Recomb-K Apparature for the Konyugatsiya (Conjugation) experiment (ISS035E030072).

is hypothesized that the main mechanism of polyresistant strain formation is linked to plasmid recombination which are determined by both the frequency of conjugative plasmid transfer and mobilization in the changed environment. In addition, it is well known that changing living environment factors (gas composition, pressure, etc.) has a significant impact on R-plasmid transfer.

### EARTH BENEFITS

The investigation results could be used to comprehensively study the effect of extreme factors of a changed living environment on the formation of antibiotic resistance in infectious agents. The results obtained have great theoretical value for all fields of gravitational biology, including for fundamental research in the fields of physiology and medicine.

### SPACE BENEFITS

Microorganism plasmids could be used as biological indicators to determine the level of impact of spaceflight factors on microbial communities in terms of assessing the risk of growth of the potential pathogenicity of human and environmental microflora. Thus, in flights of varying

duration and radius, the plasmid transfer frequency may be different, as could be the corresponding risk of formation of drug-resistant bacteria. This aspect is potentially dangerous because poly-resistant strains of potentially pathogenic microorganisms formed in enclosed living spaces such as an orbiting space station are a type of depository for plasmids of multiple drug resistance, and consuming antibiotics could lead to their selective growth.

## RESULTS

Strains of *Bacillus thuringiensis* were used as the donor and the recipient: *Bacillus thuringiensis* GBJ085 (donor) x *Bacillus thuringiensis* 4Q7 (recipient). The donor contained a plasmid resistant to tetracycline and with the nalidixic acid resistance associated with the gene chromosome. The recipient was resistant to streptomycin. During the experiment on the ISS, Rekomb-K equipment was used, in which donor and recipient liquid cultures were transferred and mixed for conjugation.

The results of the studies conducted point to the suppression of plasmid transfer frequency in gram-negative microorganisms. This was particularly clear for the mobilization of bacterial genes, where the difference in the production of transconjugants was 1000 times less than in the control. The conditions for the preliminary adaptation of donor and recipient strains to space-flight facilitated the suppression of conjugation transfer frequency and did not affect the frequency of plasmid mobilization, which in both cases remained extremely low. However, gram-negative microorganisms strains adapted to spaceflight demonstrated an increase in plasmid transfer frequency during conjugation in flight and an increase in plasmid mobilization activity on Earth.

As regards plasmid stability, the flight plasmids were much more stable than the controls. The increase in the average number of determinants of antibiotic resistance in strains was observed in virtually all the groups. This was most clearly noted for the mobilization of strains adapted to space conditions when conjugation was done on Earth. This circumstance points to the fact that in spaceflight there is the risk of strain formation with signs of resistance, despite the decrease in plasmid transfer frequency in some microorganism groups in spaceflight. The research confirmed the possibility of using plasmid transfer frequency as a biological indicator for the status of microbial communities in changed living conditions. Based on the results of the research, a mathematical model was created of the impact of microgravity on conjugation processes, and as a result on the formation of strains with signs of resistance.

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***This investigation is complete; however, additional results are pending publication.***



## STUDYING THE FEATURES OF THE GROWTH AND DEVELOPMENT OF PLANTS, AND TECHNOLOGY FOR THEIR CULTURING IN SPACEFLIGHT ON THE ISS RS (RASTENIYA), 18 INVESTIGATIONS



**Research Area:** Biology and Biotechnology: Plant Biology  
**Expedition(s):** 5-14, 16, 17, 19-22, 27, 28 – Ongoing  
**Principal Investigator(s):** Vladimir N. Sychev, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

### RESEARCH OBJECTIVES

The goal of the Studying the Features of the Growth and Development of Plants, and Technology for their Culturing in Spaceflight on the ISS RS (Rasteniya) experiment is to do research in space in order to solve fundamental problems in biology and to optimize modes of culturing plants for future greenhouses to be part of future crew member life support systems. Main scientific tasks of the experiment include studying the impact of spaceflight factors on the growth and development of plants, and impacts on the phenology of plant development and the genetic consequences of the long-term cultivation of plants in microgravity. Understanding the effects of gravity on plant life is essential in preparation for future interplanetary exploration. The ability to produce high energy, low mass food sources during space flight will enable the maintenance of crew health during long duration missions while having a reduced impact on resources necessary for long distance travel.



*ISS crewmember Sergei Volkov observing the Rasteniya investigation during Expedition 29 (ISS029E041932).*

### EARTH BENEFITS

The scientific results of the experiment enable fundamental problems in biology to be solved, and the plant culturing technologies developed could be used in the future to create greenhouse facilities to be operated in remote regions of the Earth and in various structures with an enclosed living environment. The results of the experiment could be used to draft educational materials and encyclopedic publications. It should be highlighted that during

experiments in the Lada greenhouse on the ISS, a school science program to study plants in microgravity was developed and Mikrolada equipment for participants to use was created. Students from several high schools in Moscow, Saint Petersburg, Japan (Okayama prefecture), and the USA (Utah) grew pea and super dwarf wheat plants in parallel with ISS crews. The



results obtained have been presented on many occasions by participants at municipal and Russian national conferences, and have been discussed on specially created websites and in international teleconferences.

### **SPACE BENEFITS**

In the future, the results of the experiment could be used to develop plant cultivating technologies in integrated systems for crew life support on interplanetary missions, lunar bases, etc. This work has high applied value, because in the process of creating and operating the space greenhouse, cutting-edge equipment and software was developed, making it possible to grow plants automatically. The psycho-physiological aspect of human-plant interaction was also studied in an enclosed living environment and data were obtained on the safety of growing plant biomass for human consumption on a space station. These data are of great interest in design work for the creation of productive greenhouses as parts of future life support systems of any habitable complexes beyond the Earth's biosphere.

### **RESULTS**

The equipment used, the Lada space research greenhouse (Figure-1), makes it possible to study the growth, morphogenesis, and development of model cultures of higher plants for future life support systems, and to optimize the modes of plant cultivation in long-term spaceflight. In the experiment, the following plants were used: peas, mizuna, wheat, radishes, and barley. The series of experiments on the International Space Station (ISS) in Lada demonstrated that growth, development, and the duration of the cycle of plant ontogenetic development "from seed to seed" does not depend on spaceflight conditions. No impact of spaceflight factors was identified on such crucial higher plant functions as the development of reproductive organs, formation of spores and gametocytes, fertilization, and the formation of buds and reserve seed substances. The seeds of higher plants formed in the absence of gravity were biologically fully viable, and the plants obtained from these seeds did not differ from typical "Earth" plants. The morphological and biometric indicators and indicators of the photosynthetic activity of higher plants grown in the Lada greenhouse did not differ in any significant way from similar indicators in control plants grown on Earth. In spaceflight conditions, four consecutive generations of pea seeds were obtained, whose growth and development characteristics did not significantly differ from those in control plants on Earth. Therefore, for the first time, it was demonstrated that plants can be grown for an extended time comparable to the duration of a mission to Mars without losing reproductive functions, and can produce viable seeds in these conditions. The capability to indicate contamination of the air in an orbiting station biologically was demonstrated using plants.

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***Investigation is ongoing and more results are pending publication.***

## EXPERIMENTAL TESTING OF AN EARTH-SPACE SYSTEM FOR MONITORING AND PREDICTING THE DEVELOPMENT OF NATURAL AND MAN-MADE DISASTERS (URAGAN), 17 INVESTIGATIONS



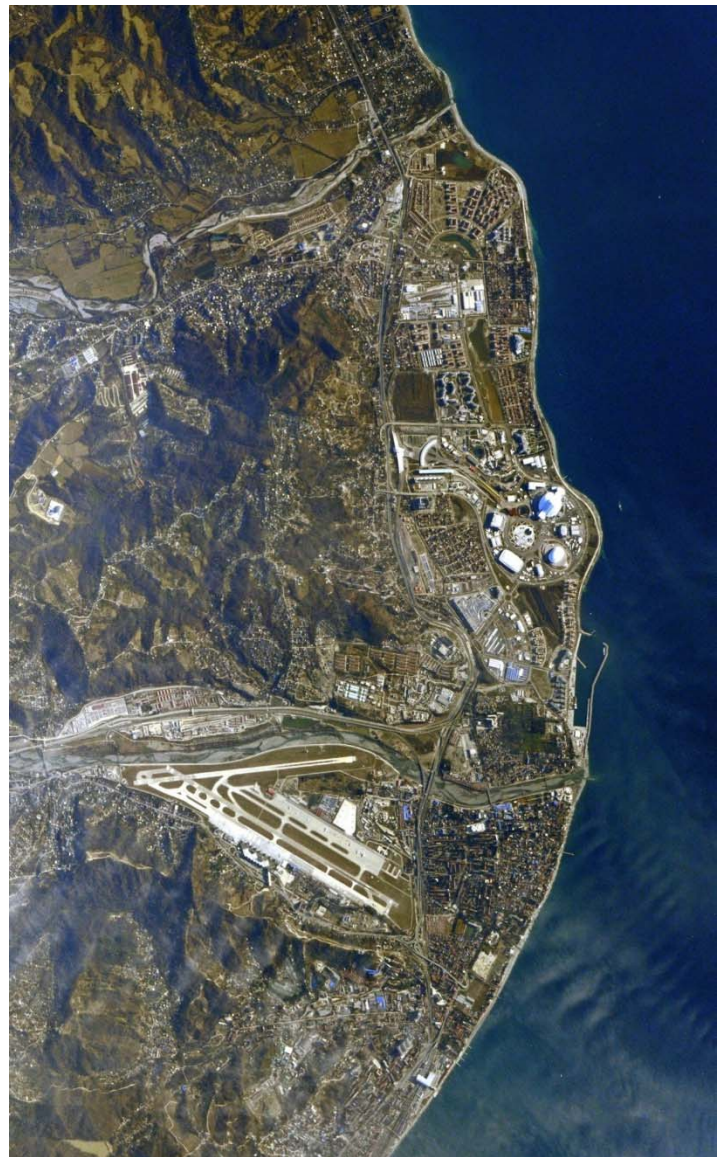
**Research Area:** Earth and Space Science: Earth Remote Sensing  
**Expedition(s):** 1 - Ongoing  
**Principal Investigator:** Mikhail Y. Belyaev, Ph.D., S.P. Korolev Rocket and Space Corporation Energia, Korolev, Russia

### RESEARCH OBJECTIVES

The Experimental Testing of an Earth-Space System for Monitoring and Predicting the Development of Natural and Man-Made Disasters (Uragan) investigation is designed to test technical equipment and methods of observing the Earth's surface from the Russian segment of the International Space Station given the actual constraints from the ballistic conditions of ISS flight, crew work and rest schedules, crew time resources, weather and lighting conditions in the imaging area, etc. The objects being observed are natural and man-made areas that are potentially dangerous for the occurrence of catastrophic events, including the mountainous areas of the Krasnodar region, with the goal of predicting the occurrence of flooding and landslides. The human impact on the environment in the area of Sochi during the construction and operation of Olympic facilities is monitored, as is the state of the environment in areas containing conservation reserves, national parks, and other nature facilities.

### EARTH BENEFITS

Space imagery and spectral information obtained during the experiment on the ISS RS will be widely used by different entities during scientific research and practical activities. At the present time, the feasibility has been established to apply them to studying events and objects, such as



Sochi, Olympic facilities from ISS on 05/21/14. Image provided by Roscosmos.



landslides, mountain rock falls, avalanches, movements of glaciers; patterns of the vegetation canopy; volcanic activity, consequences of earthquakes; catastrophic floods, patterns of snow melt, ice flows; forest fires; pollution near cities and oil spills in the water; changes in the environment; dust storms; indicators of climate change; geological structures.

The data obtained in the Uragan experiment present great value; they make a significant contribution to the worldwide data bank of observing changes in ecosystems in given regions of the Earth.

## **RESULTS**

This scientific work began to be compiled in 1977, with the first ever monitoring of the environment on the Salyut series of orbiting stations. Over 37 years, on many occasions, the achievement of goals was confirmed based on the example of conducting individual applied and scientific activities. As equipment has improved, the importance of using the ISS to monitor the Earth's surface and disasters has increased. In 2001-2006, the medium-resolution photo images from the ISS were the only ones in the overall Roscosmos Earth remote sensing program. Using the ISS to conduct this experiment is entirely justified.

During the experiment, technology was developed and used to take digital photographs and spectrometry from a manned station, and included obtaining information on catastrophic events in real time.

Approximately 400,000 photo images (~6.5 TB) were compiled of natural and man-made objects, including a large amount of new scientific knowledge on several dozen current problems concerning the study of nature, ecology, and monitoring disasters.

The results of the experiment were broadly disseminated in the mass media (newspapers, television, etc.). A report to the president of the Russian Federation analyzing the data obtained in the Uragan experiment on the disaster near the city of Krymsk received high marks.

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***Investigation is ongoing and more results are pending publication.***

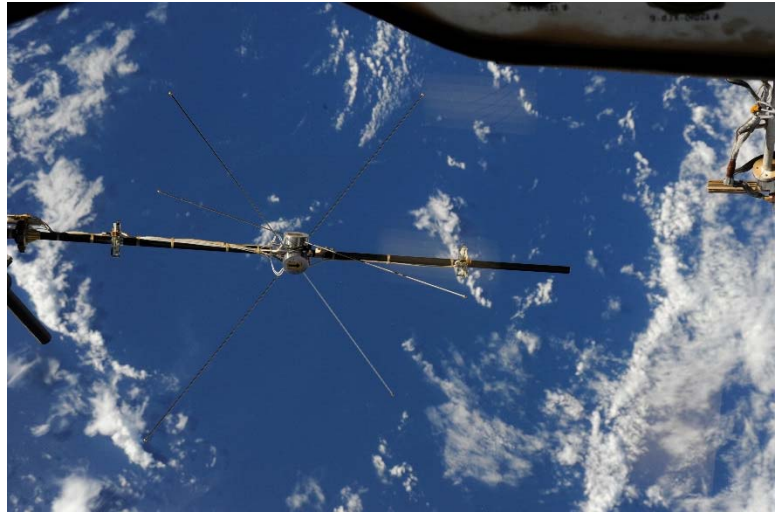
## INVESTIGATING PLASMA WAVE PROCESSES OF VERY LARGE SPACECRAFT INTERACTION WITH THE IONOSPHERE IN THE NEAR-SURFACE REGION OF THE ISS (OBSTANOVKA)



**Research Area:** Earth and Space Science: Near-Earth Space Environment  
**Expedition(s):** 35 – Ongoing  
**Investigator:** Stanislav Ivanovich Klimov, Ph.D., Space Research Institute of the Russian Academy of Sciences, Moscow, Russia

### RESEARCH OBJECTIVES

The Investigating Plasma Wave Processes of Very Large Spacecraft Interaction with the Ionosphere in the Near-surface Region of the ISS (Obstanovka) investigation is aimed at measuring the electrical potential of the ISS RS relative to the surrounding plasma and at assessing the possible negative impact of changes in the potential on the ISS operation and components. This goal can be achieved by running combined wave diagnostics to support a wide frequency-range study of the electrical, electrostatic, and magnetic field strength (including permanent fields) and plasma particle fluctuation spectrum.



View of antenna on the Obstanovka (Environment) investigation on the Service Module during ISS Expedition 37 (ISS036E048752).

### EARTH BENEFITS

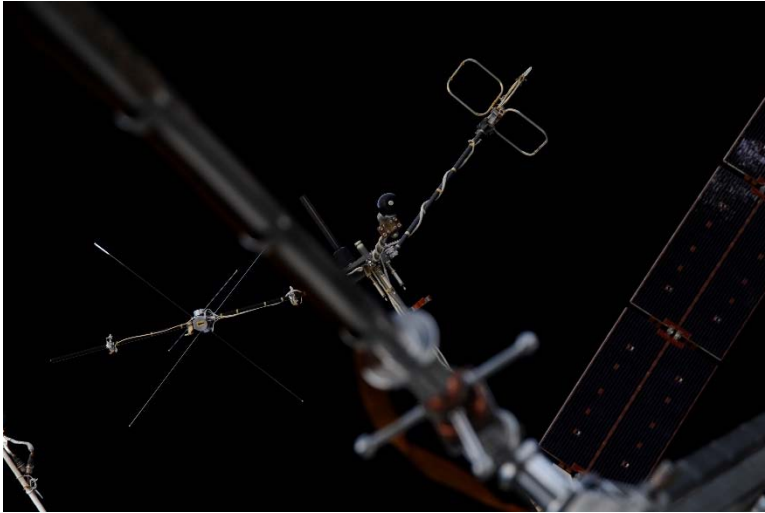
The long-term ISS-based monitoring of parameters of the ionosphere and some magnetosphere regions may provide invaluable help to users of real-time data on the ionosphere state (radio communications, GLONASS, GPS).

### SPACE BENEFITS

Plasma processes are an integral component of space weather. This approach is based on a cutting-edge physical concept where plasma, including plasma in space, is considered as a dynamic medium containing charged particles, including protons from solar flares, and a broad spectrum of plasma wave movements and discontinuities. Continuous observations are necessary in order to study the orbital, daily, and seasonal variations of the interaction processes between the ISS and the surrounding environment.

## RESULTS

The Obstanovka space experiment is being conducted in order to create a database of single-component measurements of electromagnetic fields near the ISS when exposed to spaceflight factors, including those of man-made origin. The results will be used in the fields of applied geophysics and ecology to forecast space weather and adjust the operating requirements for space technology products.



View of antenna on the Obstanovka (Environment) investigation on the Service Module during ISS Expedition 37 (ISS037E005126).

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***Investigation is ongoing and more results are pending publication.***

## MECHANISMS OF SENSORY-MOTOR COORDINATION IN WEIGHTLESSNESS (MOTOKARD)



**Research Area:** Human Research: Bone and Muscle Physiology  
**Expedition(s):** 37 - Ongoing  
**Principal Investigator(s):** Inessa B. Kozlovskaya, M.D., Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

### RESEARCH OBJECTIVES

The Mechanisms of Sensory-Motor Coordination in Weightlessness (Motokard) investigation is carried out on the treadmill and involves locomotion in various modes of running and walking during various modes of operation of the treadmill. During the test, electromyography of the thigh and calf muscles, support structure response, heart rate, and treadmill load parameters



*ISS crewmember Oleg Kononenko, wearing a harness and electrodes, is photographed during Motocard experiment operations in the Zvezda Service Module (ISS045E075856).*

(actual speed, time elapsed, distance, integrated indicators for support structure response) are recorded. Locomotor disruptions in humans are a predictable result of spaceflight. Results of research conducted after even relatively short missions (72 hours to 16 days) indicate that crewmembers gait differs in obvious instability: crewmembers walk is rickety, with their legs wide apart, swaying from side to side, and sometimes hold their arms out to maintain balance.

### EARTH BENEFITS

Data obtained in the experiment could serve as the basis for developing recommendations on selecting modes of locomotor training for patients located in an immobilized state for a long period of time.

### SPACE BENEFITS

The results of the experiment will be used to improve the Russian system of countermeasures, including developing effective methods and equipment to support human physical performance in long-term space, including interplanetary, flights.

### RESULTS

To date, preliminary results from the Motokard experiment have been received on two ISS crewmembers.

*Investigation is ongoing and more results are pending publication*



## INVESTIGATION OF THE FEATURES OF THE STRUCTURAL/FUNCTIONAL STATE OF VARIOUS SECTIONS OF THE GASTRO-INTESTINAL TRACT TO IDENTIFY THE SPECIFIC CHANGES IN THE DIGESTIVE SYSTEM OCCURRING IN SPACEFLIGHT (SPLANKH)



**Research Area:** Human Research: Integrated Physiology and Nutrition  
**Expedition(s):** 37 - Ongoing  
**Principal Investigator(s):** Boris V. Afonin, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia



ISS crewmember O. V. Kotov conducting blood biochemical studies with the Reflotron analyzer (ISS038E058100).

### RESEARCH OBJECTIVES

Investigation of the Features of the Structural/Functional State of Various Sections of the Gastro-Intestinal Tract to Identify the Specific Changes in the Digestive System Occurring in Spaceflight (Splankh) obtains data in spaceflight on the structural/functional state of different sections of the gastrointestinal tract, organs and vessels of the abdominal cavity, retroperitoneal space, and their underlying mechanisms that determine the features of changes in the digestive system in weightlessness. Stage 1 involves performance of an initial series of studies to identify the specific features of electrical activity of various sections of the

gastro-intestinal tract in weightlessness and determine blood biochemical indicators. Stage 2 involves the electro-gastro-enterography and biochemical studies to broaden to include ultrasounds of organs and vessels of the abdominal cavity and retroperitoneal space. Stage 3 involves doppler studies of vessels in the retroperitoneal space and of regional blood flow in this area are added, which will enable the features of changes in the condition of the digestive system in weightlessness and their underlying mechanisms to be identified.

### EARTH BENEFITS

The electro-gastro-enterography method and its device are promising for applications in gastroenterological clinical practice in order to conduct non-invasive medical examinations of the gastrointestinal tract.



ISS crewmember S. N. Ryazansky recording electrical activity of the gastrointestinal tract using the Splanxh gastroenterography device (Image provided by Roscosmos).

#### **SPACE BENEFITS**

The results of the experiment will be used to improve methods for diagnosing and predicting changes in the digestive system in spaceflight, and to develop ways to prevent and correct these changes. In addition, based on the experiment results, the scientific understanding of the features of changes in the condition of the digestive system in weightlessness and their underlying mechanisms will be broadened.

#### **RESULTS**

For the first time, data were obtained in long-term spaceflight on the features of the electrical activity of the main sections of the gastrointestinal tract. Given a lack of changes in biochemical indicators of the functional activity of the digestive system, a clear decrease in the electrical activity of all sections of the gastrointestinal tract was identified, apparently reflecting the specific features of its functioning in weightlessness.

***Investigation is ongoing and more results are pending publication***

**STUDYING THE VARIATIONS OF THE RADIATION ENVIRONMENT ALONG THE FLIGHT PATH AND IN COMPARTMENTS OF THE INTERNATIONAL SPACE STATION AND TIME HISTORY OF DOSE ACCUMULATION IN A SPHERICAL AND TORSO PHANTOMS LOCATED INSIDE AND OUTSIDE THE STATION (MATRYOSHKA-R), 11 INVESTIGATIONS**



**Research Area:** Human Research: Radiation Impacts on Humans  
**Expedition(s):** 8 – Ongoing  
**Principal Investigator(s):** Vladislav M. Petrov, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia  
 Vyacheslav A. Shurshakov, Ph.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

**RESEARCH OBJECTIVES**

Studying the Variations of the Radiation Environment Along the Flight Path and in Compartments of the International Space Station and Time History of Dose Accumulation in a Spherical and Torso Phantoms Located Inside and Outside the Station (Matryoshka-R) studies the field of charged particles and neutrons in a wide energy band inside the Russian segment (RS) of the ISS and on its external surface, including studying the dose accumulation in representative points of a spherical and Torso phantoms in order to determine the main characteristics of the radiation exposure of crews on manned spacecraft and to perfect methods of space dosimetry.

**EARTH BENEFITS**

The field of application of the spherical phantom used to measure absorbed doses in critical crewmember organs and the radiation environment on board spacecraft may be broadened and extended to radiation hazardous objects on Earth, where the likelihood of significant dose variations occurring in the human body is rather high, such as at nuclear waste storage facilities, nuclear submarines, etc. In addition, the chemical composition of the tissue-equivalent material (polyurethane) and the process for producing it are such that they make it possible if necessary to change within required proportion the ratios of primary elements (hydrogen, nitrogen, oxygen, carbon) responsible for the material nuclear/physical and technical specifications.



Spherical phantom in the ISS SM crew quarter (Image provided by RSC Energia)

## SPACE BENEFITS

In the future, it is planned to use the results of the space experiment for a comprehensive assessment of the integral doses received by humans exposed to ionizing radiation during long periods of time in spaceflight on the ISS to justify a system-level approach to using a spherical tissue-equivalent phantom as an phantom-witness of the radiation environment for different space missions: to the moon, Mars, and inter-planetary outer space.



*Torso phantom in the interior of the ISS RS on the left wall. (Image provided by RSC Energia).*

## RESULTS

Matryoshka-R yielded a substantial lack of uniformity in the depth-dose and surface-dose distributions for the spherical phantom (and thus in a crewmember's body) was discovered. Studies were conducted of the dose distribution in the Torso phantom both inside the station and in conditions simulating extravehicular activity. The effectiveness of the radiation protection properties of materials containing hydrogen to reduce the doses of charged particles and neutrons was demonstrated while using additional protection in crew quarters. The radiation exposure rate in ISS compartments was assessed for the period of active Sun near the maximum of the solar activity in the final stage of its growth. During 2009-2010 this investigation was carried out jointly with ESA. Currently it is being carried out jointly with JAXA.

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*Protection screen on the wall of the ISS SM crew quarter, shown with the arrow (photo presented by RSC Energia)*



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***Investigation is ongoing and more results are pending publication.***



## VIRTUAL MANUALS (VIRU)



**Research Area:** Technology Development and Demonstration:  
Communications and Navigation

**Expedition(s):** 33-44 – Ongoing

**Principal Investigator(s):** Evgeniy I. Zhuk, Ph.D., S.P. Korolev Rocket and Space Corporation  
Energiya, Moscow, Russia

### RESEARCH OBJECTIVES

Virtual Manuals (Virus) intends to increase the efficiency of training and the conductance of space experiments by cosmonauts through the use of virtual manuals aboard the ISS RS. It also intends to develop a methodology concept for creating virtual manuals and using them aboard the ISS RS.

### EARTH BENEFITS

Virtual guides can be used in various fields of science and technology as a tool that provides a visual image of the required operations performed with sound accompaniment.

### SPACE BENEFITS

Virtual guides can be used for implementation of the other space investigations, recovery operations on the ISS RS, installation and dismantlement of on-board equipment, both inside and outside ISS.

### RESULTS

The scientists found a savings of at least 37 minutes of mission time to perform a Relaksatsiya session and at least 8 minutes of mission time to perform an Uragan session. They also found that the cosmonauts could begin activities with the science hardware immediately, without preliminary preparation. However, the crew assessed the Virus as “poor.” This suggested that the Virus program must be upgraded to allow the selection of two alternatives—a verbose alternative, and a more streamlined version.

*Investigation is ongoing and more results are pending publication.*

## EXPERIMENTAL TESTING OF A SYSTEM OF PHOTO IMAGERY COORDINATE REFERENCING USING ULTRASOUND SENSORS (VIZIR)



**Research Area:** Technology Development and Demonstration: Imaging Technology  
**Expedition(s):** 31 – Ongoing  
**Principal Investigator(s):** Sergey V. Bronnikov, Ph.D., S.P. Korolev Rocket and Space Corporation Energia, Moscow, Russia

### RESEARCH OBJECTIVES

Experimental Testing of a System of Photo Imagery Coordinate Referencing Using Ultrasound Sensors (Vizir) tests the technology of automated coordinate referencing of images of the Earth's surface, and space, taken by crewmembers using "free-floating" photography



ISS crewmember Oleg Skripochka takes photos of the Earth using an ultrasound target trajectory system during Vizir experiment operations (ISS048e014038).

equipment in weightlessness. The use of an unsecured camera enables the crewmember to easily aim it at objects, track them, and quickly re-focus on other objects. The camera is equipped with small ultrasound emitters, and a small area around the window is equipped with ultrasound receivers.

### RESULTS

In September 2014, using the technology tested in this experiment, over 6,000 images of the Earth's surface taken on board the ISS were automatically

coordinate referenced. The labor intensiveness of manually processing images was significantly decreased. In addition, coordinate referencing was applied to images for which it was previously impossible.

*Investigation is ongoing and more results are pending publication.*



## STUDYING THE CHARACTERISTICS OF THE ISS AS AN ENVIRONMENT FOR RESEARCH (SREDA ISS)



**Research Area:** Technology Development and Demonstration:  
Microgravity Environment Measurement

**Expedition(s):** 12 – Ongoing

**Principal Investigator(s):** Mikhail Y. Belyaev, Ph.D., S. P. Korolev Rocket and Space Corporation Energia, Korolev, Russia

### RESEARCH OBJECTIVES

Studying the characteristics of the ISS as an environment for research (Sreda ISS) investigates microaccelerations on the ISS RS during dynamic operations. The necessary data are collected and analyzed, including calculations to determine the magnetic field of the ISS itself using a mathematical model of the Earth's magnetic field. The modes of operation of ISS system and science equipment are determined that ensure that requirements on conducting scientific research on the ISS RS are met.

### RESULTS

Fifty five experiment sessions were conducted. Measurements were taken automatically, without crew intervention. The motion control system equipment used operated nominally. In all the experiment sessions, the necessary telemetry was obtained. Studies were also conducted of the microgravity situation on the ISS during dynamic operations. The data obtained made it possible to conclude that the information, currently being received in the Sreda ISS experiment from nominal microacceleration sensors and the magnetometer, will not be sufficient in the stage of ISS operation as a scientific laboratory. Specialized science equipment needs to be created.

### PUBLICATIONS

Zavalishin DA, Belyaev MY, Sazonov VV. Determining the typical frequencies of elastic oscillations of the ISS structure. *M. V. Keldysh Institute of Applied Mathematics of the Russian Academy of Sciences*. 2007.

Babkin YV, Belyaev MY, Krikalev SK, Markov AV, Ryabukha SB, Stazhkov VM. Technical experiments on the ISS to study background micro-disturbances. *Materials of the XLII scientific readings dedicated to developing the scientific legacy and ideas of K.E. Tsiolkovsky*. Kaluga: Eidos Publishing House. 2007.

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Belyaev MY, Monakhov MI, Sazonov VV. Calculation of quasi-static microaccelerations occurring on the International Space Station. *M. V. Keldysh Institute of Applied Mathematics*. 2012;60:32 pp.

Belyaev MY, Monakhov MI, Sazonov VV. Assessment of accuracy of the readings of the magnetometer mounted on the Service module of the International Space Station. *M. V. Keldysh Institute of Applied Mathematics*. 2012;54:32 pp.

***This investigation is ongoing; however, additional results are pending publication.***

## SPATIAL ORIENTATION AND INTERACTION OF EISODIC SYSTEMS UNDER CONDITIONS OF WEIGHTLESSNESS (VIRTUAL)



**Research Area:** Technology Development and Demonstration: Nervous and Vestibular Systems

**Expedition(s):** 37 – 43

**Principal Investigator(s):** Ludmila N. Kornilova, M.D., Institute of Medical and Biological Problems of the Russian Academy of Sciences, Moscow, Russia

### RESEARCH OBJECTIVES

The Spatial Orientation and Interaction of Eisdic Systems Under Conditions of Weightlessness (Virtual) investigation allows for objective and accurate data to be obtained on how weightlessness impacts vestibular function, on the disposition of gaze and visual tracking during the course of a long-duration space mission. The results obtained over the course of the Virtual are unique for the physiology of the vestibular function and intersensory interactions and for understanding the delicate mechanisms of visual tracking.



ISS crewmember O. V. Kotov during a Virtual experiment session (ISS037E004379).

### EARTH BENEFITS

The developed procedures and hardware–software packages have already found application in high-performance sports (gymnastics, figure skating, speed skating, archery, etc.), in diagnosis and therapy of patients suffering from vertigo and balance disorders, and in the evaluation of pharmaceutical drug effectiveness. The experiment results will improve the methodology for predicting the reliability of the professional activity of persons in an operator role, and allow the expert diagnosis capabilities of physicians to be expanded for therapy and prevention of various kinds of vestibulopathies.

### SPACE BENEFITS

Using the experiment results will allow the state of cosmonaut vestibular and visual systems to be monitored during flight and the methodology for predicting the state of the cosmonaut visual–vestibular system to be improved, which will promote the reliability of their professional activity and accelerate the process of their post-flight adaptation.

## RESULTS

Research data were received on the state of cosmonaut vestibular systems in weightlessness starting from the second or third day of arrival aboard the ISS. A preliminary analysis of cosmonaut eye and head motion during vestibular tests showed there to be a verifiable reduction in torsional ocular counter-rolling and the presence of atypical reactions (lack of or inversion of the otolith reflex).

## PUBLICATIONS

Kornilova LN, Naumov IA, Glukhikh DO, *et al.* The role of vestibular and support-tactile-proprioceptive inputs in visual-manual tracking. *40<sup>th</sup> COSPAR Scientific Assembly*. Moscow, August 2–10, 2014.

***This investigation is complete; however, additional results are pending publication.***

## A STUDY OF EARTH RADIATION CHARACTERISTICS AND TESTING OF THEIR USE IN A MODEL OF THE ISS RS POWER SUPPLY SYSTEM (ALBEDO)



**Research Area:** Technology Development and Demonstration: Radiation Measurements and Shielding  
**Expedition(s):** 32 – Ongoing  
**Principal Investigator(s):** Dmitriy N. Rulev, Ph.D., S.P. Korolev Rocket and Space Corporation Energia, Moscow, Russia

### RESEARCH OBJECTIVES

A Study of Earth Radiation Characteristics and Testing of Their Use in a Model of the ISS RS Power Supply System (Albedo) studies the impact of radiation reflected from the Earth's atmosphere and underlying terrain on the operation of the ISS RS power supply system.

### SPACE BENEFITS

The results of the experiment are already used in the mission control of the ISS RS. The methods of tracking the outgoing radiation from the Earth are used in forecasting the generation of electricity by the ISS RS solar batteries at the stages of long-term and operational mission planning of the ISS RS.



At a window in the International Space Station's Zvezda Service Module, ISS crewmember Oleg Kotov uses a digital camera photospectral system to perform a session for the Albedo Experiment (ISS038E005022).

### RESULTS

Albedo has produced results for Earth-reflected radiation, and these were compared to power generation on the ISS. The experiment also measured spectra of specific areas of Earth. The science team also analyzed the feasibility of using data from the Meteosat spacecraft to perform additional processing of the Albedo results.

***This investigation is ongoing; however, additional results are pending publication.***

## **MONITORING THE STATE OF THE ISS RS INHERENT EXTERNAL ATMOSPHERE AND EXTERIOR WORKING SURFACES, AND DIAGNOSIS OF THE PERFORMANCE OF MATERIALS AND COATINGS USED ABOARD THE SPACE STATION (KONTROL)**



**Research Area:** Technology Development and Demonstration: Spacecraft and Orbital Environments  
**Expedition(s):** 36-40 – Ongoing  
**Principal Investigator(s):** Andrey N. Krylov, Ph.D., S.P. Korolev Rocket and Space Corporation Energia, Korolev, Russia

### **RESEARCH OBJECTIVES**

Monitoring the state of the ISS RS inherent external atmosphere and exterior working surfaces, and diagnosis of the performance of materials and coatings used aboard the space station (Kontrol) tests methods to monitor ISS RS environmental orbital conditions. It tests methods to monitor ISS RS environmental orbital conditions. It also refines the physical and mathematical model of the ISS RS inherent external atmosphere based on the results obtained from inherent external atmosphere parameter measurements.

### **SPACE BENEFITS**

The experiment obtained results on the dynamics of the inherent external atmosphere pressure change in different conditions of the ISS operation will make it possible to give recommendations on the conditions for carrying out technological and material research experiments, as well as to specify the operating conditions of the orbital complex with regard to its inherent external atmosphere.

### **RESULTS**

Over the first year of the experiment, 69 measurement sessions were carried out. The sensor temperature conditions were maintained within the limits of the allowable range. Pressure in background measurement modes was also found to be within the expected range.

***This investigation is ongoing; however, additional results are pending publication.***



## STUDYING THE HYDRODYNAMICS AND HEAT TRANSFER OF MONODISPERSE DROPLET STREAMS IN MICROGRAVITY (KAPLYA-2)



**Research Area:** Technology Development and Demonstration: Thermal Management Systems  
**Expedition(s):** 38  
**Principal Investigator(s):** Arnold M. Gubertov, Ph.D., M.V. Keldysh Research Center, Moscow, Russia

### RESEARCH OBJECTIVES

Studying the Hydrodynamics and Heat Transfer of Monodisperse Droplet Streams in Microgravity (Kaplya 2 (First Stage)) focused on confirming the operation of droplet generators in microgravity and vacuum. It also was to determine the main parameters of monodisperse droplet streams. It was to confirm the continuous operation of a closed hydraulic circuit.

### SPACE BENEFITS

The scientific and technical results obtained during the experiment will allow to substantiate the reality of the working process organization in the droplet refrigerator radiators and formulate recommendations on the design and development of droplet radiators' equipment for power units for space purposes. Advantages of such radiators are the minimal thermal resistance between the coolant and the radiating surface, invulnerability to meteor breakdown and low mass.



ISS crewmember Oleg Kotov setting up the Particle Cooler/Generator Module (MKXI) for the ПКЭ-5, Kaplya-2 experiment, in the Mini Research Module 1 (MRM1) (ISS038E029764).

### RESULTS

The operations of droplet generators, the droplet collector, and closed circuit were confirmed. However, the droplet collector operation did not make it possible to ensure complete heat transfer medium collection, because of the reflection of some droplets from the unmoistened surface of the spinning disk. Also, due to the reflection of some droplets from the collector, the closed circuit was not complete.

***This investigation is complete; however, additional results are pending publication.***



### **Canadian Space Agency**

<http://www.asc-csa.gc.ca/eng/iss/default.asp>



### **Japan Aerospace Exploration Agency**

<http://iss.jaxa.jp/en/>



### **National Aeronautics and Space Administration**

<http://www.nasa.gov/iss-science/>



### **European Space Agency**

<http://www.esa.int/esaHS/iss.html>



РОСКОСМОС

### **Roscosmos – Russian State Space Corporation**

<http://knts.tsniimash.ru/ru/site/CenterInfRes.aspx>

<http://www.energia.ru/english/index.html>