

→ SPACE FOR LIFE

human spaceflight science newsletter

Issue 6 | November 2014



↑ ESA astronaut Alexander Gerst preparing for the Energy experiment in September 2014

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→ EXPEDITION RESEARCH ACTIVITIES:

Overview of Science Achievements During Expeditions 40-41

ESA research was on-going during ISS Expeditions 40-41, the vast majority of which encompassed the Blue Dot Mission with ESA astronaut Alexander Gerst. Expedition 40 started on the night of 13-14 May 2014 with the undocking of Soyuz 37S, and Expedition 41 concluded on 10 November with the undocking of Soyuz 39S, bringing Gerst and his fellow crew members Maxim Suraev (Roscosmos), and Reid Wiseman (NASA) back to Earth. They had been on the ISS since 29 May as part of the six-member ISS crew.

Human Research Activities

The Energy experiment, was carried out with ESA astronaut and ISS Flight Engineer Alexander Gerst as the 7th test subject in September 2014. The experiment, which consists of an 11-day on-orbit period of data acquisition, aims at determining the energy requirements of astronauts during long-term spaceflight.

Three test subjects concluded the Space Headaches experiment during Expeditions 40-41. All three astronauts (Alexander Gerst and NASA astronauts Steve Swanson and Reid Wiseman) completed weekly questionnaires respectively before returning to Earth. The experiment is now around the half-way point with 12 test subjects having returned from

orbit (a minimum of 20 are required). Headaches can be a common astronaut complaint during space flights. This can negatively affect mental and physical capacities of astronauts/cosmonauts which can influence performance during a space mission.



↑ ESA astronaut Alexander Gerst wearing a thermal sensor on his forehead during 36-hour session of the Circadian Rhythms experiment whilst also undertaking additional research activities for a NASA combustion experiment in the Microgravity Science Glovebox

The Circadian Rhythms experiment (covered in detail in a previous newsletter) is providing a better basic understanding of any alterations in circadian rhythms in humans during long-duration spaceflight. Alexander Gerst and Reid Wiseman carried out all required (~monthly) sessions on orbit, through continuous monitoring of their temperature/activity profiles. Gerst and Wiseman are the 6th and 7th subjects of the experiment, which means it is well on its way to completion (with a minimum of eight subjects required).



↑ European Space Agency astronaut Luca Parmitano undertaking Skin-B experiment on the ISS in August 2013

The Skin-B experiment, which started in Expedition 36 with Luca Parmitano, was concluded by Steve Swanson and Alexander Gerst. This means that on-orbit activities have been concluded for the minimum required amount of test subjects (three) though a further two are in the pipeline. Skin-B will help to develop a mathematical model of aging skin and improve understanding of skin-aging mechanisms,

which are accelerated in weightlessness. The experiment which is carried out in cooperation with DLR will also provide a model for the adaptive processes for other tissues in the body. Each approximately monthly session included three different non-invasive measurements taken on the inside part of the forearm: skin moisture measurements, trans-epidermal water loss measurements to determine barrier function of the skin and surface evaluation of the living skin.

Outside of the ESA experiments Alexander Gerst was a subject of many human research experiments from ISS Partner agencies which have used facilities within ESA's Columbus laboratory. This included: NASA's Ocular Health, Microbiome, Salivary Markers, Cardio Ox, and Pro K/Biochemical Profiles protocols as well as the Canadian Space Agency's Blood Pressure Regulation (BP Reg) experiment. Additional ISS Partner human research activities undertaken by Gerst included being a subject of NASA's Body Measures and Force Shoes experiments and JAXA's Biological Rhythms 48 experiment.



↑ Alexander Gerst performing examination on the ISS as part of NASA's Ocular Health experiment

A new unit of the Portable Pulmonary Function System was transported to the ISS on ESA's fifth and final Automated Transfer Vehicle (ATV-5) and was deployed in the Destiny lab on 2 September. The old unit had undertaken more than four successful years of activities within different human research projects. The Portable Pulmonary Function System is an autonomous multi-user facility supporting a broad range of human physiological research experiments under weightless conditions in the areas of respiratory, cardiovascular and metabolic physiology.



↑ NASA astronaut Steve Swanson setting up the new Portable Pulmonary Function System (PPFS) hardware on the ISS on 2 September 2014

Biology Research

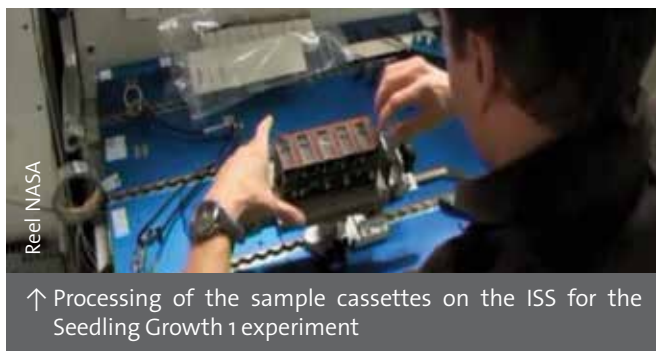
Following successful processing of the Gravi-2 experiment in the European Modular Cultivation System (EMCS) in Columbus during Expedition 39, the processed samples were returned from orbit at the start of Expedition 40. These are being analysed to determine the movement of amyloplasts in the root cap cells (at the tip of the root) as it is thought that their sedimentation under the influence of gravity sends a gravitropic signal leading to differential growth of the root tissues. The GRAVI-2 experiment (covered in detail in the last newsletter) continues the research undertaken within the GRAVI-1 experiment in determining the threshold of perception of gravity by lentil roots.



↑ Lentil seeds inside Gravi-2 culture chamber

The next experiment to take place in the EMCS is the joint ESA/NASA Seedling Growth 2 experiment. Alexander Gerst inserted experiment containers in the EMCS on 17 October 2014.

The overall Seedling Growth experiment comprises in total a series of three experiments until 2015 which builds on previous space flight experiments with *Arabidopsis thaliana* seeds and studies the effects of various gravity levels on the growth responses of plant seedlings. These experiments are done in collaboration with NASA.



↑ Processing of the sample cassettes on the ISS for the Seedling Growth 1 experiment

Astrobiology Research

The Expose-R2 facility was successfully installed outside the Russian Service Module of the ISS during a Russian-based spacewalk on 18 August 2014. A detailed article on Expose-R2 appears in this newsletter. Expose-R2 hosts a suite of four new astrobiology experiments, three from ESA and one from IBMP in Moscow some of which could help understand how life originated on Earth. The Expose-R2 payload follows up the success of the Expose-R payload (2009 –2011), the results of which are currently being published in the International Journal of Astrobiology (Cambridge University Press).

Radiation Research

The Dose Distribution inside the ISS 3D (DOSIS-3D) experiment has been on-going in Columbus since May 2012. The experiment has continued data acquisition using the two active detectors and different sets of passive detectors installed in different locations around Columbus. One set of passive detectors (installed from March –September 2014) were collected in by Alexander Gerst and returned to Earth on Soyuz 38S on the night of 10/11 September. Gerst installed a following set of passive detectors on 29 September. The passive detectors are used in order to undertake 'area dosimetry' i.e. to measure the spatial radiation gradients inside the Columbus module.



↑ Passive detector package (orange) located close to the portable breathing apparatus in Columbus following installation on 28 March 2014

The active detectors undertake time-dependent cosmic radiation measurements for the experiment. These were undertaking almost continuous data measurement during Expeditions 40-41.

The aim of the DOSIS-3D experiment is to determine the nature and distribution of the radiation field inside the ISS and follows on from the DOSIS experiment previously undertaken in the Columbus laboratory. Comparison of the dose rates for the DOSIS-3D and the DOSIS experiments has shown a difference in dose level which can be explained due to the different altitude of the Station during the measurements. The DOSIS-3D experiment will build on the data gathered from the DOSIS experiment by combining data gathered in Columbus with ISS International Partner data gathered in other modules of the ISS.

Solar Research

During Expeditions 40-41 six Sun visibility windows (77th – 82nd) for SOLAR, which is located on the external platform of Columbus, were undertaken for the facility to acquire scientific data when the ISS is in the correct orbital profile with relation to the Sun. This included an extended period of science acquisition whereby windows 78/79 were joined together by slightly rotating the ISS in the intervening period (~10/11 days) to continue science acquisition. This was the fourth such campaign and have been the only times that the attitude of the Space Station has been changed for science reasons.

As the Sun visibility windows last for around 12 days these bridging events make it possible to undertake solar measurements during a full Sun rotation cycle (which lasts around 26 days at the Solar equator and up to 36 days at the solar poles). The extended period with windows 78/79 undertook successful scientific measurements for the whole scheduled period from 17 June – 22 July.

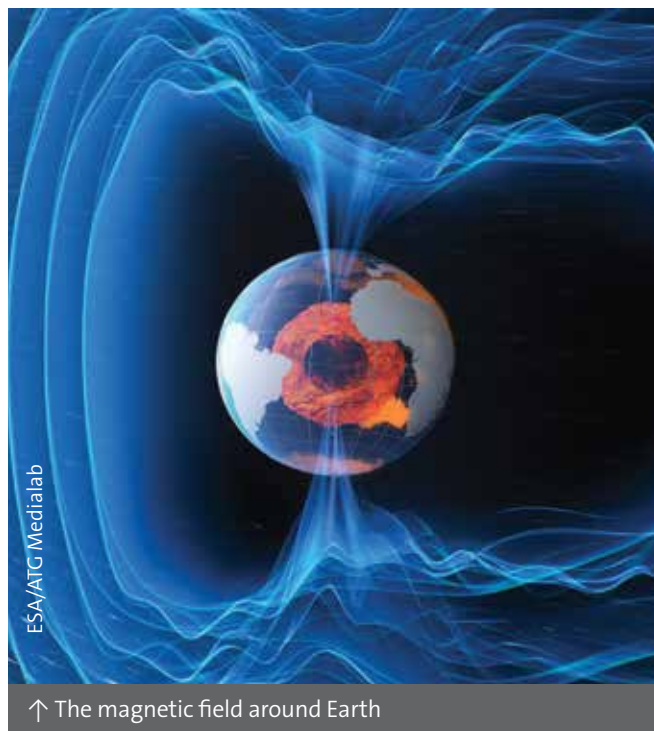
All other Sun Visibility Windows in the Expedition 40-41 timeframe successfully undertook data acquisition.

The SOLAR payload facility is studying the Sun's irradiation with unprecedented accuracy across most of its spectral range. This has so far produced excellent scientific data during a series of Sun observation cycles. An extension to the payload's time in orbit could see its research activities extend up to early 2017 to monitor the whole solar cycle with unprecedented accuracy.

Environmental Monitoring

The MagVector experiment was installed inside the European Drawer Rack in Columbus in August/September 2014. This was undertaken at different points by Alexander Gerst and ISS Expedition 40 Commander Steve Swanson with the first science run in September. An initial problem was experienced though ground analysis of the data generated tracked the problem to script files that were ultimately uplinked and updated in the MagVector payload on 16 October. This allowed for the repetition and successful completion of the first science run the following day with scientific assessment providing the approval to continue with the next science runs.

The MagVector experiment from DLR qualitatively investigates the interaction between a moving magnetic field (of Earth origin) and an electrical conductor. The experiment will help researchers gain insight into how the magnetic field influences how conductors

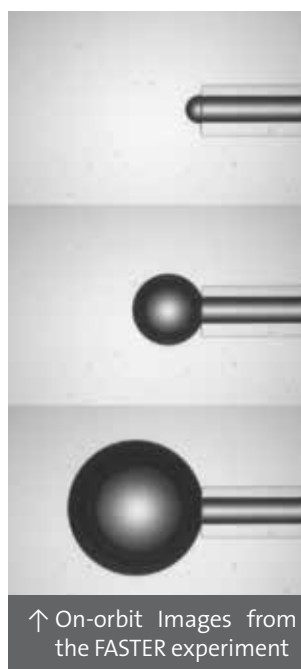


↑ The magnetic field around Earth

work. The expected changes in the magnetic field structure on different sides of the electrical conductor are of interest for technical applications as well as for astrophysical research.

Fluid Science

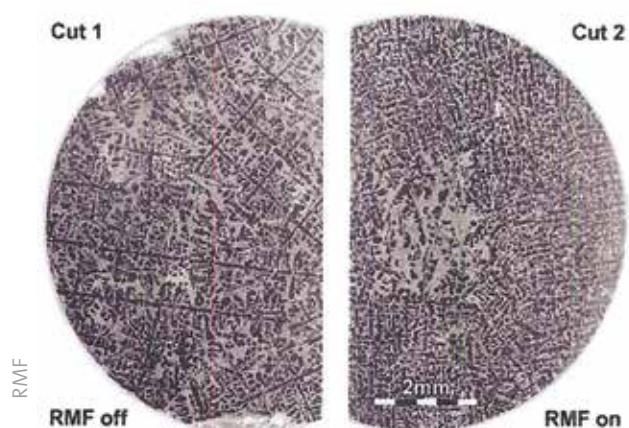
Fluid Science saw the completion of the FASES and FASTER series of experiments in Expeditions 40/41. The Facility for Adsorption and Surface Tension (FASTER) payload was installed in the European Drawer Rack in Columbus in April 2014. The experiment core is a Capillary Pressure Tensiometer developed for the study of the links between emulsion stability and characteristics of droplet interfaces, liquid films and the collective properties of an emulsion. Data will be used to generate a model of emulsion dynamics to be transferred to industrial applications.



↑ On-orbit Images from the FASTER experiment

After a number of successful runs with increasing surfactant levels FASTER did experience issues with the first experiment container in terms of droplet handling. Activities with the second experiment container with even more critical liquid combinations were successfully undertaken. Experiment container 2 was studying a hexane droplet inside a pure water bulk fluid, with increasing surfactant at different temperatures. All activities were concluded in August 2014. The FASTER science team acquired a wealth of excellent video and telemetry data which is currently being evaluated in detail off line.

The Fundamental and Applied Studies of Emulsion Stability (FASES) experiment has now been concluded following a reduced number of successful experiment runs. Some good quality images/emulsions have been assessed by the science team. Image analysis of samples processed will allow to extract the emulsion structure with deduction of droplet size and droplets cluster with respect to time. The FASES experiment investigates the effect of surface tension on the stability of emulsions. Results hold significance for oil extraction processes, and the chemical and food industries. The experiment container was returned to Earth on SpaceX-4 for engineering analysis related to thermal control issues affecting the experiment cells.



↑ Metallographic sections of a processed MICAST sample. Left: under diffusive conditions. Right: under forced convection induced by rotating magnetic field

Materials Research

Within materials research the Batch 2a experiments (including samples for the CETSOL, MICAST and SETA projects) are now on the verge of completion with only one sample cartridge waiting to be processed. Two samples successfully completed processing in ESA's Materials Science Laboratory in the US Laboratory during Expeditions 40/41 including the successful reprocessing of a sample which experienced processing problems in 2013. The Batch 2a experiments are studying different aspects of solidification in metal alloys which will help to optimise industrial casting processes. The research in the Materials Science Laboratory is covered in detail in a separate article.

More good news for expanding the possibilities within materials research is the Electro Magnetic Levitator (EML) which arrived at the ISS on ATV-5 in August. Following resolution of a protruding bolt, the installation of the EML hardware was completed between 22 – 28 October in advance of starting its checkout and commissioning run. This included installation of the EML high speed camera, sample chamber, gas filter, and harness, as well as associated configuration tasks. Successful testing followed.

The Electro Magnetic Levitator will perform container-less materials processing involving melting and solidification of electrically conductive, spherical samples, under ultra-high vacuum and/or high gas purity conditions. The EML will measure, under weightlessness, thermophysical properties

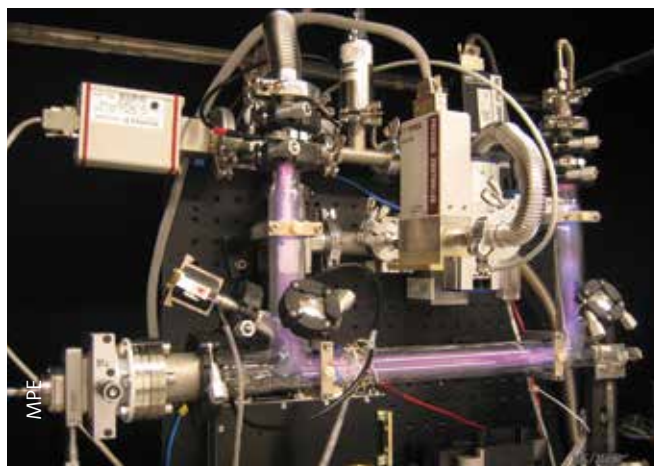


↑ Top: Alexander Gerst undertaking minor troubleshooting too remove a protruding bolt during EML installation procedures in September 2014. Bottom: EML during sample chamber and gas filter installation in October 2014

of a variety of metal alloys in liquid state with high precision, supporting basic and industrial research.

Complex Plasma Research

The European Physiology Modules (EPM) facility was reconfigured in May in preparation for the Plasma Kristall-4 (PK-4) experiment installation. Following various final checkouts on ground the PK-4 hardware was launched to the ISS on Progress 57P on 29 October. It is scheduled to be installed inside the EPM rack by the end of November, under a cooperation agreement between ESA and Roscosmos.



↑ Plasma inside PK-4 hardware: the 'positive column' of a DC glow discharge

PK-4 is an experiment for investigating complex plasmas. Plasmas are ionized gases produced by high temperatures, such as in the sun, or by electric fields, i.e., low temperature discharge plasmas like in neon tubes. In the latter case the degree of ionization is small and a large amount of neutral gas is present. Complex or dusty plasmas are plasmas which contain micro-particles, e.g., dust grains, besides electrons, ions, and neutral gas. Due to the strong influence of gravity on the micro-particles, most experiments on complex plasmas are strongly distorted or even impossible on earth and therefore require weightless conditions. The main interest lies in the investigation of the liquid phase and flow phenomena of complex plasmas for which PK-4 is especially suited.

Technology Research

Successful data acquisition is still on-going for the Vessel Identification System (commonly known as the Automatic Identification System, AIS), using its Norwegian receiver, and telemetry is still being successfully received by the Norwegian User Support and Operation Centre (N-USOC) in Trondheim via ESA's Columbus Control Centre in Germany. By the end of Expedition 41 the hardware has been operating for more than four years in orbit.

The Vessel Identification System is testing the means to track global maritime traffic from space by picking up signals from standard AIS transponders carried by all international ships over 300 tonnes, cargo vessels over 500 tonnes and all types of passenger carriers.

As part of preparations for future human exploration missions, the second part (OPSCOM#2) of the Multi-Purpose End-To-End Robotic Operations Network (METERON) experiment was undertaken on the ISS in August. ESA's METERON project is aiming to demonstrate communications and operations concepts and technologies for future human exploration missions with human robotic elements accounting for issues such as disruption in network connectivity, communications delays caused by distance, efficient bandwidth usage, human-in-the-loop operations, multi-rover operations, multi-operator interaction, supervisory control and haptic teleoperation to name a few. (A detailed article on METERON appeared in the last newsletter).



↑ The Eurobot Ground Prototype and Aouda.X spacesuit mockup being tested in Mars-like terrain of Spain's Rio Tinto mines in April 2011

Following an initial period of ground commanding to the METERON laptop from the Belgian User Support and Operations Centre (B.USOC) and the ESA centre in Darmstadt (ESOC) testing all the connectivity prerequisites the OpsCom#2 event itself was performed on 7 August 2014 with Alexander Gerst tele-operating the Eurobot rover at ESA's ESTEC facility in the Netherlands via the Delay Tolerant Network. The rover could be controlled from the ISS based on loaded maps, movements of the rover could be followed, its arms could be deployed and rover images could be received. All operations were executed flawlessly and even some bonus tasks could be achieved.

Another important technology demonstration undertaken during Expedition 40/41 involved ESA's fifth and final Automated Transfer Vehicle (ATV-5). On 8 August, a few days prior to docking, ATV-5 successfully performed a fly-under of the ISS to gather data for the Laser Infra-Red Imaging Sensors (LIRIS) experiment.



The LIRIS demonstrator is testing the feasibility of two different rendezvous sensor technologies (LIDAR and IR camera) as the ATV approaches the ISS. Testing such technologies in the actual ISS flight environment will help to advance navigation technologies such that future rendezvous sensors are valid for both cooperative and non-cooperative targets such as orbiting debris or un-manned capsules.

Alexander Gerst also undertook the first sessions of the SpaceTex experiment from 29 September – 10 October. SpaceTex is testing new fabrics on astronauts on the ISS. This research will increase our basic knowledge about the heat transfer/heat exchange from the human body to the environment under terrestrial and micro-g conditions and improve the overall comfort and well-being of the astronauts on board of the ISS. In addition new fabrics might reduce the potential microbiological/fungal contamination on board the ISS due to faster cloth drying avoiding thereby the possible development of skin problems.

Education Activities

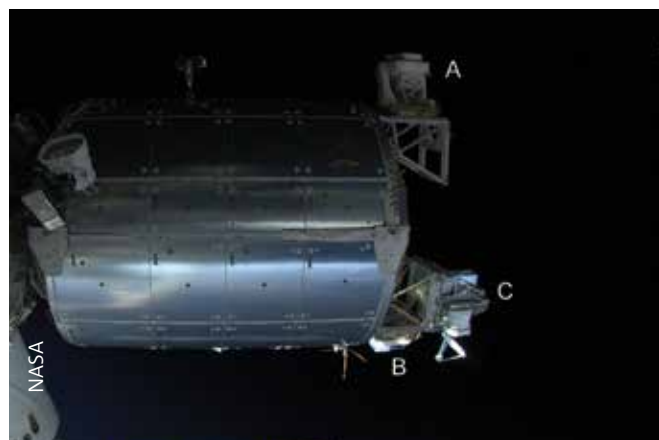
Within education Alexander Gerst performed a number of activities as part of the EPO (Educational Payload Operations) Gerst umbrella series of activities for ESA's Blue Dot mission in June/July. This included: recording a video from the European-built Cupola Observation Module as part of the Earth Guardian education activity which educates pupils (7-12 years old) about the causes of, for example, loss of biodiversity and climate

change and promotes possible solutions; the Top experiment student competition for which Gerst performed the winning experiment on the ISS to stimulate the curiosity of students and to motivate them towards STEM (Science, Technology, Engineering and Mathematics) subjects; and the “EPO Rosetta Philae Docking”, “EPO Foaming of water”, “EPO - Flying in Microgravity”, “EPO Gyrotwister”, and “EPO Marangoni” demonstrations which aim to introduce the concept of weightlessness to European children and students;

Additional ISS Partner Research

In addition to the Human Research activities mentioned, Alexander Gerst has also been involved in numerous research activities for different ISS Partners in the areas of biology (Resist Tubule, Zebrafish Muscle, VIABLE, Cell Mechanosensing-2, Micro-8, Rodent Research 1, Drug Metabolism, Plant Gravity Sensing), combustion research (FLEX-2, BASS-2), fluids research (Capillary Flow, Soret Facet), materials/crystallisation research (Ice Crystal 2, Hicari, Alloy Semiconductor, NanoRacks Module 19, Commercial Protein Crystal Growth High Density Modified), radiation research (Radi-N2), Earth Observation (Earth Rim), Exploration-related research (Comm Delay Assessment), Technology (IVA Clothing) and Education (SPHERES, NanoRacks Module-9),

Gerst was also central in the deployment of a multi-purpose experiment platform through the Japanese laboratory airlock in July/August for deploying mini-satellites (NanoRacks Cubesats) outside the ISS and cover areas such as humanitarian and environmental applications.



↑ Columbus External Payload Facility showing ESA's Solar Facility (A) and NASA's HDEV (B) and RapidScat (C) payloads

The Columbus External Payload Facility (on which ESA's Solar facility and NASA's High Definition Earth Viewing are located) also saw the installation of NASA's RapidScat external payload on 29/30 September by robotic arm after delivery to the ISS on SpaceX-4 Dragon. Following the installation, the payload was activated to start a 30-day calibration and checkout period. RapidScat is a space-based scatterometer that measures wind speed and direction over the ocean, and is useful for weather forecasting, hurricane monitoring, and observations of large-scale climate phenomena.

Other Activities

In support of the ISS research activities a fleet of ISS logistics spacecraft have helped to supply the ISS with research equipment, samples and other necessary supplies during Expedition 40/41.

ESA's 5th and final ATV (ATV-5) called 'Georges Lemaître' was launched to the ISS at the end of July from Europe's Spaceport in Kourou, French Guiana. ATV-5 transported more than 6 tonnes of cargo (including dry cargo for the ISS, oxygen and air, water, and propellants). ATV-5 docked with the ISS on 12 August. The ATV spacecraft will remain at the ISS until January 2015 after which the vehicle filled with excess equipment and garbage will undock from the ISS.

The third commercial SpaceX Dragon spacecraft flight (SpaceX-3) was unberthed from the ISS in on 18 May (after one month at the ISS), with the SpaceX-4 Dragon mission berthed at the ISS from 23 September – 25 October. Both spacecraft which transported over 2.2 tonnes of cargo to the ISS splashed down in the Pacific Ocean on landing. Dragon is one of NASA's commercial resupply spacecraft for the ISS. The second such spacecraft is Orbital Sciences' Cygnus spacecraft which had two scheduled missions to the ISS during Expeditions 40/41.

The second commercial ISS Flight of Cygnus, launched on 13 July, berthing to the ISS 3 days later with Alexander Gerst assisting robotic arm procedures, and unberthing on 15 August with Alexander Gerst as primary robotic arm operator. The third commercial flight of Cygnus unfortunately experienced a launch failure on 28 October. The failure is under investigation and ISS ground teams are assessing the impacts due to the loss of cargo which included equipment for ESA's GRIP human physiology experiment and samples for the SODI-DCMIX-3 experiment.

Following undocking of Progress 53P and 55P in June and July 2014 respectively two additional Progress flights have been launched to the ISS, Progress 56P (2.6 tonnes of cargo) in July 2014, and Progress 57P (2.6 tonnes of cargo) in October 2014. Progress 56P undocked two days prior to the arrival of Progress 57P.

Of course just as ISS supplies need renewing and refreshing, the crew also need to be rotated in order to continue the work on the ISS. Expedition 40 started with the undocking of Soyuz 37S on the night of 13-14 May 2014 leaving ISS Commander Steve Swanson (NASA) and ISS Flight Engineers Alexander Skvortsov and Oleg Artemyev (both Roscosmos) on the ISS. They were joined on 29 May by ISS Flight Engineers Alexander Gerst (ESA), Maxim Suraev (Roscosmos) and Reid Wiseman (NASA) after docking of Soyuz 39S. The situation remained in this configuration until 11 September when Swanson, Skvortsov and Artemyev undocked in Soyuz 38S signifying the start of Expedition 41 with Suraev as the ISS commander. Three more Expedition 41 Flight Engineers arrived at the ISS two weeks after in Soyuz 40S: NASA astronaut Barry Wilmore and Roscosmos cosmonauts Alexander Samokutyaev and Elena Serova. Expedition 41 concluded with the undocking of Soyuz 39S with Gerst, Suraev and Wiseman on 10 November 2014 signifying the start of Expedition 42 with Wilmore taking over as ISS Commander.

During Expeditions 40-41 five spacewalks have taken place: three Russian (June, August and October) the second of which installed the Expose-R2 facility, and two US spacewalks (in October), the first of which involved Alexander Gerst as one of the EVA astronauts, the second of which helped return one of the eight ISS power channels to full functionality.

→ STRESS AND IMMUNOLOGY RESEARCH IN ITS EXTREMES:

Uncovering the Keys of Immune System Response in Space

The Immuno experiment was successfully completed this year in collaboration with Russia and is providing important data related to human immune function in space. This data and subsequent results are helping to develop the follow-up research study, Immuno-2 which will start following launch of the first two test subjects for the experiment (Roscosmos cosmonauts Mikhail Kornienko and Gennady Padalka) in March 2015. Kornienko is undertaking a landmark mission as he will be one of the first two crew members scheduled to stay for a full year on the International Space Station (along with NASA astronaut Scott Kelly). The Immuno-2 data will therefore provide important information to help ESA with planning and preparations for long-duration missions and future human exploration missions.



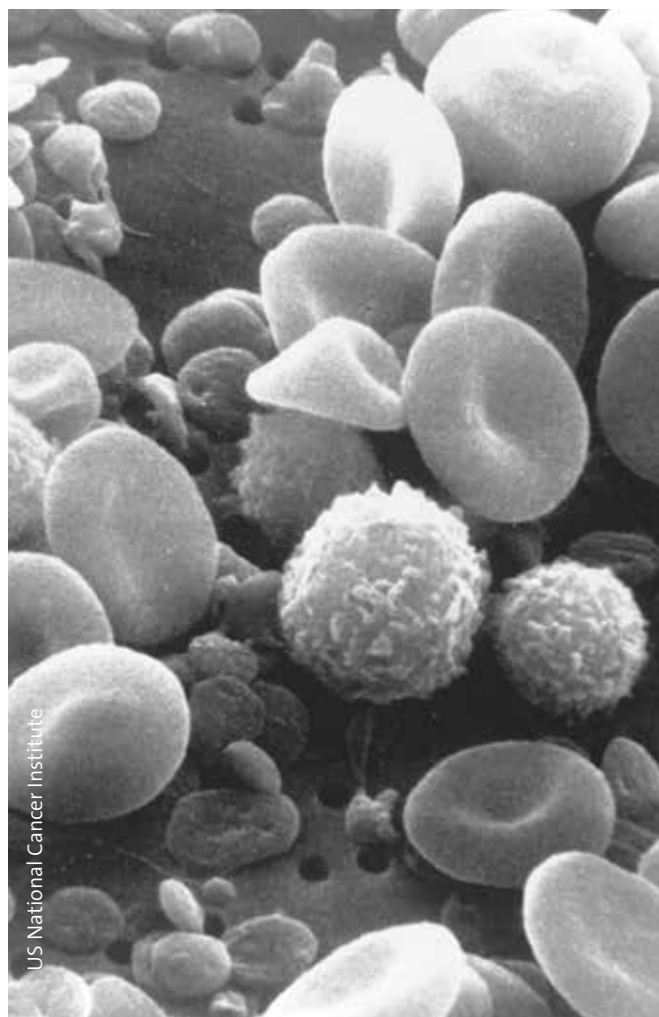
NASA

↑ Roscosmos cosmonauts Gennady Padalka (centre) and Mikhail Kornienko (right) will be the first test subjects for the Immuno-2 experiment. Kornienko and NASA astronaut Scott Kelly, (left) will also become the first two 12-month ISS crew members during Expeditions 43-46

The immune system is extremely complex and intricately interconnected with other systems of the human body. Stresses to the body can suppress immune system responsiveness, having a variety of effects such as impairing wound healing, making us more susceptible to viruses or even failing to stop the growth of cancer cells.

More than 40% of astronauts from the Apollo missions experienced sickness and more than 50% of astronauts and cosmonauts show significant signs of immune dysfunction after long-duration missions. This makes it important to determine how, and what, different elements of the immune system are affected by the various stresses of spaceflight and if this could preclude future human exploration missions.

Stresses to the body, (i.e. anything that disrupts its physiological balance) can be psychological or physical and in space the stresses are quite significant with psychological effects such as confinement and sleep disruption and physical stress factors such as weightlessness, variable oxygenation status and radiation. With human exploration missions these effects will be compounded with longer mission durations and more extreme environmental conditions outside of low-Earth orbit.



US National Cancer Institute

↑ Scanning electron microscope image from normal circulating human blood showing red blood cells, several white blood cells and many small disc-shaped platelets

When the body experiences stress, stress hormones like norepinephrine and cortisol are released which influence immune response on different levels (cortisol is an immune suppressant for example). These stress hormones are central to the activation of the Hypothalamic–pituitary–adrenal (HPA) axis that controls reactions to stress and regulates many body processes, including digestion, the immune system, mood and emotions. White blood cells (including macrophages, B lymphocytes, T lymphocytes) also release cytokines which are small proteins important in cell signalling to activate immune response. Along with stress hormones and cytokines, other substances which provide good indicators of chronic stress and immune response are endocannabinoids, two principal examples of which are anandamide and 2-Arachidonoylglycerol (2-AG).

Endocannabinoids are substances produced within the body that play an important role in the regulation of physiological functions, from stress and memory regulation to immunity. In scientific terms research into endocannabinoids is still reasonably new with anandamide and 2-AG only being isolated in the 1990s. Research on ground has shown that anandamide has an immunosuppressive effect impairing the body's ability to respond to, for example, infection or inflammation by suppressing the release of cytokines from T-lymphocytes.

The Immuno experiments are carried out under a bilateral collaboration agreement between Roscosmos and ESA. Dr. Alexander Chouker, from the Hospital of the Ludwig-Maximilians University in Munich, is the scientific lead of for the experiments which involve an international consortium of European scientists including a science team from the Russian Institute for Biomedical Problems (IBMP) in Moscow led by Dr. Boris Morukov.



↑ Russian Expedition 35 flight engineer and Roscosmos cosmonaut Roman Romanenko on the ISS in March 2013. Romanenko was the final test subject for the Immuno experiment

The first Immuno experiment series started in 2005 and finally concluded all on-orbit experiment procedures in May 2013 with final samples returned in September 2013 with Soyuz 34S. The experiment was undertaken on 12 different test subjects (11 cosmonauts and ESA astronaut Thomas Reiter) that were taking part in long-duration (six-month) ISS missions. This included a two year gap in research in order to sign a continuation agreement to increase the number of test subjects and hence the significance of the data collected.

The experiment made an analysis of all test subjects from blood/saliva assays of markers of chronic stress/immune response, combining these with stress questionnaires (KAB stress symptom questionnaire) filled out by the test subjects. Samples/measurements were taken at six different points: once before the mission, twice during the mission (at about 3-4 months and 6 months after launch) and three times after landing (just after landing, a week after landing and a month after landing).

The preliminary results from the Immuno experiment were presented by Dr. Chouker during an ISS human research workshop at ESA's ESTEC facility in Noordwijk in April 2014. The scientific results are very promising, also with some surprise findings which could point towards new research topics in this area. The full results will be published in the future once all samples are analysed.

However, initial results of the experiment related to the endocannabinoids have already been published, which have provided some clear indications of how the body reacts to the stress of spaceflight conditions. These results compared data from the ISS Immuno experiments with similar experiments undertaken on short-duration parabolic flights which use a converted Airbus A300 aircraft. During parabolic flights participants are not only exposed to short-duration (22 secs)

periods of weightlessness during each parabola but also hypergravity (1.8g) periods (about 20 secs) when going into and coming out of each parabola in addition to periods of normal gravity in between. These extreme changes in environmental conditions are perceived as stress by the human body.

During parabolic flights there was a significant increase in endocannabinoid levels in blood samples for the 14 test subjects that showed no signs of motion sickness (low stress) and an almost unchanged level of cortisol. In contrast the 7 test subjects that did experience motion sickness (high stress) showed an inverse reaction with a lack of endocannabinoid response and a large increase in cortisol levels. One interesting point for these two different groups is that the highly stressed individuals had initially lower baseline endocannabinoid levels.

Similarly, low stress subjects on the ISS had sustained higher endocannabinoid levels which returned to normal after flight. This was also mirrored by the astronauts self-perception of stress from the questionnaires in addition to an increase in cortisol levels measured in saliva samples. That said at the time these results were published only the results for the levels of the endocannabinoid anandamide could be seen as significant. Chronic stress exposure during long-duration missions therefore demonstrates a positive relationship between stress, endocannabinoid response, and activation of the HPA axis.

Comparing this with ground research, evidence suggests that enhanced endocannabinoid signalling is probably required for adaptation and tolerance under stressful conditions. Physical exercise in physically fit individuals results in elevated levels of endocannabinoids which return to baseline levels on conclusion of the activity. (Feuerecker et al., 2012; Heyman et al., 2012). Human and animal experiments have also confirmed the antinausea/antiemetic effects of cannabinoids and their receptors (Choukèr et al, 2010; Van Sickle et al., 2003; Parker et al., 2009) and cannabinoids are used to treat chemotherapy-induced emesis in cancer patients (Machado Rocha et al., 2008).

However even though acute (short-term) stress response and its effects constitute an important survival reaction of the human body, chronic (long-term) stress response can lead to maladaptation syndromes causing various disorders such as depression, chronic pain, posttraumatic stress disorder (Yehuda, 2002), or autoimmune disease (McEwen and Dhabhar, 2002).

The scientific data so far produced by the Immuno experiment on the ISS is very positive for analysis. What conclusions can be drawn concerning the patterns of endocannabinoid/cortisol levels induced during spaceflight and the implications for immune system response, cannot be stated with any significance until all data for all test subjects has been analysed though the data also points towards positive outcomes.

Measurements have already been determined for most subjects for biological markers within both the innate and adaptive immune systems. One common innate immune system response is inflammation and the analysis is looking at levels of pro-inflammatory markers from the various samples taken. For the adaptive immune system, which



↑ Immuno experiment's lead scientist, Dr. Alexander Chouker

comprises the T-cells, B-cells and natural killer cells and creates immunological memory after coming in contact with a specific pathogen, cell signalling protein levels (needed to activate immune response) have been measured.

Once the detailed analysis is complete this should provide some indications of the influence of spaceflight on response to bacterial, fungal and viral infection during and post-flight as well as any individual factors that may play a role in immune response.

This type of human research is quite unique as the majority of experiments in this area have previously relied on samples/measurements taken only pre- and post-flight, implying that no in-flight development in immune function could be determined from the data. The Immuno-2 experiment will provide even more important information by taking at least 3 assays in orbit (similar protocol to first Immuno experiment but with additional early sample collection two weeks to one month after launch)

With spaceflight seeming to increase psychoneuroendocrine stress response the understanding of the complex interactions of cognition, stress and immunity will provide a base to suggest suitable countermeasures for the prevention of the unwanted immunological effects of stressful conditions during space missions both within and outside of low-Earth orbit as well as on Earth.

The outlook is positive for this research, and it will be interesting to see what the full results will determine and what the following steps will be. Looking further into the future this type of research could provide a greater depth of information if the possibility of on-board functional analysis were made possible. This may not only be a benefit for the type of information that could be gathered but may also provide an important, if not the only, means for gathering such information during exploration missions to, for example, Mars.

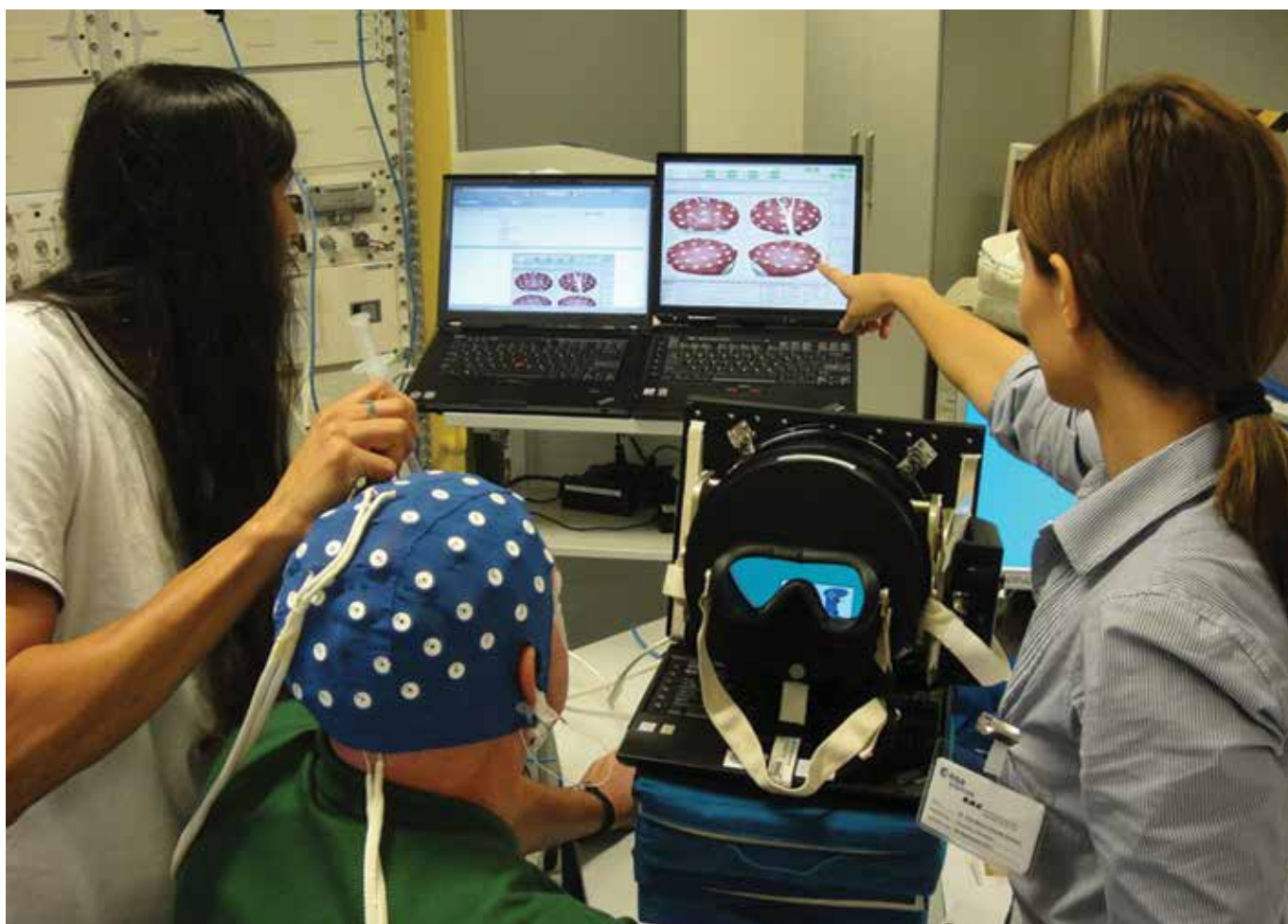
References

1. C. Strewe, M. Feuerecker, I. Nichiporuk, I. Kaufmann, D. Hauer, B. Morukov, G. Schelling, A. Choukèr (2012). Effects of parabolic flight and spaceflight on the endocannabinoid system in humans. DOI 10.1515/revneuro-2012-0057 Rev. Neurosci. 2012; 23 (5-6): 673–680
2. M. Feuerecker, D. Hauer, R. Toth, F. Demetz, J. Holzl, M. Thiel, I. Kaufmann, G. Schelling, A. Choukèr, (2012). Effects of exercise stress on the endocannabinoid system in humans under field conditions. Eur. J. Appl. Physiol. 112, 2777 – 2781.
3. E. Heyman, F.X. Gamelin, M. Goekint, F. Piscitelli, B. Roelands, E. Leclair, V. Di Marzo, R. Meeusen, (2012). Intense exercise increases circulating endocannabinoid and BDNF levels in humans – possible implications for reward and depression. Psychoneuroendocrinology 37, 844 – 851.
4. M. D. Van Sickle, L. D. Oland, K. Mackie, J. S. Davison, K. A. Sharkey, (2003). Deltag-tetrahydrocannabinol selectively acts on CB1 receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets. Am. J. Physiol. Gastrointest. Liver Physiol. 285, G566 – G576.
5. L.A. Parker, C. L. Limebeer, E. M. Rock, D. L. Litt, M. Kwiatkowska, D. Piomelli (2009). The FAAH inhibitor URB-597 interferes with cisplatin- and nicotine-induced vomiting in the *Suncus murinus* (house musk shrew). Physiol. Behav. 97, 121 – 124.
6. F. C. Machado Rocha, S. C. Stefano, R. De Cassia Haiek, L. M. Rosa Oliveira, D. X. Da Silveira, (2008). Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. Eur. J. Cancer Care 17, 431 – 443.
7. R. Yehuda, (2002). Post-traumatic stress disorder. N. Engl. J. Med. 346, 108 – 114.
8. B. S. McEwen, F. Dhabhar (2002). Stress in adolescent females: relationship to autoimmune diseases. J. Adolesc. Health 30, 30 – 36.
9. A. Choukèr, I. Kaufmann, S. Kreth, D. Hauer, M. Feuerecker, D. Thieme, M. Vogeser, M. Thiel, G. Schelling. Motion sickness, stress and the endocannabinoid system. PLoS One. 2010 May 21;5(5):e10752.

→ GROUND REFERENCE RESEARCH

Behind the Scenes of Baseline Data Collection

A fundamental element of ESA's human spaceflight research is Baseline Data Collection which is the process of taking measurements before and after astronaut missions as part of experiment protocols. These measurements provide either self-standing data for analysis or provide context for in-orbit physiology research procedures. The nature of spaceflight has wide-ranging effects on the astronauts that go into orbit even more so for long-duration missions experienced by the Expedition crew members on the ISS. We can expect that this would be even more extreme for astronauts on future human exploration missions beyond low-Earth orbit which could last up to 18 months and possibly beyond. Reference data taken before and after missions has already proved central to results coming from many experiments within ESA's human research programme which is vitally important to improve our understanding of the effects of spaceflight on the human body and in turn draw conclusions to similar conditions on Earth.



↑ ESA astronaut André Kuipers during Baseline Data Collection procedures for the Neurospat experiment

To a wider audience current human spaceflight research is connected with astronaut activities on-board the ISS. However all the data gathered on-board the ISS would not provide any useful conclusions without the extensive sequence of Baseline Data Collection (BDC) that occurs for human research experiments both pre- and post-flight. In fact, due to the specific requirements of some experiments, pre-flight and post-flight procedures can be sufficient without the need of any in-flight procedures, so-called ground experiments.

These ground reference experiments provide an added degree of cost-efficiency by optimising the finite amount of hours and resources available on orbit for ESA to fulfil its research objectives.

International Cooperation

The presence of six crew members on the ISS since 2009 has expanded the possibilities (and subjects) within human research activities. This has also required a more consolidated approach in the coordination of BDC activities and ESA has on-going agreements with NASA and Roscosmos on BDC implementation. Space physiology experiments are performed by astronauts from different ISS partners agencies (ESA, NASA, JAXA, CSA and Russian crewmembers) who are also involved in the implementation of their national programmes with most of the experiments targeting the



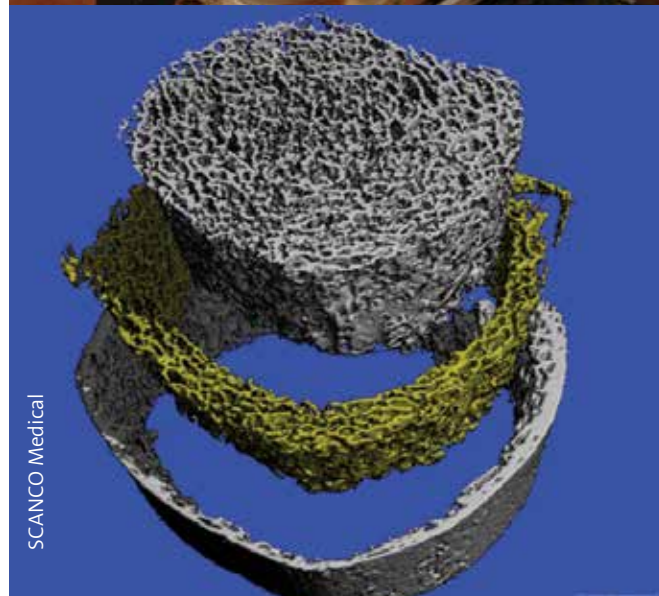
↑ ESA astronaut André Kuipers undertaking the Neurospat experiment on the ISS in 2012

recovery period immediately after return to Earth. As such the complexity of implementing BDC increases especially in the first hours/days after landing.

In order to facilitate the BDC measurements hardware unique to each experiment has to be delivered to the different BDC locations either in Europe or following complicated customs regulations and import/export controls in the US and

Russia. For some experiments BDC equipment is installed at the BDC sites for the time while the experiment is being performed along the several space missions. Such examples of ESA equipment are the Visual and Vestibular Investigation System (VVIS) rotating chair, used in the now-completed SPIN experiment, and the 3D peripheral Quantified Computed Tomography (3DpQCT) scanner, used in the almost completed EDOS experiment. Both are presently installed and operated at the Gagarin Cosmonaut Training Centre in Star City.

Many experiments include blood and urine samples collection. Samples delivery back to the European investigators from the USA and Russia is one of the most difficult parts of the BDC not only because of the specific (thermally conditioned) storage conditions but also due to the restricted regulations for transportation of the biological samples all over the world. Those and many other aspects require thorough analysis and preparation starting far in advance before the first BDC session takes place.



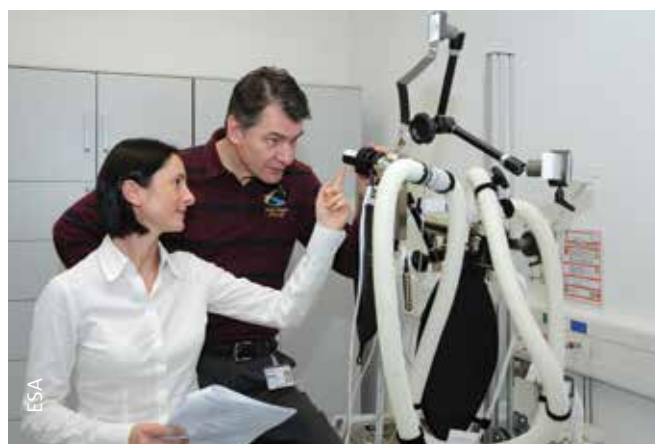
↑ Top: Final check before centrifugation of cosmonaut Yuri Malenchenko seated on the VVIS in Star City GTC – Moscow. The VVIS was the rotating chair used as a central element of the Spin experiment. Bottom: Xtreme Computed Tomography scan of the distal radius, as would be produced in the EDOS experiment

Baseline Data in Context

In order to determine the physiological effects of spaceflight on different bodily systems, a clear picture of the physiological/neurological state of an astronaut pre-flight is a necessary start point. The measurements taken depend on the requirements of the scientific protocol. For example for immunology experiments this could involve blood and urine sampling to test for white blood cell levels/activity and biochemical markers present; for research into bone and muscle degeneration this could involve either 3D-pQCT scans or muscle biopsies; for neurological protocols this may involve tests of visual perception and personal questionnaires; and for cardiopulmonary research this could involve ultrasounds of different blood vessels, blood pressure measurements and oxygen uptake measurements. These pre-flight measurements provide the baseline against which deviations from the 'norm' are measured. Depending on the specific experiment protocol, the measurements are taken at different (multiple) points before flight.

Taking measurements at different points prior to launch provides a certain degree of consistency in the data, to make sure a clear picture of the base level is obtained and possibly providing important data if any deviations occur on ground in the run up to launch i.e. increased adrenaline levels as launch approaches. Any deviations found can be factored into results when comparing to in-flight and post-flight data. These pre-flight measurement points which as a standard occur at one, two and three months prior to launch (sometimes 3, 6, 9 months) also double up as an effective means of training astronauts in procedures that take place on orbit (for experiments which include on-orbit elements) as measurements taken on-orbit and on-ground try to follow similar protocols (also for consistency). The pre- and post-flight measurements at the various different locations are undertaken by the relevant science team.

As mentioned some protocols can be undertaken using just pre- and post-flight BDCs. There are two main aspects of post-flight BDCs, which use similar measurement protocols to pre-flight for comparison purposes. Firstly BDCs taken on immediate return to ground can provide clear information on



↑ ESA astronaut Paolo Nespoli (right) during training on the Portable Pulmonary Function System (PPFS) in 2010. The PPFS has been a central element of measurements for different ESA research protocols both in orbit and for taking BDC measurements

how the body has been changed by exposure to weightlessness, by comparing the post-flight measurements to the pre-flight measurements. With this clear the continued gathering of BDCs at different points after landing provides additional data on how the body recovers to the adverse effects of spaceflight, and also how long it takes to recover. Plus, almost all experiments have a requirement for one more BDC session after the re-adaptation process is completed to ensure that the values have “returned to preflight BDC levels” (which they usually do). Standard post-flight measurements are taken at 1-5 days, one month and three months after landing. How long BDCs continue after flight very much depends on the area of research being studied. For example recovering bone loss after flight takes longer than, for example, recovery of altered perception/orientation due to transition into and out of weightlessness. For this reason research undertaken on bone demineralisation may also include measurements taken at six months and one year after return.



↑ ESA astronaut Luca Parmitano being carried just after landing in November 2013

Of course outside of ground experiments there are also research protocols that require in-flight measurements. Measurements taken in flight provide additional information about the onset and development of alterations in neurological/physiological systems of the body by taking measurements throughout a mission and can provide a clear picture of changes occurring during spaceflight for bodily systems that may be too heavily affected by the transition back to gravity on return to Earth before BDCs can be initially taken, for example the vestibular system which governs balance and orientation.

With every research protocol, whether just with pre- and post-flight BDCs or also including in-flight measurements, the combined data set provides significance to the results. This would however have no significance if taken for only one test subject.



↑ ESA astronaut Luca Parmitano wearing a temperature sensor on his forehead as part of the Circadian Rhythms experiment on the ISS (top) and post-flight (bottom). This experiment examines the hypothesis that long-term spaceflight significantly affects the synchronisation of the circadian rhythms in human beings due to changes of a non-24 hour light-dark cycle

Human research experiments performed on board the ISS are requested to be conducted, and publishable results provided, with a small number of astronaut-subjects, usually between 6 and 10 astronauts for each experiment protocol (ground research usually has a minimum of 20 and true clinical studies have 100+ participants).

Each human being is a bit different, and potentially the variation between the data values from each subject could show a wide variation, which would be a problem with the small number of test subjects. Thus, researchers ask each astronaut to function as his/her own control.

That said, initial studies with just one subject could provide a good indication as to the direction areas of research could go in. One example of this was the Skin Care experiment which started as a one person protocol with ESA astronaut Thomas Reiter, applying non-invasive skin testing methods before, during, and after a long-term mission. This was due to the fact that a thinning of the skin, increased skin sensitivity, delayed

healing of wounds and increased tendency to skin infections have been reported after a long stay in space, in addition to dry and itchy skin. Results coming from this study were then used in order to develop and initiate the current Skin-B experiment.

BDC Planning

The BDC process includes several phases. Prior to the first BDC session each ESA experiment has to pass approvals of the ESA Medical Board, NASA Committee for Protection of Human Subjects (CPHS) and ISS Human Research Multilateral Review Board (HRMRB). Depending on the crewmember participating in an experiment it is also necessary to get approval of the national space agency's medical board.

The next step is presentation of the experiments to the crews at the Informed Consent Briefings (ICB). Each astronaut receives an experiment briefing from the science team for each research protocol. After this familiarisation session an astronaut would sign an Informed Consent Form to confirm their participation in an experiment.

Based on the individual experiment requirements the BDC plan is developed for each ISS increment (or previously Shuttle mission). The BDC plan consists of the timeline and detailed sessions description (required data points, location, duration, facilities, specific equipment, procedures for sampling and sample processing etc.) so that the BDCs for the ground- and in-flight experiments with their specific requirements and constraints are well implemented into the busy crew schedule. These are put together in cooperation between ESA, the science team and Partner Agency representatives.



↑ The European Astronaut Centre near Cologne, Germany, one of ESA's main locations for Baseline Data Collection

BDC Locations

The locations where BDCs are conducted were linked to the launch and landing sites with early pre-flight (Launch-180 to Launch-60 days) and late post-flight (Return >+60 days) measurements still being able to be performed in Europe during the crew training at the European Astronauts Centre near Cologne or in the investigators' laboratories. This was due to the fact that crews did not travel in the period two months prior to launch and in the first weeks after landing. This set the locations for conducting late pre-flight and early post-flight measurements as NASA's Johnson Space Center in Houston, Texas, NASA's Kennedy Space Center in Florida or Star City, just outside Moscow, Russia. This has changed slightly over recent years. Firstly there is not a real necessity for late-

pre-flight measurements for the majority of human research protocols allowing BDC measurements for European research protocols to be undertaken in Europe. With respect to early post-flight measurements undertaken in Russia following a Soyuz landing, the introduction of direct return back to the US for (non-Russian) astronauts enabled the possibility for post-flight measurements to be undertaken both in Russia and the US. This will be opened up even further from a European perspective with the first direct return to EAC for ESA astronaut Alexander Gerst in November 2014 after his Blue Dot mission, enabling early post-flight BDC measurements in Europe.

Positive Results

Positive results have already come from ESA research based only on pre- and post-flight BDC measurements. For example the Chromosome-1 and -2 experiments (2002 – 2008) in the area of immunology elucidated a lack of chromosomal aberrations in astronauts on short- and long-duration missions. The Early Detection of Osteoporosis in Space (EDOS) experiment (2007 – 2014) within musculoskeletal research saw the successful commercialisation of a 3D scanner which ESA supported the development of for a non-invasive observation of bone structure. Results in neuroscience from purely ground experiments have also discovered that the recovery of otolith function (central to balance in the inner ear) proceeds over a period of 8-10 days with considerable inter-individual variability (Otolith experiment, 2008 – 2011) and that a simple tactile prosthesis is sufficient to bring landing day performance back to preflight levels (ZAG experiment, 2007 – 2011). This could have positive applications for example for people with vestibular disorders.

From an ESA perspective there are currently numerous research protocols on-going (as of October 2014) the latest of which is Brain-DTI, which uses only pre- and post-flight data using advanced MRI methods to study human central nervous system adaptation. The landscape of research is also evolving with many human research protocols in the pipeline. As one example, a follow up experiment to EDOS which will start with (a) member(s) of the one-year crew from 2015. As such the link to future exploration missions is becoming stronger and ESA's human research programme is growing in depth.



↑ Test subject in the Variable Radius Centrifuge (VRC) as used in the Otolith and ZAG experiments

→ MATERIALS RESEARCH:

Materials Science Laboratory: Back on Track and Approaching Completion

ESA's materials research is producing a wealth of data to help industry improve solidification models and casting processes in order to produce stronger lighter weight materials. Following extensive work by ground teams the research undertaken in ESA's Materials Science Laboratory (MSL) has been enjoying an extended period of continuous research. This culminated in the successful reprocessing of a sample thought unusable 18 months ago. With one more sample awaiting processing from the current batch of MSL experiments the outlook is looking bright for results of this research in advance of the follow up set of experiments.



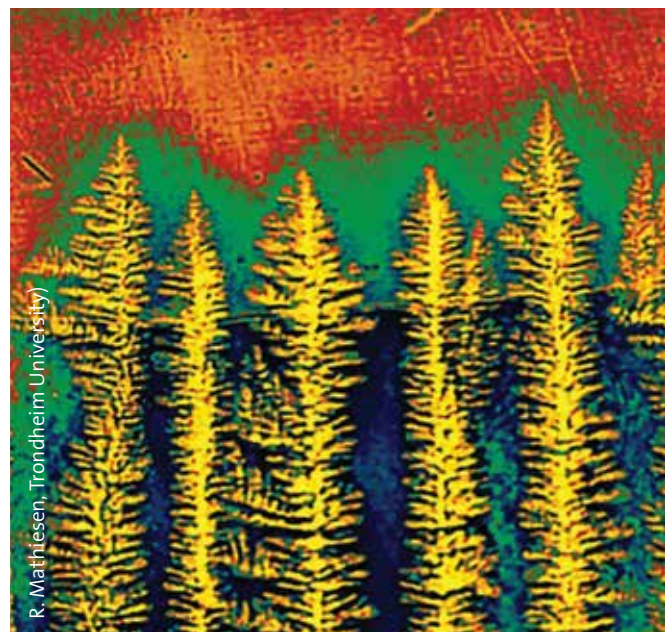
↑ ESA's materials science research is producing positive results to help improve casting processes for producing new lighter weight materials for use in for example airplane engines

ESA's second batch of solidification experiments in the MSL (for the CETSOL, MICAST and SETA projects) have been on-going on the ISS since August 2011. These continue the successful research undertaken on the ISS within the first batch of CETSOL and MICAST experiments which completed just over a year's sample processing on the ISS in January 2011.

ESA's Materials Science Research forms part of a world class programme in materials research involving hundreds of international partners from academia and industry. The goal of this research is to increase our understanding in materials

solidification processes in order to help develop new stronger lighter weight materials which will have a significant impact on industry for solving some of the most significant issues facing our planet such as fuel efficiency and consumption and recycling of materials. Results of this research will have cost reducing effects across numerous industries and in turn make these industries more competitive and attractive to investment.

The influence of convection on processes involved during the solidification of different aluminium-based alloys is being analysed with a view to validating/updating numerical models, which will enable industry to optimise casting processes. It is the determination of the internal (micro) structure in processed metal samples which is of great importance in



R. Mathiesen, Trondheim University

↑ Columnar dendritic or tree-like growth during solidification from solid (bottom) to molten metal (top) using real-time X-ray topography

these experiments as the microstructure influences an alloy's characteristic properties such as strength, flexibility and resistance to fatigue.

CETSOL (Columnar-to-Equiaxed Transition in Solidification Processing) and MICAST (Microstructure Formation in Casting of Technical Alloys under Diffusive and Magnetically Controlled Convective Conditions) are two complementary material science projects. MICAST is investigating the effect of controlled convection on the columnar dendritic or tree-like structures (see image below) that can form in solidifying aluminium-based alloys under specific conditions. For CETSOL the study is focussing on how these tree-like structures evolve into randomly oriented 'branches', the so-called equiaxed structure and the parameters that influence this transition. Having non-uniform dendritic forms in the alloy microstructure implies that the mechanical properties of the sample can vary considerably through the sample.

The SETA (Solidification along a Eutectic path in Ternary Alloys) experiment is looking into a specific type of eutectic growth in alloys of aluminium manganese and silicon.

All three of these experiments are undertaken in the Materials Science Laboratory which is the core facility inside NASA's

Materials Science Research Rack-1 (MSRR-1). This forms part of long-term collaboration with NASA comprising the launch and accommodation of MSL in MSRR-1 and the science collaboration. MSL/ MSRR-1 was delivered to the ISS on the STS-128 Shuttle mission and installed in the US Laboratory in September 2009 by ESA astronaut Frank De Winne and Christer Fuglesang. This was a milestone in materials research as it was ESA's first materials rack on the ISS specifically designed for undertaking unique long-duration solidification research in space providing data not accessible on Earth.

Following successful commissioning on orbit, the first two samples (one for CETSOL and one for MICAST) were processed in November 2009. This was followed up by 10 more Batch 1 samples between January and April 2010, bringing the total to six CETSOL and six MICAST samples processed. The MSL furnace insert was hereafter swapped out from the Low Gradient Furnace to the Solidification and Quenching Furnace by NASA astronaut Catherine Coleman and the 7th and final batch 1 MICAST sample was processed in January 2011.



↑ ESA astronaut Frank De Winne (left) and Christer Fuglesang (top right) installing the Materials Science Research Rack which includes ESA's Materials Science Laboratory as its principal payload



↑ Top: ESA astronaut Frank De Winne installing the Low Gradient Furnace into the Materials Science Laboratory as part of commissioning activities in October 2009. Bottom: De Winne installing a CETSOL sample into the Materials Science Laboratory as part of the first sample exchange on 8 November 2009

This completed successful processing of the Batch 1 samples with the final processed MICAST sample returned to Earth in March 2011. Positive results from these experiments provided a positive outlook for processing of the second batch of samples which included the follow up samples for the CETSOL and MICAST experiments along with additional samples for the new SETA experiment.



↑ Expedition 26 Flight Engineer and NASA astronaut Catherine Coleman during Materials Science Laboratory Furnace Exchange in January 2011

The first set of Batch 2 samples (two each for the CETSOL, MICAST and SETA) experiments were delivered to the ISS on STS-135/ULF-7 Shuttle Atlantis in July 2011 and processing started well with one CETSOL sample and one MICAST sample processed in August 2011.

However, on 30 September 2011 the first sample for the SETA experiment was being processed in the Materials Science Laboratory when the primary Payload Multiplexer/Demultiplexer (MDM) computer in the US laboratory (one of the principal ISS computers responsible for monitoring and allocating power/resources to different research facilities) crashed. This caused a power down of the Materials Science Research Rack/Materials Science Laboratory and cooling was cut during the sample processing at very high temperatures inside the Solidification and Quenching Furnace of the Materials Science Laboratory.

Even though this had no safety issues for the crew, some graphite foil thermal filler on the outside of the SETA Sample Cartridge Assembly became stuck to the inner surface of the furnace cooling zone and detached from the Sample Cartridge Assembly when it was removed from the MSL. As the Solidification and Quenching Furnace was not designed to be cleaned inside this posed some very significant issues, which could have jeopardised the further research in the facility.

However, due to the resourcefulness of our ground engineering and operations teams, cleaning tools were developed which were launched to the ISS on Progress 48P along with an additional nine Sample Cartridge Assemblies for the second batch of experiment samples. (The first three processed Sample Cartridge Assemblies for the second batch of experiments were returned to earth on the first SpaceX Dragon Demo flight which landed on 31 May 2012).



↑ MSL Conical Tool, one of the tools used within the scraping step of the cleaning process of the MSL (in the background)

NASA astronaut Kevin Ford successfully cleaned the Solidification and Quenching Furnace in November 2012 using the specially developed MSL Conical Tool, Protection Tube and Scraper along with a standard ISS vacuum cleaner for suction. An appropriate software upgrade also took place to avoid the possibility of a future reoccurrence of the cooling loss during experiment processing.

Successful testing was undertaken in January 2013 and research activities successfully restarted with the processing of two MICAST samples and one CETSOL sample by February 2013. (These three samples returned for analysis on the SpaceX-2 flight which landed on 26 March 2013).

However the Standard Payload Computer of MSL experienced an unplanned reboot during the solidification phase of processing of a SETA sample on 22 May 2013 while only marginally processed and it was initially thought that the SETA sample was lost for scientific purposes. Following ground analysis of the reboot, a go-ahead was given to restart the Batch 2 experiments and ESA astronaut Luca Parmitano exchanged the SETA sample in the MSL for a CETSOL cartridge in August 2013, with processing about a month later. This was followed up by processing of a further three CETSOL samples and five MICAST samples up until 20 May 2014.

While all of these activities were on-going on orbit, behind the scenes an analysis was being conducted to determine if the SETA sample, which did not complete processing in May 2013, could be reprocessed. One of the principal concerns relating to any activities undertaken on human spacecraft is that the very strict safety criteria are met. After much deliberation and analysis it was determined that the SETA sample could in fact be reprocessed to help produce data thought unobtainable. This sample was hereafter processed on 24-27 May 2014.

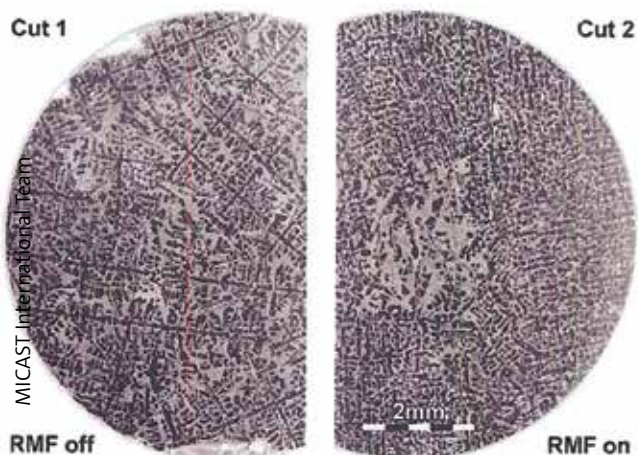


↑ View of the Canadarm2 or Space Station Remote Manipulator System (SSRMS) arm about to undock the SpaceX Dragon from the International Space Station (ISS). The Earth is visible in the background

the ISS have undergone microscopic and X-ray analysis by the relevant science teams following return to earth and very interesting preliminary results have already been presented by scientists.

The first experiments performed with the Materials Science Laboratory on the ISS proved that constant solidification conditions can be established meeting the predefined scientific requirements for sample processing.

For CETSOL, for example, experimental results were obtained in space using hypo-eutectic Al-Si7 alloys with or without a grain refiner and directionally solidified under different conditions in the Materials Science Laboratory. The analysis of the space samples confirms the occurrence of a columnar-to-equiaxed transition, especially in the refined alloy. Temperature evolution and grain structure analysis provide critical values for the position, the temperature gradient and the solidification velocity at the columnar to equiaxed transition. A sharp transition was detected when the rate of solidification was increased in contrast to the progressive transition observed when lowering the temperature gradient. In the absence of grain refiner however, triggering the transition appears to be more difficult.



↑ Metallographic sections of the processed MICAST #4 sample prepared at positions 135 mm (Cut 1) and 190 mm (Cut 2) Left: Diffusive conditions yielded periodic rectangular patterns of dendritic structures (*dark regions*) equally distributed across the sample. Right: Forced convection induced by magnetic stirring carried residual melt with eutectic composition (*bright regions*) from the mushy zone towards the centre of the sample. (RMF: rotating magnetic field)

As an example of results for MICAST, one of the first samples to return from orbit for analysis from the batch 1 samples was the MICAST #4 sample. The MICAST #4 sample was studying the directional solidification of a specific aluminium silicon alloy with a well-defined temperature gradient and a fixed solidification velocity. The first part of the sample was undertaken under purely weightless conditions while for the second part of the sample a rotating magnetic field was activated to stimulate controlled stirring in the molten metal. The basic differences of microstructures under different conditions can be seen in the image on this page.

Without the rotating magnetic field being active a very uniform microstructure is observed. Dark regions correspond to dendritic structures primarily formed from the beginning of solidification. They are arranged in periodic rectangular patterns. With ongoing dendrite formation the amount of aluminium remaining in the residual molten metal decreases until eutectic solidification occurs filling the areas between the dendrites (bright regions in images). The microstructure changes significantly when the rotating magnetic field is acting on the sample. A complex flow field between the dendrites is induced which carries the residual melt enriched with silicon from the mushy zone (consisting of both solid and liquid structures) towards the centre of the sample. Thus, the eutectic structures formed at the minimum temperature for the alloy is not equally distributed across the sample but concentrated through the centre of the sample.

With this SETA sample processed, only one more sample is currently on orbit awaiting processing prior to completion of all the second batch samples. Even more positive is the fact that six more processed Sample Cartridge Assemblies (three CETSOL and three MICAST) were returned on SpaceX-3 for analysis on 18 May 2014. This now leaves only five remaining samples (one SETA, two CETSOL and two MICAST) which will return on SpaceX-5 in December 2014.

Results from CETSOL and MICAST^[1-7]

The first batch of CETSOL/MICAST samples processed under varying parameters in the Materials Science Laboratory on

Future Prospects in ISS Materials Research

The casting industry, especially for high-end industry such as aerospace, is coming to rely more heavily on the benefits of using numerical models to determine casting methods and conditions needed in anticipation of producing materials with specific performance characteristics that are tailored to specific applications. The MSL experiments are helping to validate these casting models with the vital benchmark data needed from space experiments.

The completion of the second set of MSL samples bodes well for analysis which will build on the successful results that came from the first batch of CETSOL and MICAST experiments and pave the way for the future processing of the further MSL experiment samples which are currently undergoing development, planning and preparations prior to launch.

ESA's materials research on the ISS will also be expanded with the Electro Magnetic Levitator launched to the ISS on ATV-5 which will perform container-less materials processing involving melting and solidification of electrically conductive, spherical samples, under ultra-high vacuum and/or high gas purity conditions. With a comprehensive programme of future ISS materials research, augmented by extensive research activities on ground and on other weightless platforms such as sounding rockets, the future is looking very solid for ESA's materials research activities.

References

1. T. Enz, S. Steinbach, D. Simicic, G. Kasperovich, L. Ratke (2011). First Experiments Using the Materials Science Laboratory on Board the International Space Station. Microgravity Science and Technology. Volume 23, Issue 3, pp 345-353
2. G Zimmermann, L Sturz, B Billia, N Mangelinck-Noël, H Nguyen Thi, Ch-A Gandin, DJ Browne and W U Mirihanage, Investigation of columnar-to-equiaxed transition in solidification processing of AlSi alloys in microgravity – The CETSOL project, 2011 J. Phys.: Conf. Ser. 327 012003, IOP 1742-6596/327/1/012003)
3. W U Mirihanage, D J Browne, L Sturz and G Zimmermann, Numerical Modelling of the Material Science Lab - Low Gradient Furnace (MSL-LGF), Microgravity Directional Solidification Experiments on the Columnar to Equiaxed Transition 2012 IOP Conf. Ser.: Mater. Sci. Eng. 27 012010, IOP 1757-899X/27/1/012010
4. L. Sturz; G. Zimmermann; C.-A. Gandin; B. Billia; N. Mangelinck; H. Nguyen-Thi; D. John Browne; W. U. Mirihanage; D. Voss; C. Beckermann; A. Karma, ISS-experiments of columnar-to-equiaxed transition in solidification processing, Proceedings 141st TMS Conference 2012
5. W.U. Mirihanage, D.J. Browne, G. Zimmermann, L. Sturz, Simulation of international space station microgravity directional solidification experiments on columnar-to-equiaxed transition Acta Materialia 60 (2012) 6362–6371
6. D. R. Liu, N. Mangelinck-Noël, Ch.-A. Gandin, G. Zimmermann, L. Sturz, H. Nguyen Thi, B. Billia, Structures in directionally solidified Al - 7 wt% Si alloys – Benchmark experiments under microgravity, to appear in Acta Materialia 2013
7. G. Zimmermann, L. Sturz, B. Billia, N. Mangelinck-Noël, D.R. Liu, H. Nguyen Thi, N. Bergeon, Ch.-A. Gandin, D.J. Browne, Ch. Beckermann, D. Tourret, A. Karma, Columnar-to-Equiaxed Transition in Solidification Processing of AlSi7 alloys in Microgravity – The CETSOL project, to appear in Materials Science Forum, 2014

→ MILESTONES IN MICROBIOLOGY

Coloured Fungi In Space

The CFS-A experiment determined the changes that weightlessness and cosmic radiation have on the growth and survival of various coloured fungi species. Understanding any changes in the physiology and survivability of different microorganisms in space can help determine the effect that this may have on spacecraft, associated systems and supplies, as well as the astronauts inhabiting them. This could provide important insights for developing countermeasures to possibly deleterious micro-organisms, help to draw conclusions on how the space environment may affect similar organisms, and could also feed into biotechnology applications in the future. We take a look at some of the results that have come from this research so far [1].

The Growth and Survival of Coloured Fungi in Space-A (CFS-A) experiment was undertaken on the International Space Station (ISS) in 2011, the first time an experiment by a Romanian science team was performed onboard ISS. This research activity has been performed in the frame of the SURE project with the European Commission to facilitate access to the ISS specifically to the ESA Cooperating States (and more generally to new EU Member States).

The weightless environment and presence of radiation in spacecraft provide what can be called a 'stressful' environment

to any organisms present inside. The question is are different microorganisms negatively impacted by these stressful conditions or can they in fact adapt and thrive better than on ground?

Microbiology is a vitally important area, not only within human spaceflight but also for humans on Earth. With this microscopic world encompassing microorganisms such as bacteria, archaea, fungi and algae, that can have both a detrimental or beneficial impact on our daily lives, this research has far-reaching effects feeding into many different areas of biotechnology.



↑ Fig. 1.1 Flight Day 5 sample from the Coloured Fungi in Space Experiment

Microorganisms can spoil food but in the same respect can assist in waste and sewage treatment and processing, as well as nutrient cycling and exchange; they can assist in pollution control but can also increase greenhouse gases; they can cause disease but can be used in the manufacture of antibiotics, detergents and pesticides; they can cause deterioration in manufactured materials and buildings but can also be used in the recovery of metals in the mining sector as well as the production of biofuels and fertilisers. Different species of fungi are inherent in many of these processes. Furthermore insights into one species maybe provide insights into others and hence feed into different applications.

Several previous space research in this area (Fig 1.2) has focused on studying bacteria^[2-6] and yeast^[7-9], (yeast are themselves a unicellular fungi), while the CFS-A experiment studied multicellular fungal species with first results published in 2013^[1] and on which this article is based.

Just as on earth, a whole host of microorganisms have been found on the ISS as contaminants coming from the Earth, as part of experiments and as normal microbial populations from crew members. On the International Space Station *Staphylococcus* species (which reside normally on the skin and mucous membranes of humans and other organisms) were identified as the most dominant airborne bacterial genus. *Aspergillus* (found in almost all oxygen-rich environments), *Penicillium* (Fig 1.4, commonly present wherever organic material is available) and *Cladosporium* species (which are significant airborne allergens) were identified as the most dominant genera in the fungal population ^[10-12] (Fig. 1.3). Other opportunistic pathogens and species involved in biodeterioration of structural materials or biodegradation have been identified in food and waste storage, and recycling systems ^[13, 14].

Identifying different species on the ISS is one part of the process. The next logical step is to determine if the space environment changes the form and function of these microorganisms. The fungal species chosen for the CFS-A experiments belong to four genera selected as organic material decomposers and possible contaminants of materials destined for interplanetary travel.

The main fungal species studied in the CFS-A experiment was *Ulocladium chartarum* which is well known to be involved



↑ Fig. 1.2 ESA astronaut Frank De Winne installing Yeast experiment containers into Biolab incubator in Columbus on 2 October 2009

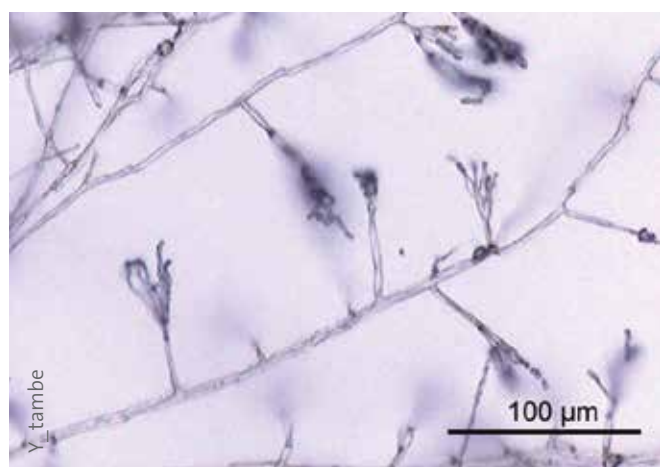


↑ Fig 1.3 Fungi on the ISS, growing on a panel of the Russian Zarya Module where exercise clothes were hung to dry

in biodeterioration of organic and inorganic materials and suspected to be a possible contaminant in spacecraft. Other species studied were *Aspergillus niger*, (which causes a disease called black mold on certain fruits and vegetables, and commercially accounts for 99% of global commercial citric acid production) *Cladosporium herbarum* (frequently the most prominent mold in air-spores and found on dead herbaceous and woody plants, textiles, rubber, paper, and foodstuffs of all kinds) and *Basipetospora halophila* (which survives in high saline environments). Along with *Aspergillus* and *Cladosporium*

species, *Ulocladium* species have also been isolated on the ISS^[10]. *U. chartarum* is also expected to be resistant to space radiation due to its melanin content^[15-17].

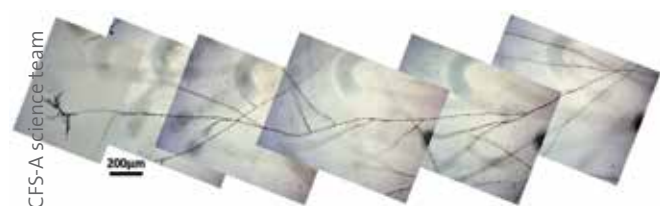
For the CFS-A experiment living cultures of *U. chartarum* were studied with spores prepared (on a culture medium) 5 days, 3 days, and 1 day prior to launch in order to analyse the effect of the space environment on the colonies at different stages of development. Dried spore samples of *Ulocladium chartarum*, *Aspergillus niger*, *Cladosporium herbarum* and *Basipetospora halophila* were placed on iron, silica and polycarbonate wafers prior to launch. All the samples were flown to the ISS on STS-133 Shuttle Discovery and stored on the ISS at normal cabin temperature. Photos of the live cultures (Fig 1.1) were taken on flight days 5 and 9 to evaluate the growth rate. On Flight day 14 the live samples were returned to earth with photos taken 4h after landing and the dry spores remained on the ISS for five months. Different post-flight analysis followed return.



↑ Fig 1.4 Microscopic image of *Penicillium* species

When most fungi grow in colonies on a nutrient source, whether this be the surface of a piece of fruit or a culture medium in a laboratory for example, the colony grows as mycelium, either submerged mycelium (growing into the nutrient source) or aerial mycelium (that grows upward or outward from the surface of the nutrient source, and from which propagative spores develop). The mycelia are made up of networks of hyphae which are long, branching filamentous structures used as the main mode of vegetative growth. Normally, submerged mycelium grows simultaneously with aerial mycelium assuring the growth of the colony.

Post-flight analysis from the live samples of the CFS-A experiment demonstrated that *U. chartarum* colonies were



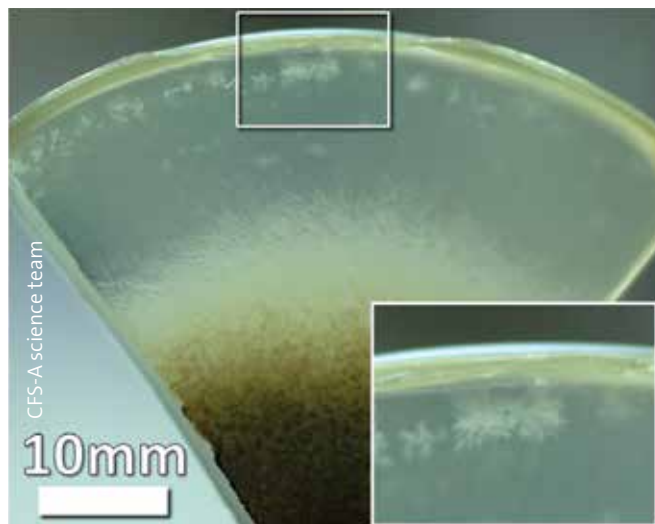
↑ Fig 1.5. Long submerged fungal hyphae ending with a microcolony

able to grow under spaceflight conditions and that the rate of growth for the first five days was significantly higher in spaceflight conditions than on ground. This showed that cell division and branching are not inhibited in space and cells proliferate faster.

Growth of the colonies in the space-flown samples were observed in the submerged mycelium, which gave rise to new colonies initially growing inside the nutrient layer (Fig 1.5). The formation of such microcolonies was not observed in the different ground samples of the same age. It seems therefore that spaceflight conditions stimulate the growth of submerged mycelium. The hyphae of space-flown samples extended in depth over long distances (Fig 1.5), in some cases very close to the edge of the culture plates and always initiating new colonies (Fig 1.6). This was not observed for both sets of ground samples during the experiment.

The microcolonies (which have the same characteristics as the initial colonies but of smaller size) started in the depth of the nutrient source as a very dense mycelium, and then submerged hyphae oriented themselves towards the surface where oxygen was available. Their development was dependent on the age of the colony. Just after landing, the live samples launched after 5 days of incubation on ground had microcolonies that had already reached the surface of the nutrient source and produced spores between flight days 9 and return. The microcolonies in the other two space-flown containers reached the surface of the nutrient source 3 and 4 days after return.

In contrast to the growth observed in the submerged mycelium, after five days of incubation on orbit the growth rate of aerial mycelium was decreasing until it totally stopped. Different factors were responsible for this, though it was discovered that the experimental setup with the biocontainer also had a negative influence on aerial mycelium growth.



↑ Fig 1.6. Colony slice showing new colonies grown from the submerged mycelium under spaceflight conditions (box magnified in inset)

It is well known that in areas rich in nutrients the mycelium branches and grows slowly maximizing the amount of nutrients it can extract. In contrast in areas low in nutrients the hyphae grow more rapidly and with little branching. Possible factors, like biosynthesis of toxic compounds, CO₂ accumulation, and

the quasi absence of air convection in space, could favour the growth of submerged mycelium as a strategy to assure the survival of the colony and to find less harsh growing conditions. There seems to be a network of sensing mechanisms and of signalling pathways able to transmit the information on aerial and nutritional status of the environment to cellular level directing the hyphal growth to the depth of the nutrient source.

For the dry spore samples flown on the ISS, *Aspergillus niger* and *Ulocladium chartarum* spores showed a very good viability grown on all types of wafers. *Basipetospora halophila* and *Cladosporium herbarum* spores had a lower viability. Both the latter species had a lower viability on the silica wafers then on polycarbonate wafers and neither had viability on the iron wafers probably due to a strong oxidation of the iron wafer in contact with salts removed from the nutrient (for *Basipetospora halophila*) and the toxic effect of iron ions (for *Cladosporium herbarum*).

The high viability of *Aspergillus niger* and *Ulocladium chartarum* spores on iron wafers showed that these species could grow on iron surfaces covered with small quantities of carbohydrates on the ISS. For *Basipetospora halophila* if by chance food preserved in salt that is contaminated with this species, is brought to space, a degradation process could start.

In summary the CFS-A experiment clearly indicated that *Ulocladium chartarum* is able to grow under spaceflight conditions, elaborating a new strategy to survive for a short time by developing submerged mycelium and for a long time by developing sporulating microcolonies on the surface of the nutrient source. By spreading the spores this species is able to survive.

Additional ground-based research suggest that weightlessness is in fact a larger driving factor and less the exposure to higher radiation or the high g-loads and vibrations experienced during launch and landing.

In spacecraft *U. chartarum* and other fungal species could find a favourable environment to grow invasively unnoticed in the depth of surfaces under the right conditions posing a risk factor for biodegradation of structural components, as well as a direct threat for crew health. This will be especially important for future long-duration missions outside of Low-earth orbit where astronauts will have to be more self-sufficient for maintaining spacecraft and systems and some food supplies would need to be preserved for longer than potentially 18 months. However, on the same line, in the future this kind of research could potentially feed into strategies for waste recycling on spacecraft and the development of biological life support systems.

With the CFS-A experiment producing positive results, further investigations are needed to determine if the changes found in *U. chartarum* are specific to this species or general for other fungal species, as well as to explain any changes at cellular and physiological levels.

References

1. I. Gomoiu, E. Chatzitheodoridis, S. Vadrucchi, I. Walther (2013). The Effect of Spaceflight on Growth of *Ulocladium chartarum* Colonies on the International Space Station. doi: 10.1371/journal.pone.0062130
2. Horneck G, Rabbow E (2007) Mutagenesis by outer space parameters other than cosmic rays. Adv Space Res 40: 445–454. doi: 10.1016/j.asr.2006.12.049
3. Lawal A, Jejelowo OA, Rosenzweig JA (2010) The effects of low-shear mechanical stress on *Yersinia pestis* virulence. Astrobiology 10 9: 881–888. doi: 10.1089/ast.2010.0493
4. Horneck G, Klaus DM, Mancinelli RL (2010) Space microbiology. Microbiol Mol Biol Rev 74 1: 121–156. doi: 10.1128/mmr.00016-09
5. Boulou P, D'Ari R (1991) Escherichia coli metabolism in space. J Gen Microbiol 137 12: 2839–2843. doi: 10.1099/00222187-137-12-2839
6. Juergensmeyer MA, Juergensmeyer EA, Guikema JA (1999) Long-term exposure to spaceflight conditions affects bacterial response to antibiotics. Microgravity Sci Technol. 12 1: 41–47.
7. Walther I, Bechler B, Müller O, Hunzinger E, Cogoli A (1996) Cultivation of *Saccharomyces cerevisiae* in a bioreactor in microgravity. J Biotechnol 47: 113–127. doi: 10.1016/0168-1656(96)01375-2
8. Van Mulders S, Stassen C, Daenen L, Devreese B, Siewers V, et al. (2010) The influence of microgravity on invasive growth in *Saccharomyces cerevisiae*. Astrobiology 11 1: 45–55. doi: 10.1089/ast.2010.0518
9. Hammond TG, Benes E, O'Reilly KC, Wolf DA, Linnehan RM, et al. (2000) Mechanical culture conditions effect gene expression: gravity-induced changes on the space shuttle. Physiol Genomics 3: 163–173.
10. Novikova N, De Boever P, Poddubko S, Deshevaya E, Polikarpov N, et al. (2006) Survey of environmental biocontamination on board the International Space Station. Res Microbiol 157: 3–12. doi: 10.1016/j.resmic.2005.07.010
11. Vesper SJ, Wong W, Kuo CM, Pierson DL (2008) Mold species in dust from the International Space Station identified by mold-specific quantitative PCR. Res Microbiol 156: 432–435. doi: 10.1016/j.resmic.2008.06.001
12. Makimura K, Hanazawa R, Takatori K, Fujisaki R, Nishiyama Y, et al. (2001) Fungal flora on board the Mir-Space Station, identification by morphological features and ribosomal DNA sequence. Microbiol Immunol 45: 357–363. doi: 10.1111/j.1348-0421.2001.tb02631.x
13. Novikova N (2004) Review of the knowledge of microbial contamination of the Russian manned spacecraft. Microb Ecol 47: 127–132. doi: 10.1007/s00248-003-1055-2
14. Ott M, Bruce R, Pierson D (2004) Microbial characterization of free floating condensate aboard the Mir space station. Microb Ecol 47: 133–136. doi: 10.1007/s00248-003-1038-3
15. Kawamura K, Tsujimoto T, Tsuge T (1999) Targeted disruption of a melanin biosynthesis gene affects conidial development and UV tolerance in the Japanese pear pathotype of *Alternaria alternata*. Mol Plant Microbe Interact 12: 59–63. doi: 10.1094/mpmi.1999.12.1.59

16. Gorbushina A, Whitehead K, Dornieden T, Niese A, Schulte A, et al. (2003) Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. *Can J Bot* 81 2: 131–138. doi: 10.1139/b03-011
17. Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, et al. (2008) Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. *Stud Mycol* 61: 99–109. doi: 10.3114/sim.2008.61.10

→ MILESTONES IN ASTROBIOLOGY

Expose-R2

ESA's Expose-R2 astrobiology payload was successfully installed outside the International Space Station in August 2014. The payload is hosting numerous experiment samples that are testing the survivability of different biological organisms such as algae, lichens, fungi and bacteria and some of the biochemical building blocks of life to the environmental conditions in open space as well as on Mars and other astronomical bodies. This is proving a very positive time for the Expose project, as the results of Expose-R, the direct forerunner of Expose-R2, in orbit during 2009-2011, are due for imminent publication.



↑ Expose-R2 payload during Russian spacewalk on 22 October 2014, with Sun shield removed

Research for this latest set of astrobiology experiments comes from a wealth of ESA-supported exposure experiments in Low Earth Orbit including the 11-month EURECA mission (1992 – 1993), Biopan facility research on five separate two-week Foton missions between 1994 – 2007, the 18 – month Expose-E set of experiments on the ISS EuTEF external payload (Feb 2008 – September 2009) and the first Expose-R mission (March 2009 – January 2011).

On 18 August 2014 the Expose-R2 payload was installed on the external surface of the Zvezda Module of the ISS during a spacewalk from the Russian segment. The payload, which is scheduled to continue for 12 to 18 months, is carrying samples for four different astrobiology experiments, three from ESA (BIOMEX, BOSS and PSS) and one from the Russian Institute of



↑ Biopan exposure facility (with ESA logo) installed on the side of Foton-M3 during launch preparations in 2007



↑ De-integration of lichens from the LIFE experiment on Expose-E after 18 months of space exposure

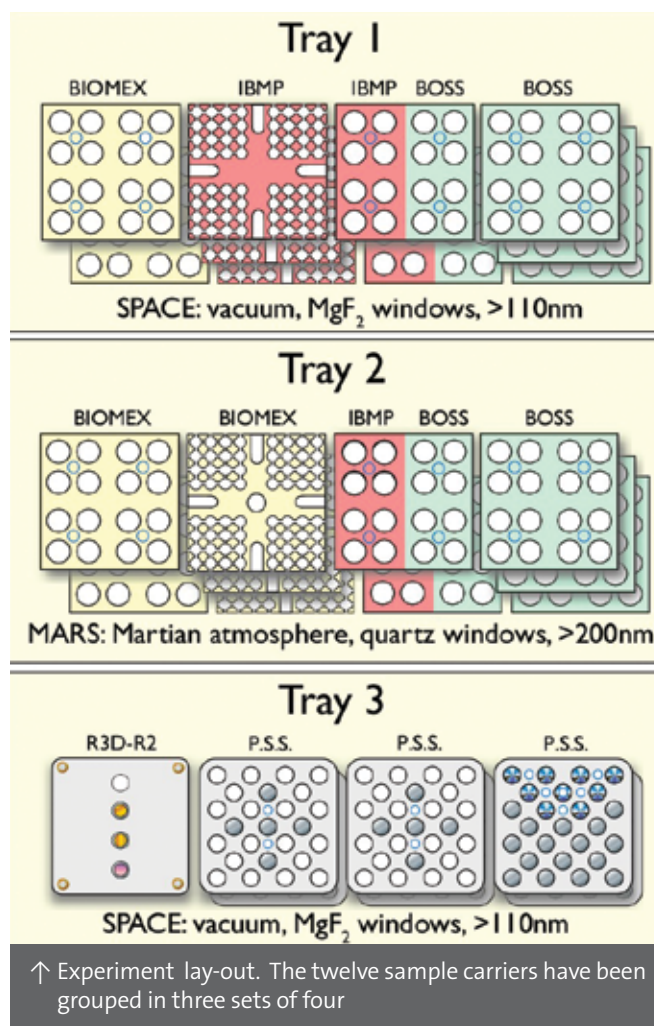


↑ The Expose-R2 payload with Sun-shield still attached

Biomedical Problems (IBMP). Astrobiology deals with the origin, development and distribution of life in space.

The Expose-R2 experiments could help understand how life originated on Earth and could survive on other planets and astronomical bodies. With good telemetry received from the payload after its installation, valves were opened two days later from ground to expose a section of biological samples to open space (vacuum) conditions. This was followed up by removal of the sun screen on 22 October in the frame of another Russian EVA to expose the top section of samples to full solar irradiation. The following day all sensors were switched on and data acquisition started.

During the Expose-R2 mission the core facility of Expose-R (which remained on board the ISS after 2011) is being used again, but equipped with three new trays loaded with fresh sample materials. Each tray contains four different sample



carrier locations, so 12 locations in total. The ESA experiments will be split across nine carriers, three carriers each. Two carriers have been allocated for the IBMP experiment. Each of the sample carrier locations has two or three levels of samples. The 12th location carries a set of active radiation sensors (R3D) which were also used for the previous Expose-E and -R missions.

Each experiment requires test samples to be exposed to the open space environment, i.e. a combination of full-spectrum electromagnetic radiation from the Sun, cosmic particle radiation, vacuum, temperature fluctuations and weightlessness. The required space conditions can only partially be simulated on ground, and then separately, not in combination. Full-spectrum solar irradiation can only be approximated, but not duplicated in the lab and only for limited periods of time. Cosmic particle radiation can also not be faithfully simulated on Earth.

Sample Setup

The BIOMEX, BOSS and IBMP experiments will expose samples to open space (Tray 1) and Martian conditions (Tray 2), with a vacuum in Tray 1 and simulated Martian atmosphere in Tray 2. The PSS experiment (Tray 3) will be in vacuum conditions (as Tray 1) though with certain differences outlined under the experiment description.

The top layer in each carrier contains the more exposed samples. For the 'open space' samples these will be placed

behind magnesium fluoride (MgF₂) windows which allow all wavelengths from deep ultraviolet to far infrared through. For the simulated Mars conditions the samples will be behind quartz windows to simulate Mars conditions by filtering out the necessary UV wavelengths.

For each of the four experiments, an additional one or two layers underneath the top, more exposed, samples remain in darkness, acting as a reference for the Sun-exposed samples and additionally simulate the samples being embedded under the surface of the astronomical body in question where protection is afforded from Solar UV.

BIOlogy and Mars EXperiment (BIOMEX)

The BIOlogy and Mars EXperiment (BIOMEX) is under the scientific lead of Dr. Jean-Pierre de Vera of DLR's Institute of Planetary Research in Berlin. The main objective of the research is to measure to what extent biomolecules like pigments and cellular components are resistant to and able to maintain their stability under space and Mars-like conditions.

The results of BIOMEX will be relevant for space-proven biosignature definition and for the formation of a biosignature data base (e.g. the proposed creation of an international RAMAN library). The library will be useful for future space missions like projects for life detection analysis on Mars.



↑ Cyanobacteria of the genus Nostoc (here imaged under an optical microscope) are among the organisms under investigation as part of the BIOMEX experiment

A second scientific objective is to analyse to what extent terrestrial extremophiles are able to survive in space and which interaction between biological samples and selected minerals (including terrestrial, lunar and martian analogue varieties) can be observed under space and Mars-like conditions. The results will provide new information about environmental extremes that can be sustained by the proposed species and about the chances for survival during a 'natural' trip in space (according to the Panspermia theory). The BIOMEX samples consist of a variety of pigments (e.g. chlorophyll, melanin), cell wall components, lichens, archaea, bacteria, cyanobacteria, algae, black fungi and bryophytes (such as common liverwort).

The results are an essential starting point for defining biotracers we might expect to find on other planets. A follow-on topic is to investigate the likelihood of lithopanspermia (the transfer of organisms in rocks from one planet to another either through interplanetary or interstellar space) with replicate experiments and a new set of microorganisms. Results will provide information about the space resistance of numerous microorganisms and will tell if the current list of microorganisms that can probably survive to space and Martian conditions has to be enlarged.



↑ The specimens being prepared for the BOSS experiment at the DLR Laboratory

Biofilm Organisms Surfing Space (BOSS)

The Biofilm Organisms Surfing Space (BOSS) experiment is under the scientific lead of Dr. Petra Rettberg from DLR's Radiation Biology Department in Cologne, Germany. In the experiment the hypothesis being tested is that biofilm-forming microbes, embedded in self-produced polymeric substances, are more resistant to the environmental conditions in space and on Mars than planktonic cultures. The BOSS samples are being exposed under open space and Mars-like conditions.

In their natural environment, most bacteria live on surfaces as slime-encased biofilms and microbial mat communities (the fossils of the latter represent the earliest clear signs of life on Earth). In comparison to their planktonic counterparts, these fixed bacterial populations are significantly more resistant to environmental stresses like chemical pollution, antibiotics and predation. The hypothesis being tested is that biofilm-forming microbes, embedded in self-produced extra-polymeric substances are more resistant to the environmental conditions in space and on Mars than planktonic cultures.

The results of BOSS will contribute to our understanding of the capability of life to persist in extreme environments on Earth, in space and on other planetary bodies. The results will help to answer numerous questions such as: Are micro-organisms in biofilms more resistant to different harmful environmental parameters than single cells? Could biofilms survive in space or on Mars? What is the possible role of mineral particles for further protection? Are there additive or synergistic effects from a combined treatment of biofilms with vacuum or martian atmosphere and pressure and solar UV radiation? Do biofilms favour the survival of particular metabolic groups when exposed to extreme conditions?

Photochemistry on the Space Station (PSS)

The Photochemistry on the Space Station (PSS) experiment is under the scientific lead of Prof. Hervé Cottin from the Interuniversity Laboratory of Atmospheric Systems of the Université Paris-Est Créteil and the Université Paris Diderot and CNRS.

Solar ultraviolet photons are a major source of energy to initiate chemical reactions in the solar system. The PSS experiment is a photochemical study of the evolution of organic molecules to improve our understanding of chemical evolution in organic-rich astrophysical environments (comets, meteorites, Titan, interstellar medium) and where organic matter is being looked for (Martian surface and subsurface). A wide range of non-



↑ Processed image of Titan taken by the ESA's Huygens probe of the joint NASA/ESA/ASI Cassini-Huygens mission

gaseous and gaseous organic compounds is being tested. This includes different amino acids, DNA and RNA nucleobases, hydrocarbons thought to be important as possible starting materials for the formation of life, and synthetic organic residues from comets and Titan.

Such studies are essential. Many experimental programmes on Earth are devoted to photochemical studies of the evolution of organic molecules. However, the solar spectrum below 200 nm is hard to reproduce in the laboratory, therefore the validity of these Earth-based studies and their applications to extra-terrestrial environments can be questioned as long as experiments conducted in a genuine space environment have not been carried out.

The samples are placed behind windows of different materials. This includes magnesium fluoride (MgF₂), quartz and potassium bromide (KBr) for various filtering properties.

In addition, as part of the PSS experiment, some biochips are being tested for survival during space travel. These are foreseen to be used during future exploration missions, for instance to detect specific organic compounds on Mars.

The results from the PSS experiment will provide data about the degradation of organic molecules when exposed for a prolonged period of time to solar UV. These results are expected to be significantly more reliable than the ground-based results obtained so far under simulated test conditions. The expected accuracy of the results is mandatory for a good understanding of chemical evolution in astrophysical environments rich in organic matter and of astrobiological relevance (comets, meteorites, Titan, interstellar medium) or where organic matter is looked for (Martian surface and subsurface).

R3D package

The R3D (Radiation Risks Radiometer-Dosimeter) will occupy one sample carrier location. R3D is a low-mass, low-size automatic device which measures, with time resolution, the solar irradiance over four wavelength ranges. In addition, it can measure the fluence of cosmic particles. R3D is equipped with a flash memory of 256 MB, capable to store data collected during the whole mission. In addition, the measured data can be downlinked by telemetry.

The solar irradiance is measured over four wavelength ranges: UV-C (170-280 nm), UV-B (280-315 nm), UV-A (315-400 nm), PAR (= photosynthetic active radiation, 400-700 nm).

Ground Experiment

In parallel to the flight experiment, a mission ground reference experiment is being conducted. This experiment will use the

environmental history of the flight samples (coming from R3D, thermal sensors, pressure sensors etc.) to simulate on-orbit conditions as far as technically feasible, except for cosmic particle radiation and weightlessness. As such, the results from the ground experiment will aid to interpret the results stemming from the flight experiment.

Results

The Expose-R2 experiment package successfully undertaking data acquisition is only one of the positive aspects of the Expose research. In addition the results of the previous set of samples that were exposed on the Expose-R payload from March 2009 – January 2011 are currently in the process of being published (14 papers).



↑ The Expose-R payload situated on the external surface of the ISS during March 2009 – January 2011

This includes a number of separate publications on the Amino experiment which was a photochemistry experiment (similar to PSS). One of these publications is by Prof. Hervé Cottin *et al.* providing an overview of the Amino experiment. Another by Marylène Bertrand *et al.* is confirming that resistance to irradiation is a function of the chemical nature of the exposed molecules and of the wavelengths of UV light with dipeptides and aspartic acid being the most altered compounds while compounds with a hydrocarbon chain were the most robust. Another Amino experiment publication from Jacques Vergne *et al.* is showing that exposure of various catalytic RNAs strongly supports the hypothesis that self-replicating RNA molecules are a crucial step of the origins and early life evolution.

A publication on the 'Spores in artificial meteorites' (SPORES) experiment (Corinna Panitz *et al.*) discloses the limits of Lithopanspermia for spores located in the simulated upper layers of impact-ejected rocks due to the presence of harmful extraterrestrial solar UV radiation. This will help to determine if meteorite material offers enough protection against the harsh environment of space for spores to survive a long-term journey in space.

Further publications cover the use of biological dosimeters for measuring UV dose in the harsh extraterrestrial radiation conditions (Attila Bérces *et al.*) as well as discussing the radiation profiles during the Expose-R mission (Thomas Berger *et al.* and Tsvetan Dachev *et al.*).

These are only a selection of the papers that are being published from the Expose-R research. The publication date is expected to be around the end of 2014, start of next year within a special edition of the International Journal of Astrobiology (Cambridge University Press - <http://journals.cambridge.org/action/displayJournal?jid=IJA>).

