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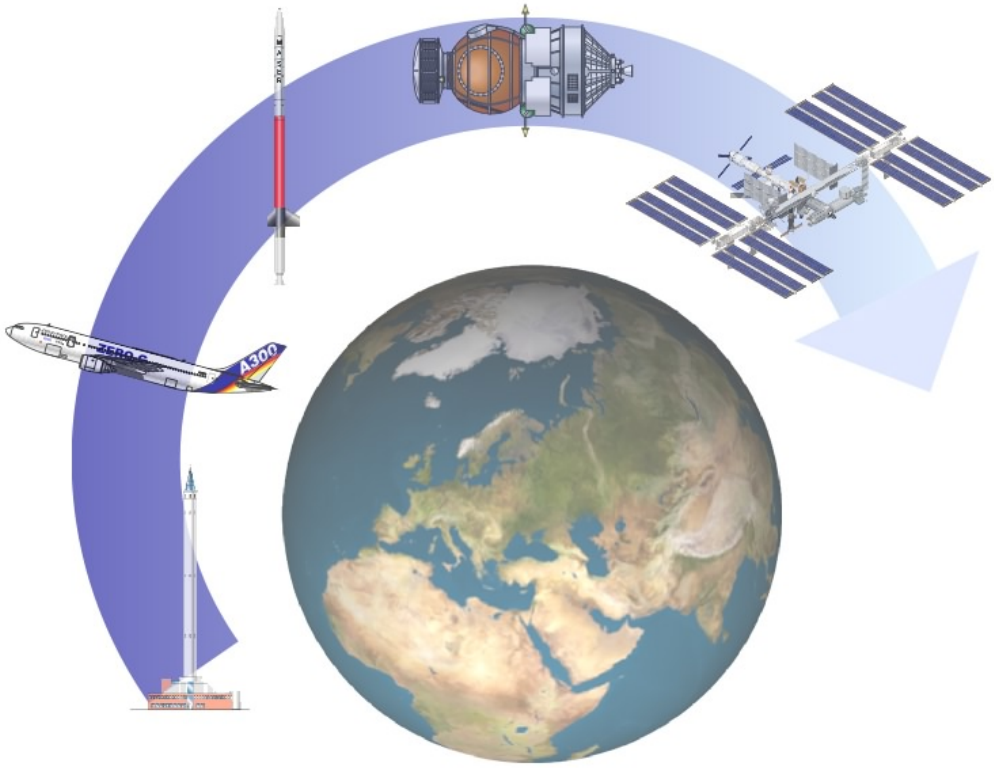
Summary Review of the European Space Agency's Low Gravity Experiments

Volume 1: ISS Increment 5



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PURPOSE OF DOCUMENT

The Summary Review of the European Space Agency's Low Gravity Experiments is intended to provide a concise, but clear, overview of the objectives and scientific results obtained from ESA sponsored low gravity research, executed on/in the five low gravity platforms and other ground based facilities supported by ESA.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

1.1 Background to ESA Low Gravity Research

European involvement in low gravity research began approximately 30 years ago, with nationally funded programmes (in particular those of France and Germany) and US collaborations. Later, in January 1982, a European Space Agency (ESA) funded programme was initiated by the ESA Member States, who agreed to a small programme to which governments could contribute according to their interests and budgets. The first phase of this new ESA programme (Microgravity Programme: Phase-1) was established for the period 1982-1985. This allowed ESA to participate in the German Texus Sounding Rocket programme (later extended to include Swedish Maser Sounding Rockets) to perform short duration microgravity experiments. The Phase-1 programme also covered the development of a first set of multi-user experiment facilities to be flown on the Space Shuttle Spacelab and SpaceHab missions.

Since then, ESA has sponsored more than 1500 experiments, payloads and facilities, which have been integrated and operated on various types of low gravity platforms, including:

- Drop Towers;
- Parabolic Flights;
- Sounding Rockets;
- Retrievable Capsules;
- Space Shuttle;
- MIR Space Station;
- International Space Station.

1.2 The Five Major Low Gravity Platforms

This document mainly covers the research executed on/in the 5 major low gravity platforms currently supported by ESA, which are:

- the ZARM (Zentrum für Angewandte Raumfahrt Microgravitation) Drop Tower, located in Bremen, Germany, which was officially declared an ESA External Facility on 2 October 2003;
- the Novespace Airbus A-300 "Zero-g" aircraft based at the Bordeaux-Mérignac airport, which has been used by ESA since 1997;
- the four ESA supported sounding rockets (miniTexus, Texus, Maser and Maxus), which are launched from the Esrange base near Kiruna, Sweden;
- the Russian Foton retrievable capsule, an unmanned Earth-orbiting spacecraft offering microgravity and space exposure, that ESA has used since the early 1990's;
- the most complex platform currently accessible through ESA, the International Space Station (ISS).

Besides the five major low-gravity platforms presented above, ESA also supports access to specific facilities and environments on Earth that simulate low gravity and the confinement of long duration space missions. Extensive and timely use of the research capabilities offered by these facilities, will not only improve the preparation of spaceflight experiments, but will also increase the level of scientific knowledge of the influence of gravity and/or extraterrestrial environments on life, physical and interdisciplinary processes.

Specific ground facilities that simulate space and planetary conditions like climate, physical and psychological isolation, low gravity, extreme environments, high velocity impacts, etc., are available in a wide range of scientific disciplines. Recent examples of these are Long Term Bed Rest Studies (refer to the following web site http://www.spaceflight.esa.int/users/file.cfm?filename=miss-gbfac) and Antarctic Isolation Studies (see http://www.esa.int/esaCP/SEMOS4T1VED_index_0.html). Both types of studies are aimed at investigating the physiological and psychological problems that may arise in conditions of isolation and confinement, such as those that will be experienced during a long duration space mission.

More detailed information regarding the above-mentioned platforms/facilities and how to access them can be found in the ESA publication "European Users Guide to Low Gravity Platforms", which can be viewed at the following web site http://www.spaceflight.esa.int/guide. A hard copy of the Users Guide can also be requested from:

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1.3 Release and Structure of Summary Review Document

This Summary Review document will be released in separate volumes, where each individual volume will cover the research carried out during one or more campaigns (Drop Tower, Parabolic Flight, Sounding Rocket, Ground-based), missions (Foton) or increments (International Space Station). The document will be comprised of two main parts:

- Section 1 will provide general information and a background to ESA's low gravity research, including a summary of the Research Cornerstones.
- Section 2 and beyond will introduce the platform or facility being covered, before providing an experiment-by-experiment summary, broken down per research cornerstone, for each specific campaign, mission or increment.

1.4 Research Cornerstones

In 2000, ESA prepared a comprehensive Research Plan defining the scientific priorities in the life and physical sciences for a 5-year period, with a horizon of 10 years. The compilation of this Research Plan was initiated by a bottom-up analysis of all the research proposals received at that time by ESA. As a next step, ESA asked the European Science Foundation (ESF) to assess the research priorities in a dedicated user consultation meeting, which took place in Bischenberg, France in November 2000. At this meeting and in the subsequent ESF recommendations, the concept of Research Cornerstones was defined.

The Research Cornerstones describe areas of research where concerted efforts at the European level have already produced, or are promising to lead to, eminence if not a leading position on a global level. They provide therefore, an excellent basis for ensuring that new proposals will address issues that have been recognised as constituting a particular strength in Europe. A particular advantage of this will be that the research objectives of the ESA programme will be better harmonised with those of other research funding agencies or entities in Europe, leading to a more efficient and complete coverage of the research efforts involved. It will also further promote the teaming of research groups at European level, thus combining strengths and increasing European knowledge and competitiveness. Finally, it will allow ESA to streamline and optimise the available and future research infrastructure to sustain those objectives.

Already at Bischenberg it was identified that the Research Plan is by definition a living document. Research priorities may shift, new promising research fields may emerge, or new results taken into account. For that reason, it was envisaged that the process of user consultation should be repeated at regular intervals.

Following this, a second user consultation on Life and Physical Sciences in Space was organised again by ESF at Obernai, France in May 2004. On this occasion a larger number of scientists participated and more time was available to discuss the individual disciplines during two workshops. After this consultation ESF recommended

updated Research Cornerstones, which ESA and its advisory committees analysed. After a full investigation, ESA produced an updated Research Plan, in which also the new Research Cornerstones were defined.

It should be stressed, however, that the Research Cornerstones are **not** used as a selection criterion in the evaluation of research proposals. In other words, the final selection of projects is based on scientific quality, regardless of the research topic addressed. This, in the view of ESA, is the only way to ensure that promising new research is identified and pursued. The Research Cornerstones should therefore be seen as a guideline to potential users who wish to carry out research in the life and physical sciences on the ISS.

1.4.1 Life and Physical Sciences Research Cornerstones

The following tables summarise the updated Life and Physical Sciences Research Cornerstones defined in 2004 for the period 2005-2009.

Table 1-1: Fluid Physics Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Fluid and Interface Physics	Study of multiphase systems (their phase transitions and related dynamics), critical and supercritical fluids, granular materials, liquid-solid interface phenomena and complex fluid phases. Geophysical fluid flows.	Quantify heat transfer, mass exchange and chemical processes in multiphase systems and supercritical fluids; Measure diffusive processes in mixtures; Study the stability of foams and emulsions; Describe dynamic coupling in granular materials under vibration.	Develop reactors for supercritical oxidation of industrial contaminants; Develop high-efficiency heat exchangers; Improve reactor design in industrial plants; Design improved oil recovery techniques.
Combustion	Study combustion phenomena that are dominated on the ground by buoyancy convection.	Quantify fuel droplet and spray evaporation, autoignition and combustion processes; Detail the process of soot formation in flames and the conditions for flammability of solid fuels.	Improve efficiency of electrical power plants; Reduce emissions of engines; Fuel-efficient and safe spacecraft for human exploration; Improved flammability test procedures.

Table 1-2: Fundamental Physics Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Physics of Plasmas and Solid/Liquid Dust Particles	Understand the three dimensional behaviour of particles in complex plasmas and aggregation processes that require weightlessness.	Enhance theoretical description of complex plasmas, including self-ordering and phase transition phenomena; Improve modelling of the interaction of protoplanetesimals, their optical properties and of the behaviour of pollutants in the atmosphere.	Develop novel plasma coating techniques; Nucleation and growth of novel substances for solar cells and plasma screens; Improved modelling of Earth climate and environment.
Cold Atom Clocks, Matter Wave Interferometers and Bose-Einstein Condensates	Study properties and applications of cold atoms, including Bose-Einstein condensates.	Develop and operate a cold atom clock in space; Check limits of validity of theories of relativity and quantum electrodynamics.	Improved accuracy of absolute time measurements; Increased accuracy for navigation and geodesy systems.

Table 1-3: Material Sciences Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Thermophysical Properties of Fluids for Advanced Processes	Utilise the extended possibilities of containerless processing in space to measure critical properties of fluids for processes that are required as input parameters for adequately describing balances in volume phases and at interfaces.	High accuracy measurements of the properties of stable and metastable (undercooled) liquid metals.	Increase the reliability of numerical simulation and control of casting facilities in the metallurgical industry.
New Materials, Products and Processes	Understand the physics of solidification and crystal growth of metals, organic and inorganic materials and biological macromolecules.	Quantify the influence of the growth conditions on the homogeneity and the defects in crystals, including protein crystals; Enhance numerical models of the microstructure formation in metals and alloys.	Improve and validate models for predicting grain structures in industrial castings; Develop processes towards new metallurgical products; Improve efficiency of production of industrial crystals.

Table 1-4: Biology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Molecular and Cell Biology	Study the impact of gravity at the cellular and molecular levels.	Study gene expression in an altered gravitational environment in relation to cellular phenomena; Improve understanding of the impact of gravity on signal transduction and the specific properties of cellular entities such as the membrane; Clarification of the role of mechanical forces including those derived from gravity in triggering proliferation, differentiation, apoptotic processes and tissue formation.	Provides the basis for other disciplines, including developmental biology, physiology, health science and biotechnology; Develop artificial functional tissues and targets for drugs screening; Depression of the immune system; Identify pharmacological substances for tissue regeneration; Develop bio-regenerative life support systems for human exploration missions; Develop novel microencapsulated drugs and cells.
Plant Biology	Understanding the impact of gravity on plant systems; Study mechanosensory elements involved in mechanisms of graviorientation and gravishaping.	Identify molecular and cellular elements of mechanosensory mechanisms and gravity-related signalling pathways; Study how gravity shapes plant morphology; Identify gene interactions important in the gravistimulus response chain.	Improvement of plant growth and mechanical properties of plants; Develop and improve biological life support systems; Provide the basis for biotechnological applications utilised on future long-term human spaceflight; Develop techniques for plant survival and growth in space.
Developmental Biology	Study the effect of gravity on whole-body developmental and reproductive processes.	Study altered gene expression in an altered gravitational environment; Study the impact of the cytoskeleton architecture on signal transduction e.g. functional genomics; Identify gravity-sensitive phases in multicellular organisms; Understand the effect of gravity on the development of the vestibular and sensori-motor systems in vertebrates.	Design pharmacological relevant substances for animal and human applications relevant to human development; Evaluation of the possible outcome of extraterrestrial colonisation attempts; Develop techniques and pharmacological substances for tissue regeneration.

Table 1-5: Physiology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Integrative Gravitational Physiology	Explore, in an interdisciplinary way, systems that are sensitive to gravity, e.g. cardiovascular system, pulmonary system, nervous system, fluid-electrolyte homeostasis, skeletal system, immune system, etc.	Study cardiovascular control and regulation; Study the mechanisms for fluid regulation by the kidneys; Investigate the interaction of the vestibular system with other inputs relevant to locomotion and posture (e.g. vision, proprioception); Study effects of changes in load on muscle atrophy and plasticity; Understand and quantify bone mass turnover as a function of e.g. local blood perfusion and mechanical stress; Study the mechanisms of osteoporosis.	Improve techniques and devices for medical applications e.g. sports medicine; Improve rehabilitation after long-term incapacitation, particularly involving bed rest; Improve treatment of patients with decreased lung-function; Develop improved approaches for the treatment of neurological diseases; Improve means for diagnostics, prevention and treatment of osteoporosis, and reduce bone loss in astronauts for future long duration missions; Improve treatment of diseases like hypertension.
Non-Gravitational Physiology of Spaceflight	Explore the effects of the non-gravitational extreme environment of space, e.g. radiation, isolation, nutrition, confinement, noise, disruption of circadian rhythms, hypobaric conditions (e.g. EVA), etc.	Study effects of isolation, group dynamics, cultural differences, etc.; Study effects of radiation on DNA damage; Study close coupling between nutrition and health, e.g. testing new space foods; Investigate effects of dust inhalation on airway inflammation; Investigate possibilities of decompression sickness in connection with EVA.	Improve crew selection techniques for future long duration missions; Develop new nutritional methods for the improvement of health; Develop new protection measures for people exposed to radiation; Improve prevention and treatment for patients suffering from decompression sickness.
Countermeasures	Develop physiological, pharmacological, psychological, and mechanical countermeasures.	Understand the mechanisms leading to various problems such as: spatial disorientation (nausea, imbalance), orthostatic intolerance, bone loss and microarchitectural deterioration, muscle atrophy and weakness, cardiac atrophy, etc.	Develop improved approaches, treatment and countermeasures for a variety of Earth and space based disorders and maladies.

Table 1-6: Exobiology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Origin, Evolution and Distribution of Life	Study the survivability of organisms under extreme conditions on Earth (extremophiles) and in space.	Investigate the contribution of space conditions, including radiation, to the formation of prebiotic molecules; Identify the conditions for survivability of micro-organisms from and in space, including planetary surfaces; Identify markers and tools to search for extinct and extant life.	Identify novel enzymes and bacteria from extreme physical and chemical environments with industrial application e.g. biocatalysis.

Table 1-7: Exploration Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Human Planetary Exploration	Study novel aspect of human planetary expeditions.	Quantify radiation risk for human beings and understand the specific biological action of space radiation; Study effects of isolation in high-stress environments; Quantify needs for consumables during missions; Perform simulation tests on in-situ resource utilisation potential.	Develop advanced radiation sensors and countermeasure devices; Develop technology for telemedicine/telesurgery in remote areas; Develop protocols for handling stress effects; Develop methods for in-situ resource utilisation; Develop life-support systems for use in space and other isolated environments; Develop the technologies for identification and utilisation of in-situ resources.

For more details regarding Life and Physical Sciences research, please contact:

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1.5 Erasmus Experiment Archive (EEA)

An important resource for low gravity research scientists and users is the Erasmus Experiment Archive (EEA), maintained by the Erasmus Centre (HME-UC). The EEA is a database of ESA funded or co-funded experiments covering a wide range of scientific areas, which were performed during missions and campaigns on/in various space platforms and microgravity ground-based facilities over the past 30 years. The archive is continuously being updated and as of February 2007, contained more than 1900 experiment records. The major items of information covered in the EEA include:

- Research cornerstone;
- Date of experiment;
- Mission name;
- Team members and institutes;
- List of publications/references;
- Experiment objectives;
- Experiment procedures;
- Experiment results;
- Attachments (figures, graphs, videos, etc.).

The EEA depends highly on the support provided by users; therefore users are encouraged to send inputs to the contact coordinates below, once they have executed an experiment. In fact, users who perform ESA funded experiments have the obligation to provide an abstract to the EEA. Failure to meet this obligation will be taken into account when deciding on new experiment opportunities/proposals from the user team in question.
Users are invited to visit the database, from which they can, among other things, obtain further information regarding experiments of their field of research already carried out in the past. The EEA web address is the following: http://www.spaceflight.esa.int/eea. For further details regarding the EEA, please contact the following by phone, fax, mail or e-mail:

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1.6 General Information and Advice

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2 THE INTERNATIONAL SPACE STATION (ISS)

2.1 ESA Utilisation Rights and Additional Flight Opportunities

The National Aeronautics and Space Administration (NASA) provides the overall leadership of the ISS programme development and implementation, and together with Russia provides the major building blocks of the ISS. The European Space Agency (ESA), together with the Japan Aerospace Exploration Agency (JAXA) and the Canadian Space Agency (CSA) are providing additional elements, which significantly enhance the Space Station. The overall ISS utilisation rights are divided among the Partners, according to the elements and infrastructure they provide (e.g. Columbus Laboratory for ESA). The main principle is that each International Partner may utilise equipment and facilities in or on each other Partner's elements in accordance with their respective "utilisation rights". Those rights are defined in the Intergovernmental Agreement (Article 9) and the different Memoranda of Understanding signed by all of the Partners.

In return for its contribution to the ISS, ESA has a resource allocation of 51 % of the internal and external user accommodation of the Columbus Laboratory. Other allocation rights to ESA comprise 8.3 % of the total ISS utilisation resources and 8.3 % of the total crew time. Note that this excludes all of the Russian accommodations and resources, as this is retained by Russia for its own use.

In May 2001, ESA and the then Russian Aviation and Space Agency (Rosaviakosmos), now Roscosmos, signed a Framework Agreement for the provision of Russian ISS flight opportunities. The Agreement documents the principles, terms and conditions for the cooperation between ESA and Roscosmos concerning ISS operations and utilisation, through the provision by the latter of fare-paying ISS flight opportunities in the period 2001-2006, for members of the European Astronaut Corps. The actual commitment for a specific flight opportunity is entered by ESA upon signature of an ISS Flight Order Contract (IFOC) for a specific flight.
The Framework Agreement, establishes a solid and stable basis for the strategic planning of the European Astronaut Corps, and it represents an important step towards the further development of operational expertise of the ESA astronauts prior to the full European utilisation of the ISS with the launch of Columbus.

Two types of flight opportunities are considered under the Agreement as ISS flight opportunities:

- ISS "taxi flights" (this term is reported in the original agreement, but is no longer used), which are defined as short duration Soyuz flights to the ISS for the purpose of exchanging the ISS docked Soyuz, including a short duration stay (approximately 7-8 days) on-board the ISS;
- ISS increment flights, which are defined as ISS crew exchange flights, including a 3-6 months (one increment) stay on-board the ISS.

The assignment of back-up astronauts/cosmonauts for ISS flight opportunities, involving ESA astronauts, is agreed upon between ESA and Roscosmos for each flight.
On-board activities are not restricted to the mandatory system operations and maintenance activities, but also allow for the conduct of activities or experimental programmes in the interest of ESA and national organisations of the ESA Member States. The terms and conditions of such activities are agreed upon in each specific IFOC. The IFOC defines the terms and conditions specific to the implementation of an agreed ISS flight opportunity. Such terms and conditions take precedence over the terms and conditions defined in the Framework Agreement.

The following table (Table 2-1) summarises the Russian ISS flight opportunities that have thus far included an ESA astronaut on-board, following the signature of the Framework Agreement in May 2001.

Table 2-1: ESA Russian flight opportunities deriving from ESA/Roscosmos Framework Agreement (May 2001)

ISS MISSION	ESA MISSION NAME	VEHICLE ID	LAUNCH DATE	LANDING DATE	ESA ASTRONAUT	ASTRONAUT NATIONALITY
ISS 3S	Andromede	Soyuz TM-33	21/10/2001	31/10/2001	Claudie Haigneré	French
ISS 4S	Marco Polo	Soyuz TM-34	25/04/2002	05/05/2002	Roberto Vittori	Italian
ISS 5S	Odissea	Soyuz TMA-1	30/10/2002	10/11/2002	Frank De Winne	Belgian
ISS 7S	Cervantes	Soyuz TMA-3	18/10/2003	28/10/2003	Pedro Duque	Spanish
ISS 8S	DELTA	Soyuz TMA-4	19/04/2004	30/04/2004	Andre Kuipers	Dutch
ISS 10S	Eneide	Soyuz TMA-6	15/04/2005	25/04/2005	Roberto Vittori	Italian
ISS ULF1.1	Astrolab	Shuttle STS-121	04/07/2006	22/12/2006	Thomas Reiter	German

2.2 Increment Timeline

The summary review of experiments carried out on board the ISS will be presented per Increment, i.e. the period of time between the launch of a vehicle carrying an exchange crew to the ISS, and the undocking of a vehicle for return of that crew. The length of an increment ranges anywhere from 3 months to about 6 months.
The Summary Reviews of European ISS experiments will be covered as from the Belgian Soyuz Mission ("Odissea"), i.e. as from the end of Increment 5.
The following schematic (Figure 2-1) presents a basic timeline of launch events and Increments of the ISS programme, and serves as a quick reference for users of this document.

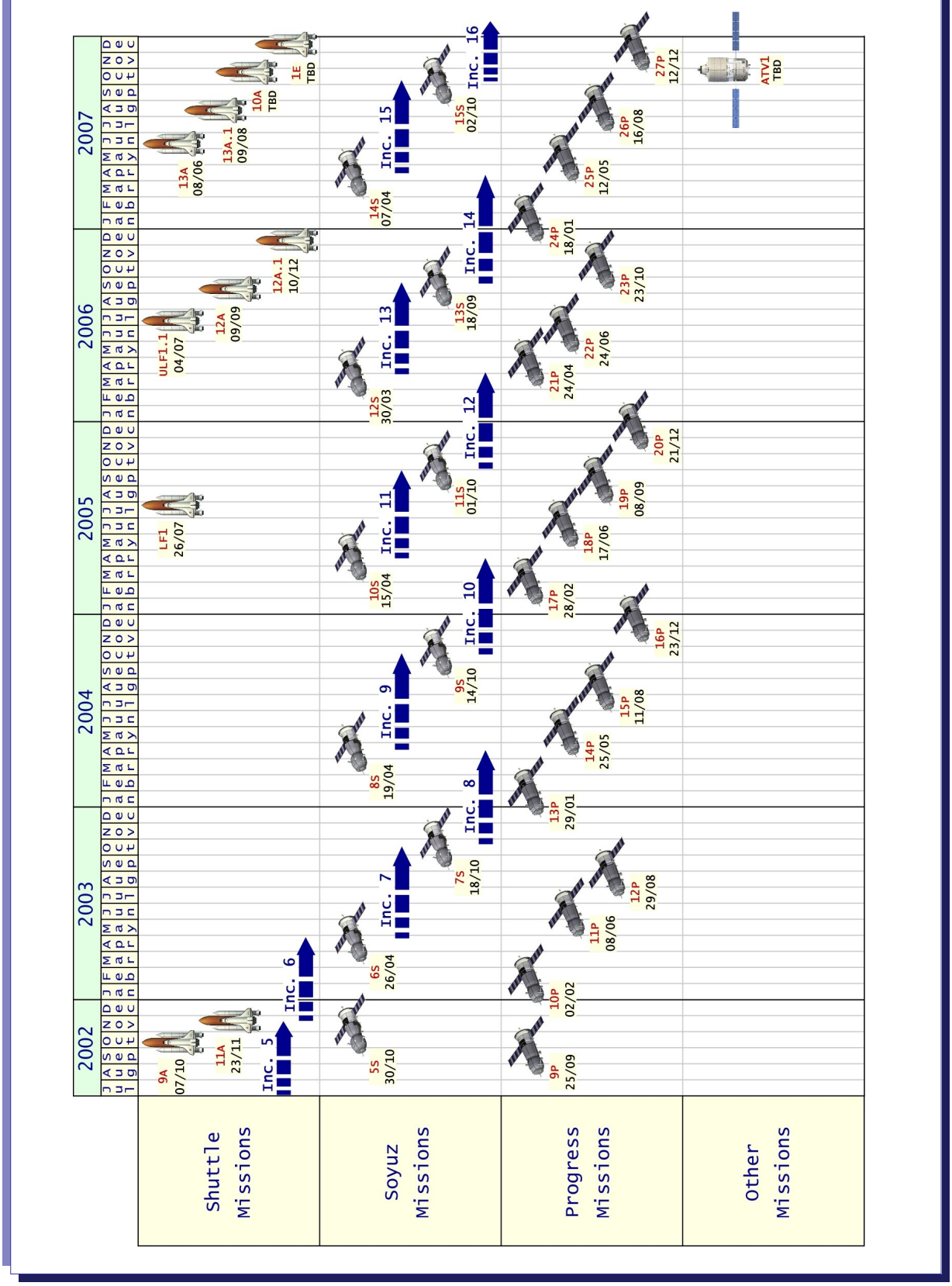

Figure 2-1: ISS Programme Launch Events and Increments (July 2002 - December 2007)

2.3 Increment 5: ESA experiments

The 9 ESA experiments carried out during Increment 5 formed part of a larger scientific programme (23 experiments) that was developed for the Belgian Soyuz Mission, "Odissea", launched on October 30th, 2002, carrying the Belgian ESA astronaut Frank De Winne to the ISS for an 8-day stay on the Station.

The following table (Table 2-2) lists the 9 ESA experiments that will be covered by this report.

Table 2-2: List of ESA experiments for Increment 5

NAME OF EXPERIMENT	RESEARCH CORNERSTONE
LIFE SCIENCES	
Characterisation of the effects of microgravity on the mechanism of action of Vitamin D in osteoblasts **(VITAMIN D)**	Biology: Molecular and cell biology
Chromosomal aberrations in blood lymphocytes of astronauts **(CHROMOSOME-1)**	Biology: Molecular and cell biology
Cosmic radiation and microgravity related oxidative stress **(RAMIROS)**	Biology: Molecular and cell biology
Directed attention brain potentials in virtual 3-D space in weightlessness **(NeuroCOG)**	Physiology: Integrative gravitational physiology
PHYSICAL SCIENCES	
Combustion synthesis under microgravity conditions **(COSMIC)**	Fluid Physics: Combustion
Diffusion coefficients in crude oils **(DCCO)**	Fluid Physics: Fluid and interface physics
Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the International Space Station **(PromISS)**	Material Sciences: New materials, products and processes
Granada Crystallisation Facility **(GCF)**	Material Sciences: New materials, products and processes
Study of aggregation mechanism and kinetics of ZSM-5 and Silicalite-1 nanoslabs into ZSM-5/Silicalite-1 hybrid phases under microgravity conditions (**NANOSLAB**)	Material Sciences: New materials, products and processes

2.3.1 Life Sciences

2.3.1.1 Biology: Molecular and cell biology

2.3.1.1.1 Characterisation of the effects of microgravity on the mechanism of action of Vitamin D in osteoblasts (VITAMIN D)

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2.3.1.1.1.1 Background, Objectives and Procedures

Spaceflight-induced osteopenia (a decrease in bone mineral density), as observed in astronauts and in-flight animals, is a result of decreased bone formation in association with normal or increased bone resorption. The hypothesis of this research project is that the response of bone to a weightless environment is partially osteoblast-mediated and that characterization of functional alterations at the cellular level may help to understand the role of gravity in complex biological processes. As has been shown during previous space flight experiments, osteoblast differentiation and function in vitro is impaired under microgravity. More precisely, gene expression for collagen Iα1, alkaline phosphatase and osteocalcin following treatment with 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and transforming growth factor β (TGFβ) is reduced in human osteoblastic MG-63 cells exposed to microgravity. A plausible explanation for this observation is that the intracellular signalling pathways by which $1,25(OH)_2D_3$ and TGFβ regulate gene expression are altered under microgravity. To test this hypothesis the effect of microgravity on the $1,25(OH)_2D_3$ signalling cascade in osteoblasts was investigated. $1,25(OH)_2D_3$ interacts with the vitamin D receptor (VDR), a ligand-inducible transcription factor that binds as a heterodimer with one of the three retinoid X receptors (RXRs) to vitamin D response elements (VDRE) in the promoter region of target genes, hereby stimulating or suppressing gene transcription. One of the best characterized $1,25(OH)_2D_3$-inducible genes is osteocalcin which is expressed at the onset of mineralization of differentiating osteoblasts.

The present study focuses on one aspect of the $1,25(OH)_2D_3$ signalling pathway, namely the interaction of VDR with its response element and the concurrently induced gene transcription. To this end, mouse osteoblastic MC3T3-E1 cells were stably transfected with a construct containing multiple VDREs of the rat osteocalcin promoter fused to growth hormone as reporter gene (Figure 2-2). Treatment of these cells with $1,25(OH)_2D_3$ resulted in increased expression and release of growth hormone in the culture medium. Growth hormone was chosen as reporter gene as freezing temperature is not required for its stability and it is therefore compatible with space flight conditions.

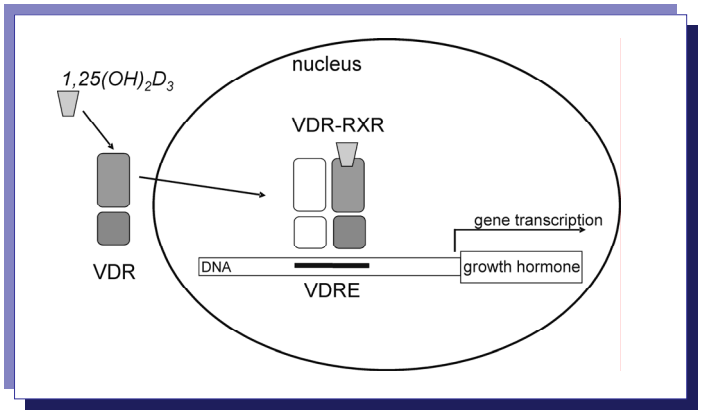

Figure 2-2: Scheme of the 1,25(OH)2D3 signalling pathway investigated during the Odissea Mission

The Vitamin D experiment was carried to and executed on the ISS during the ESA supported Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, within the Aquarius B Transport/Ascent Incubator (CTA-B) together with 2 other experiments, RHO Signalling and Ramiros. The CTA-B is a thermally controlled and isolated container for storage of biological experiments, with a Peltier element actively controlling the temperature of the inner chamber. There are 2 possible temperature settings: 22 °C and 37 °C. The CTA-B contains 3 biology containers (B-container) (Figure 2-3), one for each of the 3 experiments performed using the CTA-B. The B-container is a vacuum tight housing made in aluminium, providing 2 levels of containment for the plunger box units (PBU) and their contents. The B-containers are never opened on-orbit. The 5 Vitamin D PBUs (Figure 2-4) are fully automated and contain 2 culture compartments and six cylindrical storage compartments each (2 of them filled with fixative). Each storage compartment contains a rubber membrane in which a liquid can be stored, as well as a plunger sub-assembly. The biological samples are loaded in the culture compartments, filled with the required culture medium. The liquids in the culture compartment can be exchanged with the liquids in the storage compartments. The transfer of the liquid is performed by means of a plunger, driven by a pre-loaded spring, which is released by a heater-wire system (mini pyro-cutter) according to a pre-programmed experiment activation time. The gas permeable membrane permits the gas exchange between the culture compartments and external ambient (that is the air volume inside the B-container).

Figure 2-3: Biology container (B-Container)

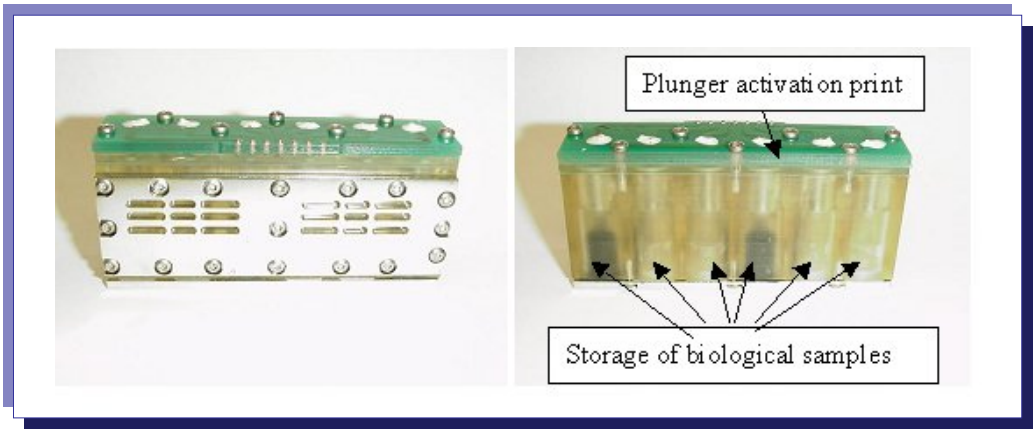

Figure 2-4: Vitamin D plunger box unit (PBU)

The CTA-B was moved from the Soyuz to the ISS after docking (approximately L+2.5 days). Subsequently, the Aquarius B Transport/Return Incubator (CTR-B) was retrieved from the Russian segment of the ISS and installed next to the CTA-B. The CTR-B is a passive version of the Aquarius B Incubator, thermally isolating biological samples that are returned to Earth. The experiment was activated automatically via the electronic control unit (ECU) (Figure 2-5) and required no crew intervention until the completion of the experiment. Five-days after ECU activation, the CTA-B was opened, the VitaminD experiment B-container status was inspected, and the VitaminD experiment B-container removed. The VitaminD container was then attached to the outside of the CTA-B, after closure of the lid, to allow it to cool from 37 °C to ambient temperature. After cooling, the container was placed inside the CTR-B. The fully loaded CTR-B was then transferred to the Soyuz vehicle for its return to Earth.

Figure 2-5: Electronic Control Unit (ECU) with 3 B-containers

2.3.1.1.1.2 Results

Stable transfection of MC3T3 cells and responsiveness to 1,25(OH)$_2$D$_3$
MC3T3-E1 cells were stable transfected with the VDRE-GH construct, cultured in selection medium and one good-responding colony was selected for further experiments. Prior to the space flight experiment, a time and dose response curve for 1,25(OH)$_2$D$_3$ was determined. MC3T3-VDRE cells were treated with vehicle or increasing doses of 1,25(OH)$_2$D$_3$ (10^{-11}M to 10^{-7}M) for 24h, 48h and 72h where after growth hormone levels in the culture medium were measured (Fig. 3). Treatment with vehicle or 10^{-11}M 1,25(OH)$_2$D$_3$ had no effect on growth hormone release. On the other hand, treatment with 1,25(OH)$_2$D$_3$ at 10^{-10}M or higher induced growth hormone release after 24h, with maximum induction already seen at 10^{-9}M 1,25(OH)$_2$D$_3$. An additional increase in growth hormone release after 48h and 72h was only observed for 10^{-10}M 1,25(OH)$_2$D$_3$ (Figure 2-6). From this data it was decided to use an incubation period of 72h with 10^{-9}M 1,25(OH)$_2$D$_3$ as treatment protocol for the flight experiment.

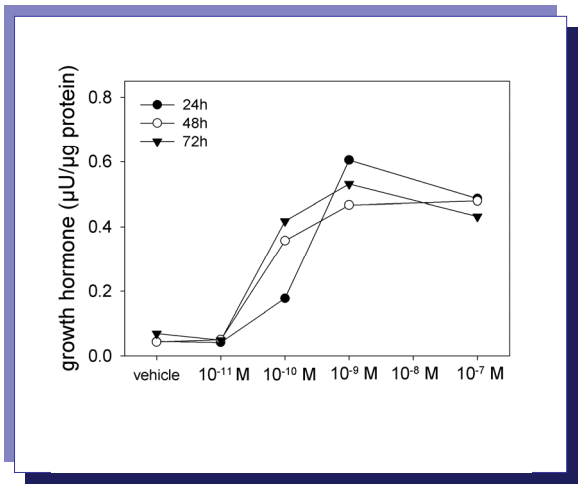

Figure 2-6: Dose response curve of growth hormone release by MC3T3-VDRE cells after 1,25(OH)2D3 treatment.

In addition, whether the cell culture conditions and temperature profile inherent to the space flight experiment altered the response of these cells to $1,25(OH)_2D_3$ treatment, was also tested. MC3T3-VDRE cells were cultured during 5 days at 20°C, after which the temperature was raised to 37°C. Three days later, cells were treated with vehicle or $1,25(OH)_2D_3$ for 72 h, after which cell culture medium was collected and stored for an additional five days at 4°C. As a control, cells were cultured during 5 days at 37°C, after which they were treated with vehicle or $1,25(OH)_2D_3$ for 72h. Cell culture medium was stored at -20°C before analysis. No differences were observed in the responsiveness of the MC3T3-VDRE cells to $1,25(OH)_2D_3$ although the levels of growth hormone release in both vehicle and $1,25(OH)_2D_3$ treated cultures were considerably lower when the temperature profile of the flight was used (Figure 2-7).

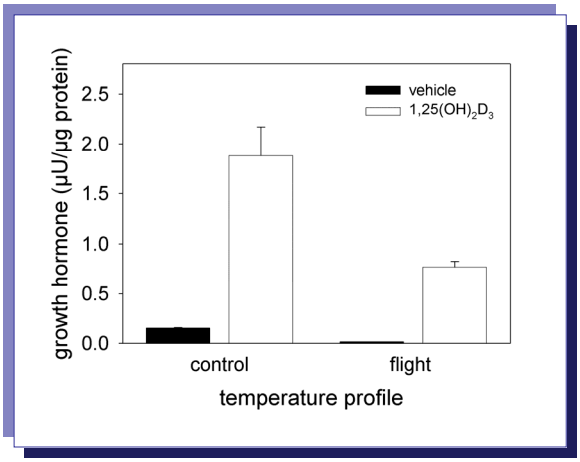

Figure 2-7: Effect of space flight temperature profile on 1,25(OH)2D3 responsiveness

Space flight experiment
During the first days of the Odissea mission, MC3T3-VDRE cells were kept at 22°C until integration of the Aquarius CTA in ISS. Shortly thereafter, the temperature of the B container was increased to 37°C and the medium was changed hereby starting the experiment. After 2 days, the cells were stimulated with 10^{-9}M $1,25(OH)_2D_3$. After a total experiment time of 5 days, cell culture medium was collected and cell cultures were stopped by adding lysis buffer. The transport of the samples to the laboratory and their retrieval was uneventful. The ground control experiment was performed 4 weeks later using the same equipment and mimicking the flight temperature profile. Biochemical analysis showed that the release of growth hormone in the medium of the ground cultures increased nine-fold after $1,25(OH)_2D_3$ treatment (Figure 2-8). The levels of growth hormone in the untreated flight cultures were comparable to their ground correlates and a similar response to $1,25(OH)_2D_3$ treatment was observed (13-fold increase). These data indicate that microgravity for 5 days did not alter the interaction of VDR with the rat osteocalcin VDRE nor the subsequent gene transcription.

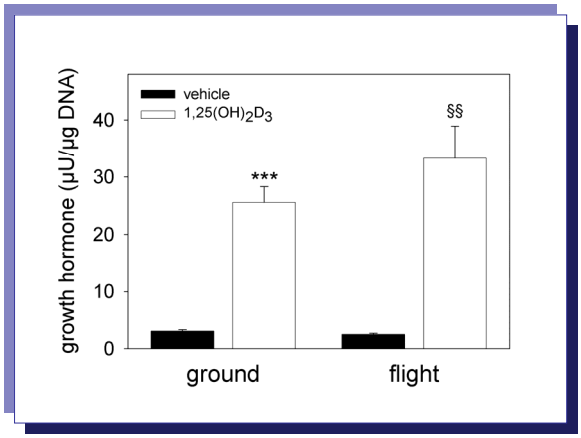

Figure 2-8: Growth hormone release after 1,25(OH)2D3 treatment of MC3T3-VDRE cells cultured during space flight (Odissea mission) or on the ground.

2.3.1.1.1.3 Conclusions and Recommendations

Previous space flight experiments showed that osteoblast differentiation induced by vitamin D and TGFβ treatment is impaired under microgravity. To elucidate whether this decrease is due to altered interaction of the vitamin D receptor with its DNA response element, osteoblastic MC3T3-E1 cells were transfected with a VDRE-reporter construct. Since cell culture and storage conditions are more restricted during space flight than on the ground, cells needed to be stable transfected and the reporter protein had to withstand storage at room temperature. Growth hormone was chosen as reporter as it is secreted in the cell culture medium, its concentration is not affected by storage at room temperature and it could easily be determined by radioimmunoassay (RIA).
After transfection, the selected MC3T3-VDRE cell line showed a time- and dose-dependent increase in growth hormone release following treatment with $1,25(OH)_2D_3$. The growth hormone response of the selected MC3T3-VDRE cell line was not manifestly altered when cells were cultured in the plunger box units and when the temperature profile of the space flight was applied. The preparation and course of both space flight and ground experiment went smoothly.
Five days of microgravity during the Odissea mission did not affect $1,25(OH)_2D_3$-induced release of growth hormone used as reporter. It has however to be remarked that no 1g centrifuge was present during the flight. An in flight 1g experiment remains the ideal control as gravity is at that moment the only parameter differing between the two conditions. These data indicate that several aspects of the $1,25(OH)_2D_3$ signalling cascade are not altered by microgravity, namely: the passage of $1,25(OH)_2D_3$ through the plasma membrane, its binding to the VDR and transport to the nucleus, the binding of the heterodimer VDR-RXR to its response element and the induction of gene transcription. Recently, it has however become evident that chromatin modifications outside the binding sites for transcription factors contribute to gene transcription and this has also been observed for vitamin D regulated gene expression. It therefore remains possible that $1,25(OH)_2D_3$-induced gene transcription is impaired under microgravity due to chromatin modifications.

2.3.1.1.1.4 Publications

1. L. Coenegrachts, I. Stockmans, N. Smets, R. Bouillon, G. Carmeliet, (2005), "VITOS: Characterization of the effects of microgravity on gene expression in osteoblasts: preliminary ground experiment", *Poster presentation at ESA symposium for Life Sciences, 26 june-1 july 2005, Cologne (Germany)*
2. L. Coenegrachts, I. Stockmans, I. Segers, R. Bouillon, G. Carmeliet, (2006), "The effect of microgravity on 1,25-dihydroxyvitamin D_3 signalling in osteoblasts", *Presentation at the Symposium Science on European Soyuz Missions to the ISS (2001-2005), 27-30 june 2006, Toledo (Spain)*
3. L. Coenegrachts, I. Stockmans, I. Segers, R. Bouillon, G. Carmeliet, (2007), "The effect of microgravity on 1,25-dihydroxyvitamin D_3 signalling in osteoblasts", *Microgravity Sciences and Technology (in press)*

2.3.1.1.2 Chromosomal aberrations in blood lymphocytes of astronauts (CHROMOSOME-1)

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2.3.1.1.2.1 Background, Objectives and Procedures

Cosmic radiation is a major risk factor in human space missions. During space flights astronauts are chronically exposed to radiations of solar and galactic origin. The space radiation field consists of electrons, protons, heavy particles, and secondary radiation like bremsstrahlung, neutrons, and charged particles created by interactions of primary radiations with nuclei of spacecraft shielding material or the human body. The contribution of the dose of single radiation types depends on altitude and inclination of the spacecraft, effective shielding thickness and solar activity during the mission. Although it can be assumed that radiation plays a major role in mutation induction in astronauts, synergistic influences such as weightlessness, acceleration, vibration hyperthermia, noise microwave radiation, physical exercises, trauma, and infections cannot be ruled out.

The objective of this experiment was to study chromosomal aberrations in human blood lymphocytes to assess the mutagenic potential of space radiation in man. It is hypothesised that cosmic radiation induces chromosomal aberrations in space flight crew members. This effect should depend on the radiation dose received, e.g. short flight crew members are if at all less affected than long duration crew members. The association of chromosomal aberrations with an enhanced cancer risk stresses the importance of the planned research. The data obtained will be helpful in order to carefully plan space flight missions.

In the CHROMOSOME-1 experiment, 4 astronauts on ESA missions to the ISS were analysed for the induction of chromosomal aberrations during the flight. The flight durations ranged from 10 to 11 days. CHROMOSOME-1 was conducted as part of the experimental package of the ESA supported Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, and the Spanish Soyuz mission, "Cervantes" (ISS 7S mission), which was executed in October 2003, during increments 5 and 8 respectively. CHROMOSOME-1 was also carried out during increments 6-11 with NASA support. For more information about the NASA supported increments regarding CHROMOSOME-1 please go to:
http://exploration.nasa.gov/programs/station/Chromosome.html

To assess the genetic impact of the radiations, 10 ml of blood was drawn a few days before launch and directly after flight (R+1) by venous puncture. Whole blood cultures were set up with phytohemagglutinin to stimulate lymphocytes to enter the cell cycle. Forty-eight hours after start of incubation, chromosome preparations were performed using colchicine to arrest mitotic metaphase stages. Three different staining procedures were performed to assess all types of aberrations induced by ionising radiations:

1. Classical Giemsa block-staining to score dicentric and ring chromosomes, and excess fragments in the whole chromosome set (Figure 2-9):
2. 24 colour fluorescence in-situ hybridisation (FISH) to score reciprocal translocations and complex aberrations involving two or more chromosomes with at least three breaks (Figure 2-10);
3. Multi-colour high resolution banding FISH of chromosome 5 to score for inversions and translocations between homologous chromosomes (Figure 2-11).

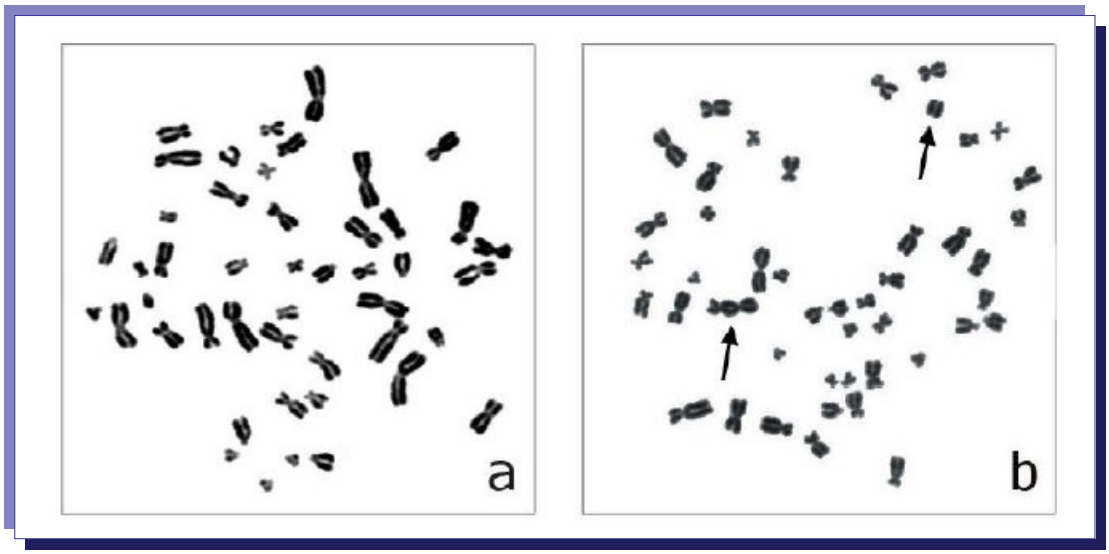

Figure 2-9: A normal set of 46 chromosomes prepared from a cultured human peripheral lymphocyte following Giemsa-staining (a) and a metaphase showing a radiation-induced dicentric chromosome with an associated fragment (b).

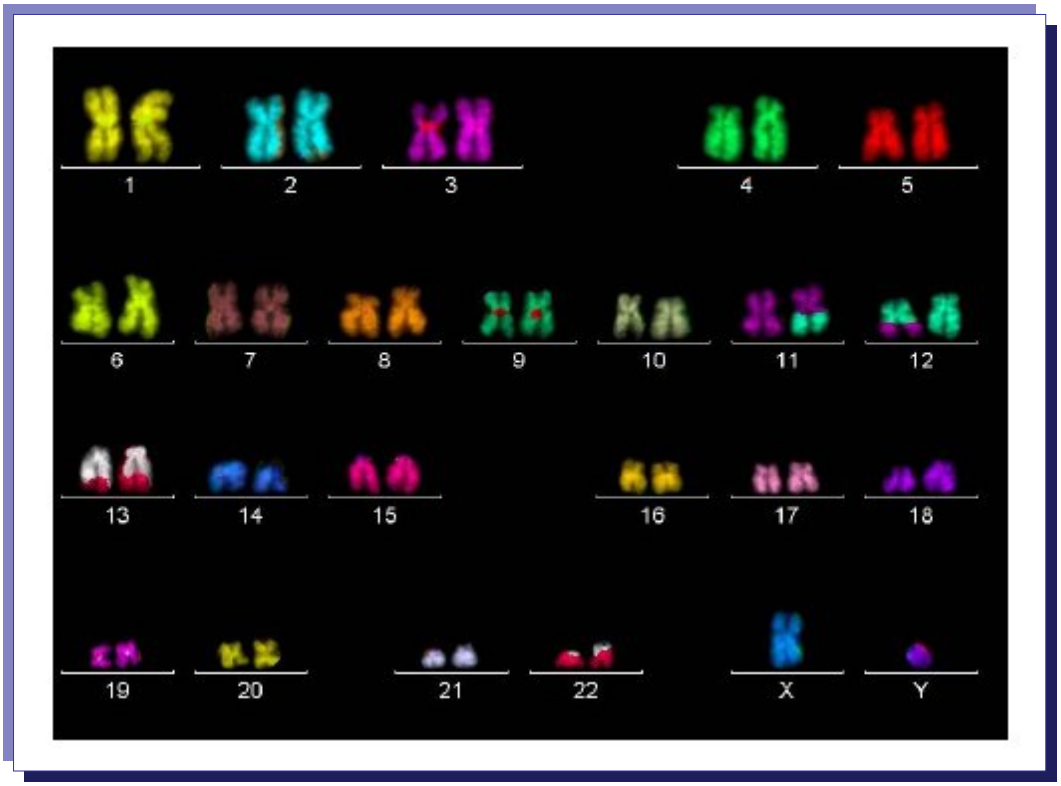

Figure 2-10: Human karyotype following 24 colours in situ hybridisation (mFISH) with a reciprocal translocation between chromosome 11 and 12.

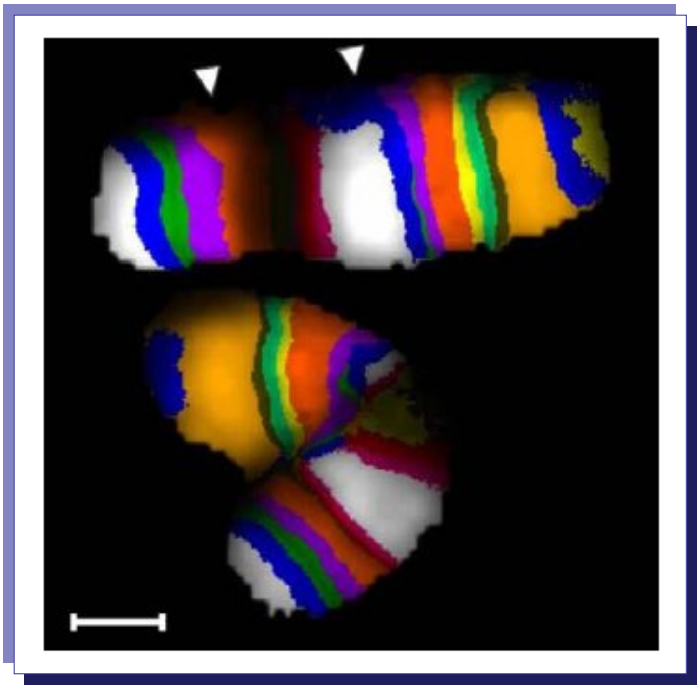

Figure 2-11: Human chromosome 5 following banding FISH (mBAND) showing an inversion in the lower chromosome. The inverted segment is indicated by arrows in the normal upper chromosome 5.

A quantitative comparison between pre- and post flight aberration values will provide information about chromosome breaking effects of cosmic radiation in blood lymphocytes of astronauts correlated with flight duration and extra vehicular activity (EVA) duration. In the ESA missions presented here the mutation rates from 4 short duration mission astronauts were determined. The results from the study were compared with results obtained from crew members of long duration missions.

2.3.1.1.2.2 Results

CHROMOSOME-1 was carried out during both the Belgian Soyuz mission, "Odissea" and the Spanish Soyuz mission, "Cervantes". The lymphocyte cultures from the blood samples of the 4 short duration crew members were grown well and gave good preparations for scoring of chromosomal aberrations.
The analyses of the blood samples of the 4 short duration crew members of the two missions mentioned above revealed no overall increase of aberrations. In Figure 2-12 the mean percentage of aberrant cells before and after flight is shown. For comparison, the mean values of 11 long duration flight crew members are included. While the increase of mutated cells by a factor of 1.4 was significant for the latter group no such increase was found for the 4 short duration subjects.

The detailed results of all assays employed are shown in Table 2-3. Before the flights only chromosomal fragments were detected (all values in control range). After the flight few dicentrics could be observed. No other aberration types which are considered to be radiation induced were found. These results are in contrast with the observations in long duration crew members (data not shown). Following missions of approximately 6-month periods, various types of aberrations increased after return to Earth.

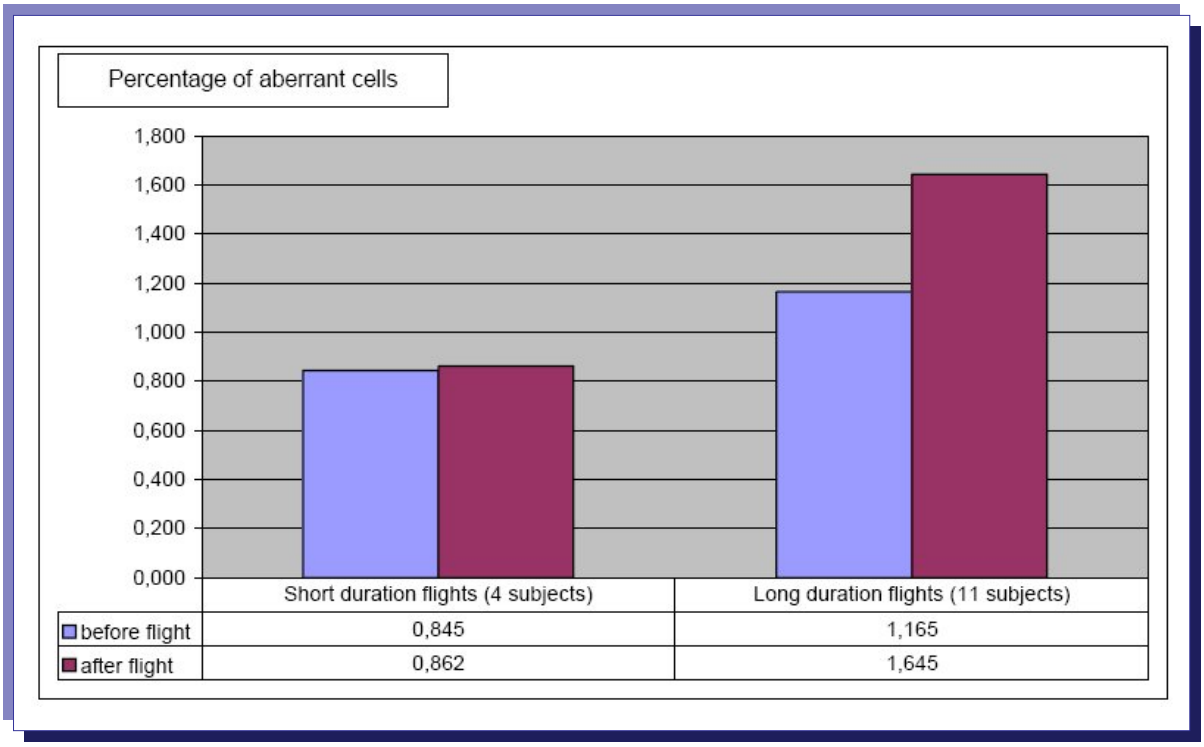

Figure 2-12: Mean values of aberrant metaphases in the 4 ESA mission astronauts and the respective values for the long duration ISS crew members. Data are compiled from all stainings.

Table 2-3: Results of all assays employed before and after flight.

BEFORE FLIGHT					
Astronaut Code Number	**Total number of metaphases analysed (all assays)**	**Dicentric chromosomes (all assays)**	**Fragments (all assays)**	**Reciprocal translocations (mFISH/mBAND)**	**Intrachromosomal aberrations (mBAND)**
0	773	0	7	0	n.d.
1	1928	0	5	0	n.d.
2	2543	0	5	0	n.d.
3	2419	0	9	0	0
Total	**7663**	**0**	**26**	**0**	**0**
AFTER FLIGHT					
Astronaut Code Number	**Total number of metaphases analysed (all assays)**	**Dicentric chromosomes (all assays)**	**Fragments (all assays)**	**Reciprocal translocations (mFISH/mBAND)**	**Intrachromosomal aberrations (mBAND)**
0	952	1	0	0	n.d.
1	1842	0	5	0	n.d.
2	1181	1	5	0	n.d.
3	1244	2	7	0	0
Total	**5219**	**4**	**17**	**0**	**0**

2.3.1.1.2.3 Conclusions and Recommendations

The compilation of results obtained in the ESA missions (and future short duration Soyuz missions) and of long term missions is expected to provide almost complete information about chromosomal aberrations in peripheral lymphocytes of crew members exposed to space radiation. The results will enable a better assessment of the genetic risk of humans in space and in consequence, will help to optimise radiation shielding. The data will also allow calculations of aberration frequencies expected during deep-space missions.

2.3.1.1.2.4 Publications

1. M. Horstmann, M. Durante, C. Johannes, R. Pieper, G. Obe, (2005), "Space radiation does not induce a significant increase of intrachromosomal exchanges in astronauts' lymphocytes", *Radiat Environ Biophys Vol. 44, Issue 3*, pp. 219-224
2. M. Horstmann, M. Durante, C. Johannes, G. Obe, (2005), "Chromosomal intrachanges induced by swift iron ions", *Adv Space Res Vol. 35, Issue 2*, pp. 276-279
3. M. Horstmann, M. Durante, G. Obe, (2004), "Distribution of breakpoints and fragment sizes in human chromosome 5 after heavy-ion bombardment", *Int J Radiat Biol Vol. 80, Issue 6*, pp. 437-443
4. M. Durante, K. Ando, Y. Furusawa, G. Obe, K. George, F.A. Cucinotta, (2004), "Complex chromosomal rearrangements induced in vivo by heavy ions", *Cytogenet Genome Res Vol. 104, Issue 1-4*, pp. 240-244
5. C. Johannes, M. Horstmann, M. Durante, I. Chudoba, G. Obe, (2004), "Chromosome intrachanges and interchanges detected by multicolour banding in lymphocytes: Searching for clastogen signatures in the human genome", *Radiat Res Vol. 161, Issue 5*, pp. 540-548
6. M. Durante, G. Snigiryova, E. Akaeva, A. Bogomazova, S. Druzhinin, B. Fedorenko, O. Greco, N. Novitskaya, A. Rubanovich, V. Shevchenko, U. von Recklinghausen, G. Obe, (2003), "Chromosome aberration dosimetry in cosmonauts after single or multiple space flights", *Cytogenet Genome Res Vol. 103, Issue 1-2*, pp. 40-46
7. O. Greco, M. Durante, G. Gialanella, G. Grossi, M. Pugliese, P. Scampoli, G. Snigiryova, G. Obe, (2003), "Biological dosimetry in Russian and Italian astronauts", *Adv Space Res Vol. 31, Issue 6*, pp. 1495-1503

2.3.1.1.3 Cosmic radiation and microgravity related oxidative stress (RAMIROS)

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2.3.1.1.3.1 Background, Objectives and Procedures

The main objectives of this experiment were to refine the knowledge about the space radiation environment on mammalian cells and to better understand fundamental biological processes, most importantly the occurrence of different DNA lesions (hallmarks of cancer and ageing), in relation with the particular conditions of HZE radiation: long duration space flights, and heavy particle radiation therapy.

The RAMIROS experiment was carried to and executed on the ISS during the ESA supported Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, within the Aquarius B Transport/Ascent Incubator (CTA-B) (Figure 2-13, Figure 2-14), together with the experiments Vitamin D and Rho Signalling. The CTA-B is a thermally controlled and isolated container for storage of biological experiments, with a Peltier element actively controlling the temperature of the inner chamber. There are 2 possible temperature settings: 22°C and 37°C. The CTA-B contains 3 biology containers (B-container) (Figure 2-3), one for each of the 3 experiments performed using the CTA-B. The B-container is a vacuum tight housing made in aluminium, providing 2 levels of containment for the plunger box units (PBU) and their contents. The B-containers are never opened on-orbit.
The 5 RAMIROS PBUs (Figure 2-15) are based on the design of the Vitamin D PBU (see Vitamin D experiment). The main modification is the additional dry compartment with a dosimeter detector stack. The detector stack is located on the position that originally was occupied by three fluid storage bags. The cell culture is fixed on a membrane in the wet culture compartment. During flight, the medium was refreshed by activation of the plunger medium. At the end of the experiment, some fixative was added by activation of the fixative plunger. At the same time, the detector stack in the dry compartment was shifted partially by the shift mechanism (third plunger).

After docking of the Soyuz vehicle to the ISS at L+2.5 days, the CTA-B was transferred from the Soyuz to the ISS. The Aquarius B Transport/Return Incubator (CTR-B) was then retrieved from the Russian segment of the ISS and installed next to the CTA-B. The CTR-B is a passive version of the Aquarius B Incubator, thermally isolating biological samples that are returned to Earth. The experiment was activated automatically via the electronic control unit (ECU) (Figure 2-5) and required no crew intervention until the completion of the experiment.

Five-days after ECU activation, the CTA-B was opened, the RAMIROS experiment B-container status was inspected and then removed. The container was then attached to the outside of the CTA-B, after closure of the lid, to allow it to cool from 37°C to ambient temperature. After cooling, the container was placed inside the CTR-B. The fully loaded CTR-B was then transferred to the Soyuz for return back to Earth.

Figure 2-13: Aquarius B Transport-Ascent Incubator (CTA-B)

Figure 2-14: Internal view of CTA-B with B-containers

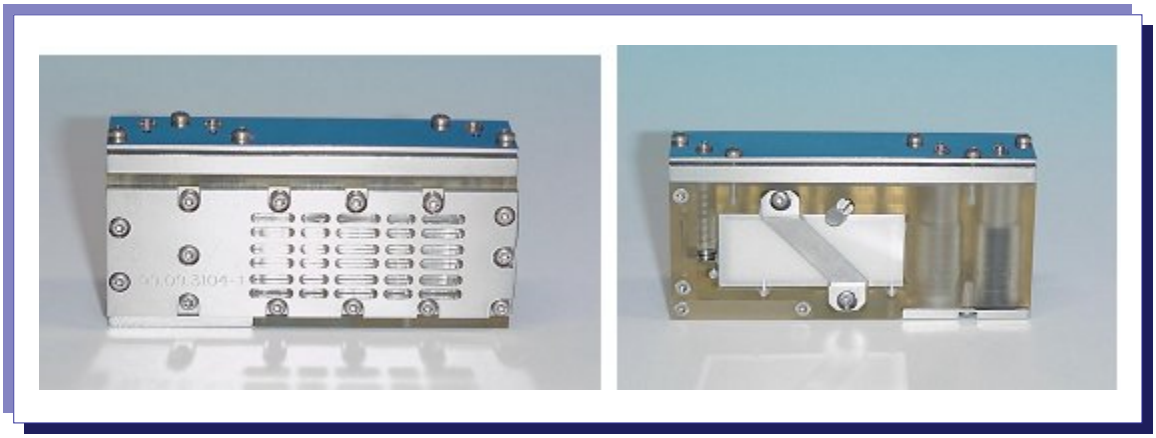

Figure 2-15: RAMIROS Plunger Box Unit (PBU)

2.3.1.1.3.2 Results

Not available.

2.3.1.1.3.3 Conclusions and Recommendations

Not available.

2.3.1.1.3.4 Publications

Not available.

2.3.1.2 Physiology: Integrative gravitational physiology

2.3.1.2.1 Directed attention brain potentials in virtual 3-D space in weightlessness (NeuroCOG)

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2.3.1.2.1.1 Background, Objectives and Procedures

The NeuroCOG experiment was designed to further investigate modifications in the perception of whole-body motion in space found during the French Soyuz Mission, "Andromede", in October 2001. The NeuroCOG experiment went a step further in understanding the neural mechanisms underlying the perceptual processes by combining the psychophysical experiments with measurements of visually-evoked EEG potentials.

The human being in his natural environment moves, because of the constraints of gravity, over a relatively flat two-dimensional surface. During Earth-bound navigation, only yaw rotations are typically used when moving from one place to another. Even when moving through three-dimensional structures, human beings tend to remain upright with respect to gravity. In weightlessness, astronauts can translate and rotate in any direction, thus their trajectory is no longer ascribed to two-dimensional surfaces. In contrast with Earth-bound navigation, astronauts can freely use pitch and roll rotations when moving through three-dimensional space. The semi-circular canals measure relative rotations around all three axes (roll, pitch and yaw). This provides relative information about the amplitude of a rotation, but does not provide absolute information about orientation. The otoliths and other graviceptor cues (tactile sensors, proprioception, etc.) can potentially indicate the absolute orientation of the head and body with respect to the vertical axis. Neural processes that allow us to perceive, interact and navigate within this world may thus be specialised for the internal representation of spatial relationships with respect to gravity.

The novel conditions of microgravity might therefore place an increased load on the cognitive capacity of the human brain because sensory signals must be processed and interpreted in a new context. By placing electrodes on the scalp of a human subject one can get a glimpse at the electrical activity underlying perceptual processes in the brain. Through the analysis of the variations in electrical potential between different locations on the scalp, one can make inferences about various neural processes such as the sensitivity to sensory information, the attention state of the system and the decision making process.
This project studies how the brain functions with respect to gravity through the use of these techniques known as electroencephalography or EEG. In this experiment the role of gravity in the perception of self-motion is tested. In a series of psychophysical tests, a comparison is made on how human subjects interpret visual-flow information

both on the ground and in the weightless conditions of orbital flight. Also, evoked potentials through surface electrodes applied to the scalp are measured in order to determine the spatial and temporal components of information processing in the brain in the absence of gravity. Through these experiments observations were made on how the CNS (Central Nervous System) adapts from its habitual environment in which gravity plays an ever-present and dominant role, to a novel environment in which the movements of our bodies no longer adhere to the constraints imposed by gravity.

The hypothesis is that gravity should influence the perception of pitch but not yaw turns. Performing perception tasks in 0g should evoke different cognitive responses and should activate different cortical circuits, depending on whether the information to be interpreted by the subject involved turns around a pitch or yaw axis.

Subjects performed a set of 2 psychophysical tasks with simultaneous recording of EEG activity. For each subject, the performance of these tasks was compared to a set of pre-flight, in-flight and post-flight procedures to test for an effect of weightlessness on the visual perception of orientation and movement and on the ability to navigate in three dimensions. Backup crewmembers were asked to perform all pre-flight training and baseline data collection (BDC) tests and were asked to work in parallel with the orbital crew during and after the flight to provide a matched control group for comparison.
Subjects take up the position and postural support depending on the gravitational conditions (ground or in-flight) and on the instructions for a particular protocol:

1. *Ground Seated*: The subject sits upright in a chair, with the elbows resting on adjustable-height elbow supports of the ground support stand. The ground support stand is adjusted to position the mask/tunnel/laptop at the level of the eyes for viewing. The height of the elbow pads is adjusted to allow the subject to comfortably grasp the grips on the laptop support.
2. *In-flight Restrained*: The subject sits in front of the laptop, which is attached to a mechanical support. Waist and foot straps are used to hold the subject securely in a seated posture.
3. *In-flight Free floating*: The subject adopts a free-floating posture and has no rigid contact with the Station structure during the performance of the experiment in this mode. A second cosmonaut assists the subject to stabilise his/her posture at the beginning of this phase of the experiment.

In all cases, the subject places his/her face into the facemask and attaches an elastic band behind the head to hold the head in place. By manipulating the buttons and trackball, the subject launches the experiment program on the laptop, identifying him/herself to the program and performs a set of experimental trials consisting of the following:

- FO1: Virtual Turns – The subject is situated in a visually-presented 3-D virtual tunnel. On the press of a button, the subject appears to either move through a tunnel at constant speed, passing through a single bend between two linear segments or the subject appears to undergo a rotation in place (no apparent translation). At the end of the trial, the subject indicates the extent of the turn (i.e. how many degrees) in one of two ways:
    1. The subject observes a bird's eye view of a planar workspace with two cylindrical tunnels connected by a variable angle. By manipulating the trackball, the subject adjusts the magnitude of the turn to reconstruct a planar representation of the virtual tunnel just experienced.
    2. The subject sees a pictogram indicating his/her starting orientation in the plane. By manipulating the trackball, the subject changes the orientation of the pictogram to indicate the amount of rotation that is perceived.
- EEG Recordings – EEG signals from 14 locations on the scalp are recorded during the above trials. The subject performs a total of 48 trials for either stimulus type, for a total of 96 trials per session. Trials are broken into blocks of 12 trials each, with pauses programmed between blocks. At a nominal rate of 4-5 trials per minute (including pauses), one complete execution of this protocol (turning in-place or passage through the tunnels) is performed in 20-25 minutes. EEG is also recorded under four control conditions:
    1. The subject relaxes and does nothing, first with his/her eyes closed, then while looking at a neutral screen.
    2. An alternating checkerboard is presented to the subject on the screen, with the colours switching between black and white every 3 seconds.
    3. The subject follows the movement of a luminous spot as it makes a sinusoidal movement across the screen.
    4. Subjects blink their eyes in synchrony with an audible metronome. Control recordings last no more than 5 minutes.

2.3.1.2.1.2 Results

After subjects emerged from the end of the tunnel, they were asked to report the perceived turn angle by adjusting a visual indicator with a trackball. Figure 2-16 shows the difference between left and right turns (left–right) and between upward and downward turns (downward–upward) as a measure of this asymmetry. On Earth, yaw turns led to equal, symmetrical errors in the estimation of the perceived angle change, but the estimation of pitch turns was greater for forward (nose-down) versus backward (nose-up) turns. The interest of this experiment lies in this asymmetry. One can observe a clear reduction in the asymmetry of vertical turns in 0g. In summary, it appears that the microgravity conditions of orbit reduce the asymmetry of vertical turn estimation, but only in the free-floating condition. The NeuroCOG experiment revealed interesting EEG correlations of these effects observed via psychophysics. Alpha rhythms were analysed in response to a standard alternating checkerboard pattern (visual evoked potential, VEP) and in response to the initial presentation of the virtual 3D tunnel (event related potentials, ERP). It was demonstrated for the first time that the VEP responses are conserved in the absence of gravity and that the phase locking of alpha rhythms is preserved in the ISS environment. In contrast, the ERP evoked by the presentation of the tunnel was dramatically perturbed in the ISS. Unspecific factors such as a noisy environment in the ISS, anxiety, stress, muscular artefacts and basic physiological factors (brain and body blood circulation differences) seem to be unlikely culprits because the classical VEP in response to the reversing checkerboard pattern is maintained. This latter phenomenon occurs through the conservation of the phase locking mechanism of the VEP in the alpha band frequency, as seen on the ground. These results (see Figure 2-17) can be interpreted in light of the specific informational content of the different visual stimuli (checkerboard vs. tunnel) that the associated task demands. The major difference between the classical checkerboard testing and the virtual tunnel task is that in the former situation the subject was mainly passive (only looking at the computer screen) while in the latter situation the subject was involved in a 3D spatial perception task. This task directly contained directional information related to the gravitational frame of reference, which may play the role of a top-down control. In the presence of gravity, this neural context implicitly contributed to the evoked response. Thus a change of perceptual context or a basic interference in the dynamics of the neural networks could be expected, resulting in the different patterns in EEG measurements between the 2 tasks.

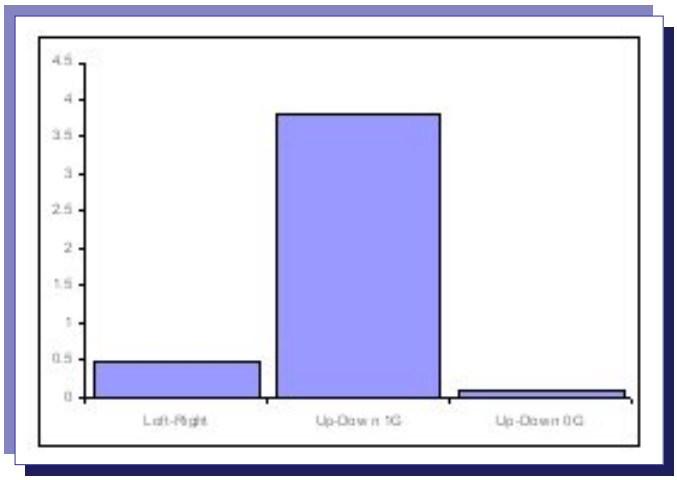

Figure 2-16: Asymmetry in the estimation of turn angles for virtual rotations around horizontal and vertical axes

In the NeuroCOG experiment before the navigation task, the arrest reaction of the alpha rhythm was used (Berger, 1929) because it is a highly stable reaction, which occurs over a large part of the brain and provides two distinct physiological states induced by opening or closing the eyes. The head figurines of Figure 2-18 illustrate the difference in the power gain of 10 Hz activity between the recordings performed in the ISS and on Earth (with data recorded before and after flight pooled together) for cosmonauts (A) and for control subjects (B). Statistical analysis revealed that the gain values recorded in parieto-occipital (O1, O2, Pz, P3, P4) and central (C3, C4, Cz) loci were significantly increased in weightlessness. The three latter electrodes are situated over the sensorimotor cortex, which is the site of the mu rhythm. In contrast, the 10 Hz gain value of the frontal recordings (Fz, F3, F4, F7, F8) remained unchanged in the absence of gravity. The same analysis performed in the control subjects showed a great stability of the gain value throughout the same period of time in all recorded channels. The

findings demonstrate that the power of the spontaneous EEG alpha rhythm recorded in the parieto-occipital regions and in the sensorimotor areas (mu rhythm) are increased and that the spectral perturbations of these rhythmic activities produced by eye-opening/closure state transition, increase in the absence of gravity. This demonstrates the influence of the absence of gravity on alpha oscillation, which is likely to be linked to the gating of sensory input. Alpha and mu rhythms may also participate in memory and cognitive processing. In this context, the finding of enhanced alpha and mu rhythm in weightlessness supports their physiological implication in the gain-field mechanism allowing the adaptation of the neural representation of space.

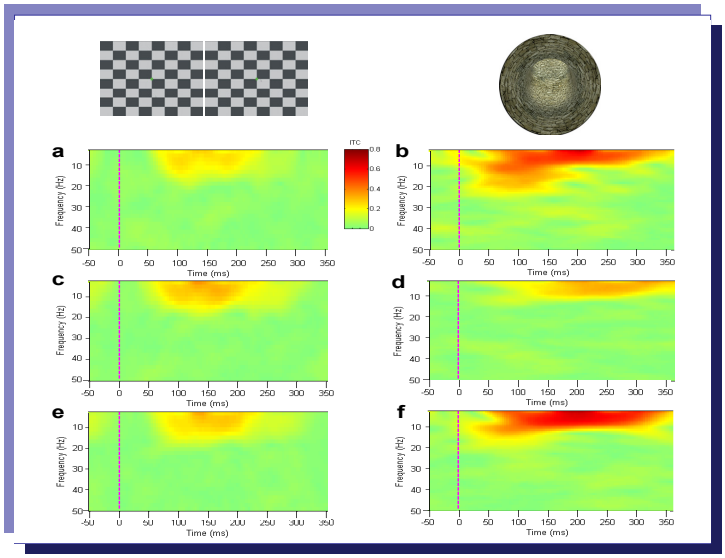

Figure 2-17: Inter-trials coherence of theta and alpha rhythms in response to a standard checkerboard pattern (a, c, e) and to the presentation of a curved tunnel (b, d ,f) on the ground before flight (a, b) in flight (c,d) and on the ground after flight (e, f)

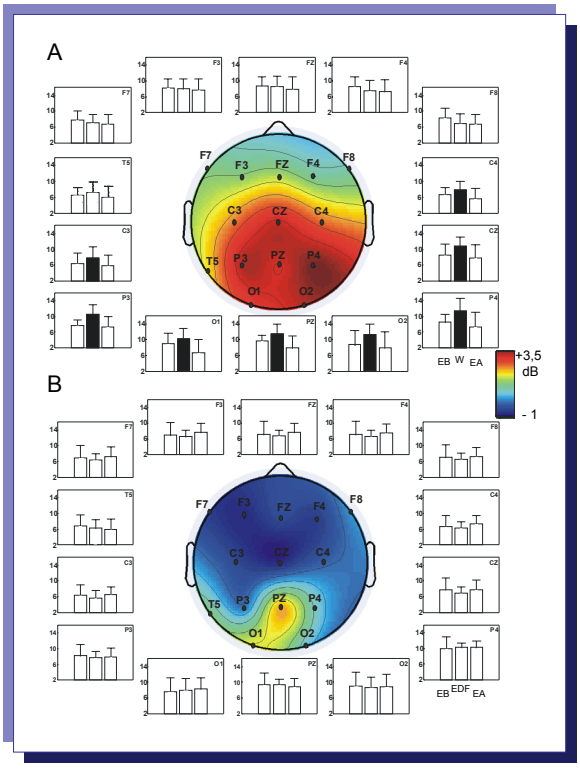

Figure 2-18: Difference in the power gain of 10 Hz activity between the recordings performed in the ISS and on Earth

Motion-onset related visual evoked potentials (M-VEPs) were recorded at a latency of ~200 ms (N200) when the first in depth motion appeared during the virtual navigation. The N200 was supported by a very significant phase locking in the theta range oscillation. It was shown for the first time that this N200 and the related phase locking in theta oscillation are suppressed during the first days in weightlessness and that this effect is reinforced in free-floating condition. Interestingly, this M-VEP reappeared with the time passed in weightlessness and will be carefully followed in the long-term ISS missions.

2.3.1.2.1.3 Conclusions and Recommendations

Three main conclusions can be made from the results obtained:

1. Weightlessness specifically affects visual evoked potential related to the presentation of a virtual 3-D navigation tunnel.
2. Weightlessness increases alpha rhythm gain during transition between eyes-closed and eyes-open states.
3. Moving in virtual navigation induced midfrontal N200 event related potentials supported by a transient theta ringing altered in weightlessness.

In any given EEG recording session a complete loss of signal (flat line) was sometimes observed on one or more of the 14 EEG channels. This loss of signal is often accompanied by a zero impedance level during the impedance check prior to the start of recording. This may be due to changing characteristics of the conductive cream with time, differences in environmental conditions between ground and flight (humidity, temperature, etc.) or due to the application of a greater quantity of cream during in-flight sessions than on the ground. Post-flight debriefing with the cosmonaut suggested that the latter may have been the case.
Future experiments using EEG should provide real-time or quasi-real-time monitoring of EEG signals on the ground. Downlink should be timely enough to allow for the correction of problems during the course of a data collection session, or at least soon enough to allow for the repetition of a session in the case of data loss.
The assurance of adequate training time should be a critical factor in the planning of future experiments.

2.3.1.2.1.4 Publications

1. M. Lipshits, A. Bengoetxea, G. Cheron, J. McIntyre, (2005), "Two reference frames for visual perception in two gravity conditions", *Perception*, *Vol. 34, Issue 5*, pp.545-555
2. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, L. Servais, B. Dan, A. Berthoz, J. McIntyre, (2006), "Effect of gravity on human spontaneous 10-Hz electroencephalographic oscillations during the arrest reaction", *Brain Research, Vol. 1121* , pp. 104-116
3. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, J. McIntyre, (2006), "Les neurosciences spatiales : l'électroencéphalographie dans la navigation virtuelle", *Sciences Connection*, pp. 25-29
4. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, A. Berthoz, J. McIntyre, (2005), "Alteration of the visual evoked potentials related to the presentation of a virtual 3D tunnel in weightlessness", *Life in Space for life on Earth (Cologne2005),* pp. 213-214
5. J. McIntyre, G. Cheron, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, A. Berthoz, M. Vidal, D. Chaput, E. Lorigny, (2005), "Effects of gravity on perceiving the angle of turns during virtual movement", *Life in Space for life on Earth (Cologne2005),* pp. 236
6. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, J. McIntyre, (2005), "The effects of gravity on human alpha rhythm during the transition between eye-closed and eye-opened state", *15th Humans in Space Symposium, Benefits of human presence in space. I. 4.1,* pp. 39
7. A. Bengoetxea, A. Cebolla, C. De Saedeleer, A. Leroy, A. Berthoz, J. McIntyre, G. Cheron, (2006), "Weightlessness effects on visual evoked potential related to virtual in-depth motion", *Proceedings of the Science on European Soyuz Missions to the International Space Station (Toledo, Spain),* pp. 63
8. A. Cebolla, C. De Saedeleer, A. Bengoetxea, A. Leroy, A. Berthoz, J. McIntyre, G. Cheron, (2006), "Microgravity specifically affects visual evoked potential related to a virtual 3D navigation tunnel", *Proceedings of the Science on European Soyuz Missions to the International Space Station (Toledo, Spain),* pp. 64

2.3.2 Physical Sciences

2.3.2.1 Fluid Physics: Combustion

2.3.2.1.1 Combustion synthesis under microgravity conditions (COSMIC)

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2.3.2.1.1.1 Background, Objectives and Procedures

Self-propagating high-temperature synthesis (SHS) occurs when a reaction, once initiated by an external energy source, is able to propagate through the sample as a combustion wave without any further supply of energy. The interest devoted to SHS is due to its simplicity, short reaction time, easy-to-build equipment, low-energy

consumption and ability to produce advanced materials such as ceramics, intermetallics, composites, graded materials, etc.

Many parameters influence the combustion synthesis process and thus the microstructure and finally the properties of the product. This project is dedicated to the investigation of the influence of gravity on the relationship between the general physico-chemical mechanisms of the combustion process (SHS) and the formation of the microstructure and product composition of an intermetallic matrix composite (IMCs) based on the Al-Ti-B system. The initial powder mixtures are chosen such that two types of matrices are studied (TiAl and $TiAl_3$) as well as different amounts of the particular reinforcing phase TiB_2, these phases being formed during the combustion process.

To study the influence of gravity, an especially dedicated reactor ensemble was designed and used in the Microgravity Science Glovebox (MSG) on board the ISS. The following geometry (see Figure 2-19) was used for combustion synthesis on the ISS: a free standing cylindrical pellet (16 mm diameter and 20 mm high) combined with a conical part inserted in the cavity of a cylindrical copper block. The conical part, 40 mm high, is used to quench the combustion front during its propagation in order to study the mechanism of the microstructure formation sequence. This technique (combustion front quenching (CFQ) technique) is based on the rapid extinction of the reaction front. Heat removal is favoured by conductive heat losses in the copper block, while heat production decreases as the front proceeds in the conical region due to shrinkage of the cross-section. Consequently the combustion front ceases to advance so that the original material, the intermediate and the final product are frozen and the evolution of the structure during preheating, combustion and post-combustion can be investigated.

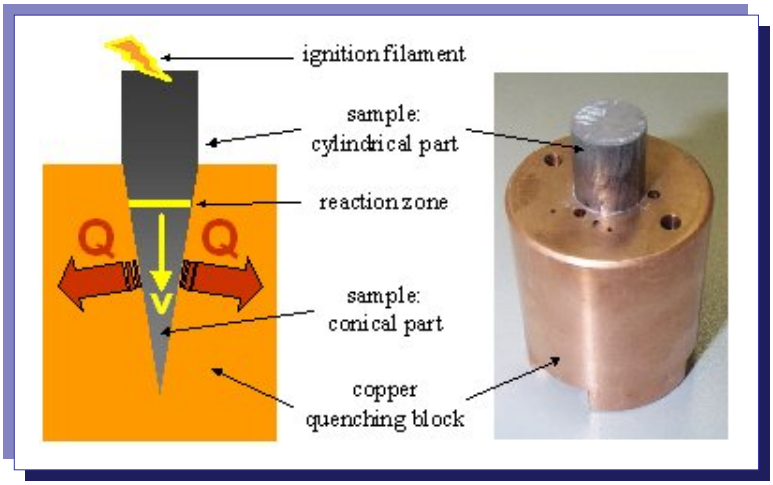

Figure 2-19: Sample geometry (cylinder coupled with a quenching cone)

The experiments conducted on board ISS used samples that were die compacted to similar green density of about 65% of the theoretical value (%TD). The set-up consisted of 6 individual combustion reactors (Figure 2-20) containing the compacted pellets inserted into an outer container (Figure 2-21).

Each individual reactor contained a sample inserted in a copper block, an electrically heated Philips tungsten ignition coil and a tungsten heat reflector sheet, used to provide the heat needed for igniting the sample. Six W-Re C-type thermocouples placed into the sample were used to measure the temperature evolution during the experiment and estimate the reaction front velocity. For direct experiment observation, each reactor contained an optical window located at the side of the reactor in order to follow and record the combustion process of the sample using the MSG video camera. Inside the reactor, a halogen lamp served as the light source for the visual inspection of the sample integrity before ignition. Each reactor contained an AISI 303 stainless steel filling valve and a pressure sensor that were used for flushing and filling each reactor with argon (experiment atmosphere) and for controlling and measuring the pressure inside the reactor respectively. For safety reasons, the outer surface temperature of each sample reactor container was measured using a thermocouple. If the temperature reached a value higher than 200 °C, the MSG would switch off automatically thus preventing overheating of the system and ensuring the crew's safety.

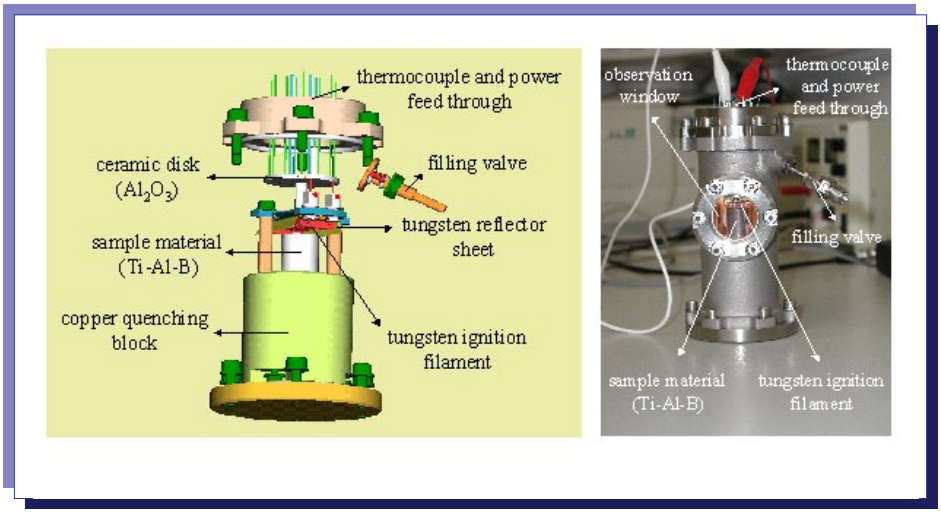

Figure 2-20: (Left) Inner view of an individual COSMIC reactor showing the main constitutive elements; (Right) External view of a single COSMIC reactor

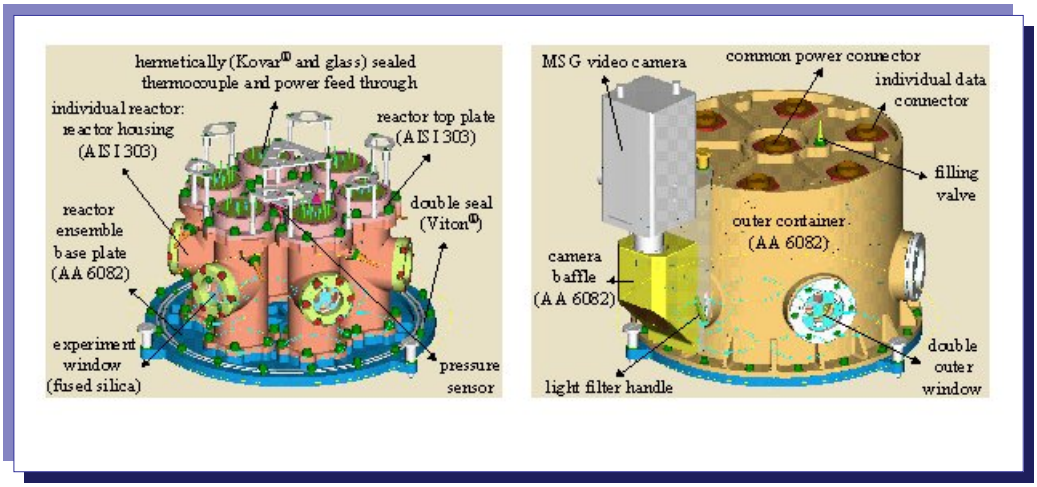

Figure 2-21: COSMIC reactor ensemble (Left) Inner view; (Right) Outer view

2.3.2.1.1.2 Results

During all experiments the self propagating reaction occurred. Typical thermocouple profiles were obtained (Figure 2-22) and allowed for the estimation of the speed of the reaction front. Knowing the position of the thermocouples inside the sample and the time at which the reaction front reached them (sharp increase of the temperature) allowed for the calculation of the velocity of the reaction front (Figure 2-23). The jump in the thermocouples at around 15s is due to an electrical artefact induced by the shut down of the current in the ignition coil. The decrease of the reaction front velocity and the penetration depth (quenching distance inside the copper block) when the amount of TiAl matrix increases is due to the decrease of the overall exothermicity of the initial mixture. This is estimated theoretically by considering the heat of formation of the different phases, the geometry and the green density of the sample for the various mixtures stoichiometry considered.

A detailed microstructural study of the samples obtained in microgravity and the corresponding ones on Earth was conducted. In order to have a synthetic view, the results obtained for only two different matrix compositions will be discussed. Observations have shown three different scales of microstructure heterogeneity: the pores correspond to a scale of 1 mm, the "white" areas to a scale of 100 µm and the reinforcement particles to a 1 µm scale. For both types of matrices and for both experimental conditions (µg and 1g), the porosity size distribution (Figure 2-24) is quite large ranging from around 10 µm up to 1 mm. The morphology of the porosity is influenced

by the composition of the matrix. The samples with a TiAl matrix (Figure 2-24 (a) and (c)) exhibit a more rounded porosity compared to the two other samples having an Al_3Ti matrix (Figure 2-24 (b) and (d)). The influence of gravity on the porosity size and morphology seems to be very difficult to characterise.

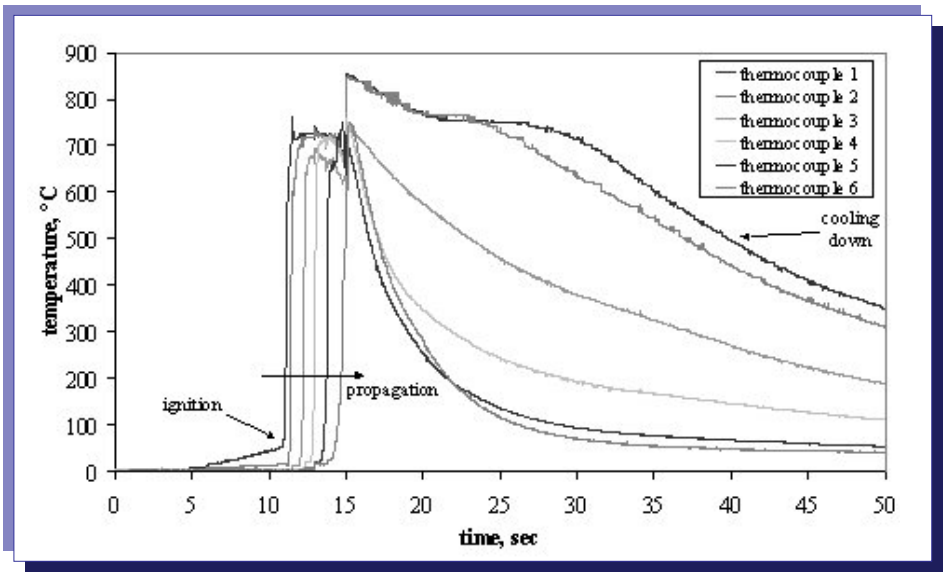

Figure 2-22: Real-time thermocouple data (temperature-time profile) from reactor CSM-ISS-06 (TiAl3/TiB2)

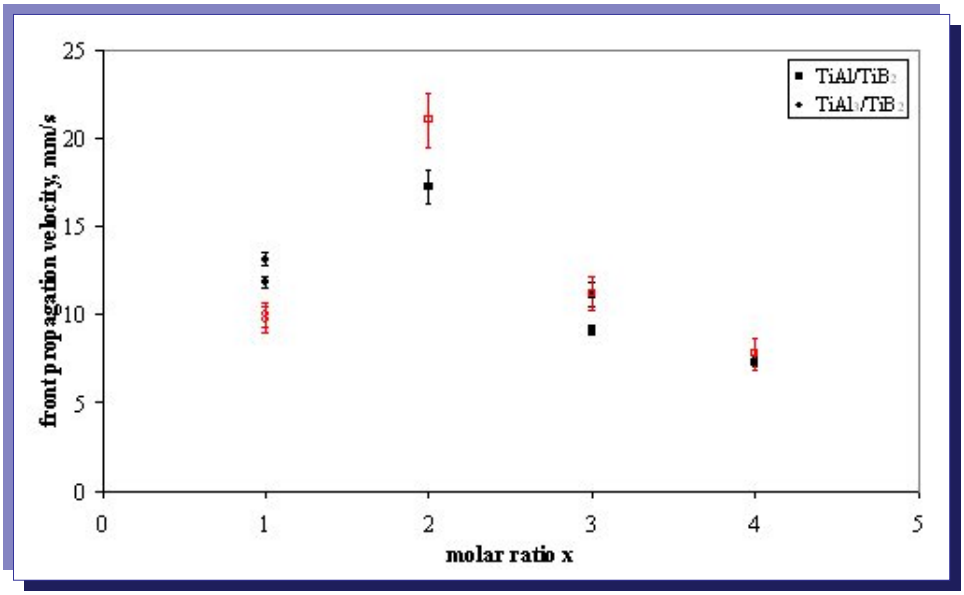

Figure 2-23: Average front propagation velocity in the cylindrical pellet between thermocouple 1 and thermocouple 3 as function of the molar ratio x

Quantitative image analysis has also been used to characterise the proportion of the boride reinforcing phase for the Al_3Ti matrix. This analysis shows that the proportion of borides remains constant along the axis of the cylindrical part, i.e. as the distance from the initial top plane increases, for experiments conducted in both gravity conditions. This indicates that no long distance sedimentation of the borides occurs, contrary to what could be expected especially under normal gravity conditions. This could be due to the relative high amount of the particle phases inducing a geometrical interlocking of the particles hindering their motion. Similar results are obtained by a qualitative analysis of the samples with a TiAl intermetallic matrix. For both compositions of the composite, it was difficult to show a significant influence of the gravity level on the microstructural features studied (boride

amount, morphology and size, matrix amount and morphology, pore amount and morphology) along the axis of the cylindrical part in the steady state zone. However, a preliminary characterization of the microstructure near and at the surface of the cylindrical part of the sample has shown the "unexpected" presence of a discontinuous layer of phase Ti_3Al, where thickness depends significantly on the gravity level.

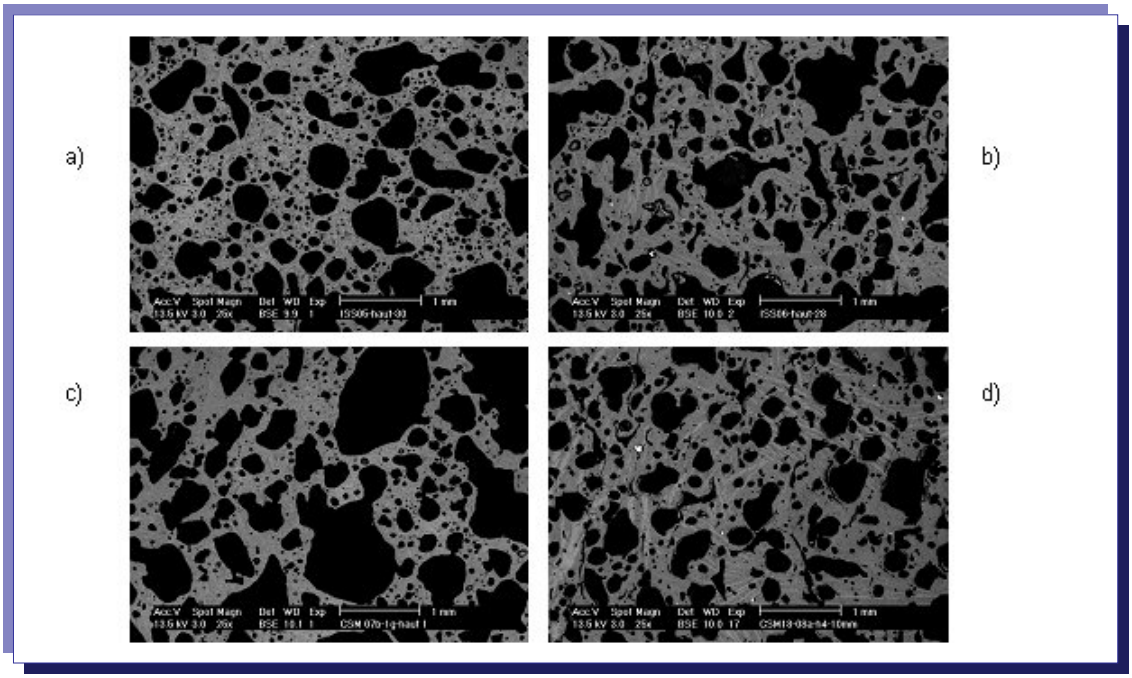

Figure 2-24: Size and morphology of the porosity at 10 mm from the initial top plane on the axis of the cylindrical part (microgravity samples (a) and (b); ground samples (c) and (d))

2.3.2.1.1.3 Conclusions and Recommendations

The comparative analysis performed between samples processed in reduced gravity and normal gravity shows that there is only a limited influence on microstructural features such as boride content, morphology and size, matrix amount and morphology, and porosity. The results of the present experiments combined with the findings of previous low gravity experiments (parabolic flights, sounding rockets, Space Station) show that the SHS process still needs more research for a better understanding of the combined effect of several interacting parameters on one hand and physical and chemical mechanisms on the other. More low gravity experiments will complement the insight in the complex and dynamic process of combustion synthesis of materials. The typical required microgravity time per experiment is in the order of minutes, offering the possibility to use sounding rockets.

2.3.2.1.1.4 Publications

1. R. Licheri, R. Orrù, G. Cao, J. De Wilde, I. A. Beloki, D. J. Jarvis, L. Froyen, (2003), "Sintesi autopropagante ad alta temperatura in condizioni di microgravità", *La Metallurgia Italiana, Vol. 7-8*, pp. 45-52.
2. J. De Wilde, L. Froyen, R. Orrù, G. Cao, I.A. Beloki, A.E. Sytschev, A.S. Rogachev, D.J. Jarvis, L. Vautmanas, F. Preud'homme, R. Licheri, (2003), "Self-propagating high-temperature synthesis of Al-Ti-B in the ISS: reactor design and preliminary evaluation", *International Journal of SHS, Vol.12, Issue 3*, pp. 165-177
3. A.S. Rogachev, J.C. Gachon, H.E. Grigoryan, A.N. Nosyrev, P.A. Tsygankov, J.C. Schuster, D. Vrel, (2005), "Phase evolution during gasless combustion of the micro- and nano-heterogeneous systems: Time resolved study by synchrotron radiation diffraction analysis", *Nuclear Instruments & Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors, and Associated Equipment, Vol. 543, Issue 1*, pp. 175-179

2.3.2.2 Fluid Physics: Fluid and interface physics

2.3.2.2.1 Diffusion coefficients in crude oils (DCCO)

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2.3.2.2.1.1 Background, Objectives and Procedures

Matter appears in our daily life and in human activities under the form of mixtures that are being transformed. These transformations involve chemical reactions, phase changes, convection and diffusion. Such processes most often combine together, and diffusion is always present. Diffusion is the general term used to describe the motion of one species with respect to the other, whatever the cause that produces this relative motion. Diffusion is effective in all solutions with non-homogeneous concentration distribution. The ratio between the mass flux and the concentration gradient is proportional to the diffusion coefficient.
However, there is a lack of diffusion coefficient data for most of the mixtures of interest to the oil industry. For multicomponent mixtures, the formalism is more complicated and the interaction theory of all chemical species present in the mixture is still open. Much work is presently being performed to understand species behaviour in systems far from concentration equilibrium and to determine the diffusion process in multicomponent mixtures. The use of numerical models in oil reservoir characterisation requires reliable values for the relevant diffusion coefficients.

The principle of the experiment is to introduce liquid A into a column of the ternary mixture B. The main reason for using a microgravity environment is to produce an environment free of natural (gravity induced) convection allowing thus to accurately measure the diffusion coefficients of ternary mixtures with a maximum accuracy without the disturbing effects of the gravity induced convection. Diffusion through the experimental cell is monitored with a specially configured Mach-Zehnder interferometer, which consists of two laser diode light sources, optics and a Microgravity Science Glovebox (MSG)-provided camera. The interferometer measures the gradient of optical paths through the experimental cell imposed by concentration gradients. The liquid whose refractive index has to be measured is placed in a transparent cell. It is illuminated by a coherent plane wave beam that is combined with a reference beam passing through a reference cell filled with premixed liquid. The two beams give rise to an interference pattern containing the information about the refractive index changes in the measurement cell. In order to have an interferometer insensitive to mechanical disturbances, the optical mirrors and beam splitters of the interfererometer are combined in prism systems. The use of a reference cell in the reference beam of the interferometer allows a good compensation of the temperature variations. From the resulting interferograms recorded with the camera the concentration gradients are derived as a function of time.

The DCCO experiment delivers images of fringe patterns that have been recorded by the dual wavelength interferometer working at 790nm and 650nm. Two wavelengths are used because in ternary liquids, measurements of refractive index with one wavelength give rise to ambiguous results in the determination of the concentrations. The diffusion coefficient accuracy determination is directly related to the accuracies on the measurements of the optical path changes due to the diffusion process. In this way, an image analysis sequence that measures the optical paths with sub-fringe accuracy is quite better than the usual fringe counting.

2.3.2.2.1.2 Results

During the execution phase, gas bubbles blocked the orifice in which diffusion between the liquid mixtures was to take place. This meant that no useful data could be obtained from this experiment, and therefore no scientific results could be derived.

2.3.2.2.1.3 Conclusions and Recommendations

Not applicable.

2.3.2.2.1.4 Publications

Not applicable.

2.3.2.3 Material Sciences: New materials, products and processes

2.3.2.3.1 Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the ISS (PromISS-1)

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2.3.2.3.1.1 Background, Objectives and Procedures

One of the best-identified crystallisation methods for proteins on Earth and in space is the contra-diffusion technique that has been invented and developed by the team of Professor Garcia-Ruiz of the Granada University. With this technique, the protein solutions are placed in capillaries and the crystallisation occurs thanks to a precipitating solution that progressively diffuses along the capillaries. As the capillaries are small, the monitoring requests microscopic visualisation that leads to a very limited depth of focus. The result is that the direct observation of crystals in capillaries can give rise to unfocused images. In order to overcome this classical limitation of optical microscopy, the Microgravity Research Centre of the University of Brussels developed a digital holographic microscope that allows for an in-depth reconstruction, by numerical means, of the samples being analysed. This instrument benefits from partial coherent illumination to eliminate the classical sources of noise inherent to the use of a laser source and provides an image quality that is comparable to the one obtained with the best optical microscopes. As the 3D refocusing is made by numerical means, the technique is very interesting for automated experiments in space because it eliminates the need to focus on the image of each crystal and it also compresses in a very important way the data to be stored. As the method is a true holographic one, it also records the optical phase information of the observed field. Therefore, this method gives an accurate measurement of the refractive index changes in the solution surrounding the crystals. For protein applications, this is a very important point as the refractive index changes around a crystal and is a direct measure of the depletion zone that is expected to have a deep impact on the crystallisation quality.

The major objective of the present experiment was to produce a detailed analysis and a quantitative interpretation of the relationship between the quality of the obtained crystals and the environment in which they are produced by the method of digital holography.
The experiment aimed to investigate the protein growth processes in weightless conditions using the counter diffusion technique, in order to measure:

- The parameters of the growing protein crystals,
- The composition changes (depletion zone) of liquid around the growing protein crystals.

Protein crystallisation is initiated when a protein solution is brought to supersaturation, most often by raising the precipitant (salt, polyethyleneglycol, organic solvent) concentration, so as to lower the protein solubility. In counterdiffusion experiments the precipitant and protein solutions are initially in different volumes, and diffuse toward each other through a separating gel layer. In PromISS experiments both capillary and full reactor geometries (Figure 2-25) were used. The length of the diffusion path through the separating layer determines the delay in the start of the crystallisation.

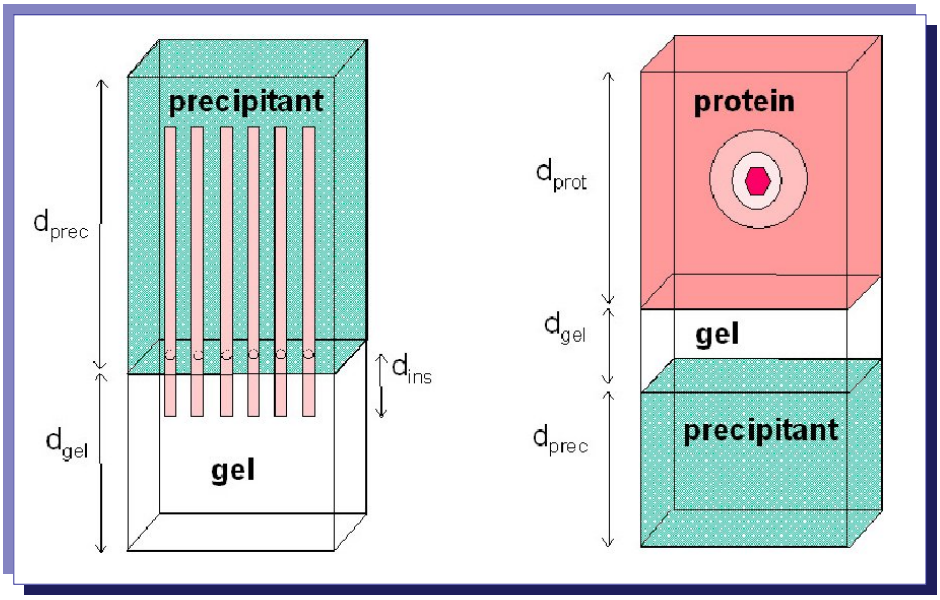

Figure 2-25: Geometry of the PromISS experiments. Internal volume of the reactors is 52 mm high, 19 mm wide, and 34 mm deep (left: capillary geometry; right: full reactor geometry)

The crystallisation experiments were observed by digital holography. This technique yields the in depth reconstruction of the field of view by numerical methods, without a mechanical focusing stage. The principle consists in the combination of the intensity and the optical phase of the object and to compute, by implementation of wave optics equations, the amplitude in planes that are parallel to the detection plane. In that way, out of focus objects can be brought into focus. Digital holography is attractive for automated experiments in space because it eliminates the need to scan the reactor, and reduces the amount of data to be stored. As the method also records the optical phase, it also gives an accurate measurement of the refractive index changes in the solution surrounding the crystals. For protein applications this is a crucial point as the refractive index is a measure of the composition of the solution.

The PromISS instrument (Figure 2-26) comprises a Mach-Zehnder interferometer where the sample is illuminated in transmission. For each image four interferograms were recorded at different phase shifts, induced by a piezo-transducer. PromISS comprised 6 experimental cells that were disposed on a rotating plate. During the experimental run, the rotating wheel sequentially placed the experimental region of interest (ROI) in the optical channel of the microscope. It was possible to define by tele-operation 6 ROI per cell. A complete rotation of the wheel was achieved in one hour. The PromISS instrument was accommodated in the Microgravity Science Glovebox (MSG) in the Destiny (US-Lab) Module of the ISS. The MSG provides power, containment, video recording, data transfer and commanding capability from ground.

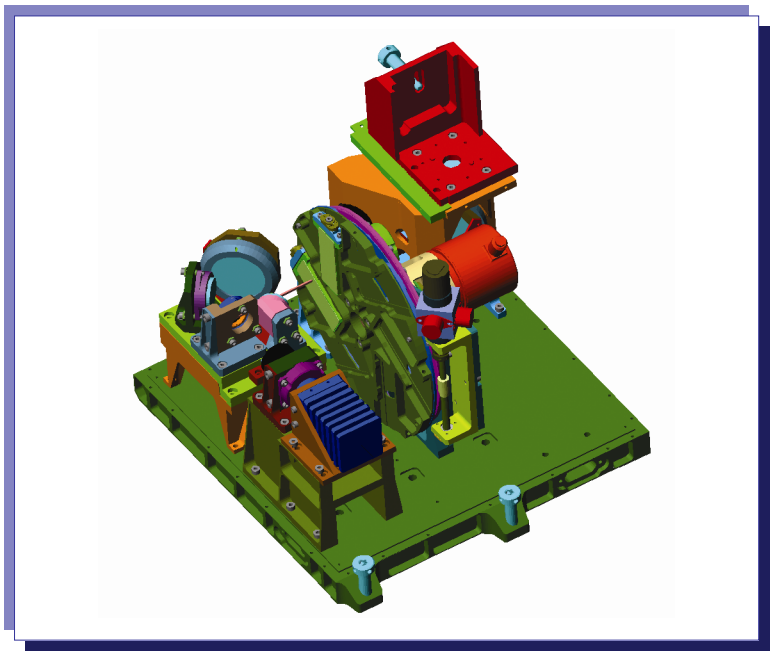

Figure 2-26: An internal view of PromISS without electronic boxes

2.3.2.3.1.2 Results

The following results are based on the first three series of PromISS experiments that have been performed: PromISS I in the context of the Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, PromISS II during the Spanish Soyuz mission, "Cervantes" (ISS 7S mission), which was executed in October 2003, and PromISS III as part of the Dutch Soyuz mission, "Delta" (ISS 8S), which took place in April 2004. In each space experiment six reactors were observed in PromISS, so a total of 18 individual crystallisation experiments were performed on 6 proteins (the complex of the variable domain of a camelid heavy chain antibody with lysozyme (cablys3*lysozyme), Thermotoga marirtima triose phosphate isomerase (TIM), pike parvalbumin, hen egg white lysozyme, equine spleen ferritin, and lumazine synthase).

The data generated by the PromISS experiments consisted first in the interferometric data produced by the instrument, and second in data generated by the post-flight analysis of the quality of the crystals, and for certain experiments of the composition of the crystals and their surrounding solutions. For all three PromISS experiments

the raw interferometric data has been treated. These data consist in successive sequences of about 2.5 seconds of recording at a frame rate of 30 images/sec. In this set of 75 images, 4 images (corresponding to the four phase shift) were selected and amplitude and phase fields were computed (specific algorithms have been developed to automatically select the four images). Images have been digitalised, automatically classified, backups have been made, and amplitude and phase images (when possible) have been computed. The reconstruction in depth has been performed when necessary (crystals out of focus in the field of view). Figure 2-27 represents a numerical propagation of the optical beam on a distance of 200 µm in the direction of the optical path (an example of refocusing of an out of focus crystal is illustrated).

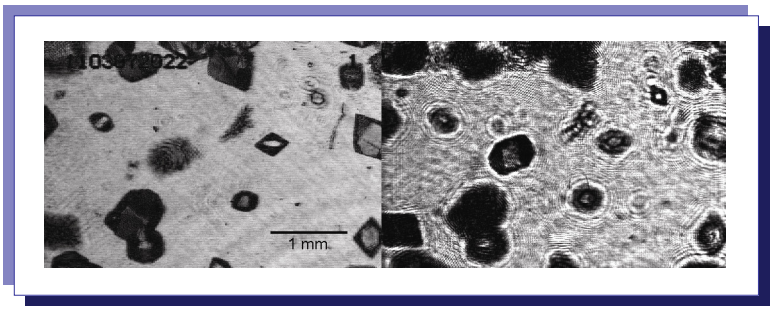

Figure 2-27: Example of amplitude computed image

Tools were developed to analyse the overall growth rate and position of the crystals. These tools are for the moment applied manually by the user. The automation of the segmentation and tracking of all the crystals contained in an image (or sequence of images) is being developed.

The first PromISS experiment was developed in less than 9 months, and performed surprisingly well in this regard, registering interferometry images every hour for the six reactors in 6 different positions. This made it possible to evaluate the moment of appearance of crystals, their growth rates, and the movement of crystals. A second instrument was built and uploaded in the context of the Spanish Soyuz Mission. This instrument has an external electronic box, and can thus be operated at the temperature of the MSG (average temperature in the PromISS II experiment 26.05 °C). The vibration sensitivity was reduced by reinforcing the holder for the beam splitter.

For 12 out of the 18 reactors that were flown for PromISS I-III, crystals were obtained in the right time frame, i.e. during observation by PromISS. In three reactors of PromISS I the high temperature caused the crystals to grow only after the end of the experiments. It was often not trivial to optimise the experiments as the timeline was very constraining. The constraint that crystals should not grow during the first five days (upload and installation of the experiments) could only be satisfied using relatively low supersaturations. On the other hand, careful analysis of the experiments showed that the use of counterdiffusion techniques reduced nucleation rates significantly for many of the proteins (TIM, parvalbumin, cablys3*lysozyme, lumazine synthase). It was thus difficult for the protein to obtain crystals before the end of the observation time. For these three proteins these problems were overcome by pre-treating protein solutions to induce intermediate phases that acted as hidden seeds. The solutions yielded crystals rapidly after supersaturation was raised, but did not produce crystals by themselves.

One of the aims of the experiments was to visualise the events occurring during a capillary counterdiffusion experiment in microgravity conditions. TIM experiments in capillary geometry were performed during PromISS I and II. Figure 2-28 shows that TIM crystals grew during the observation time and thus the crystallisation conditions and crystal growth rates could be determined.
The analysis of crystal movement showed that most TIM crystals remained stationary in the capillary during the experiment. This is important, as the aim of capillary counterdiffusion experiments is to have crystals growing in conditions that are best suited to them within a continuum of conditions created by a supersaturation wave passing through a capillary. The crystals are only weakly associated with the capillaries, as after the return of the reactors to ground the crystals could be moved out of the capillaries by induced convection. In the majority of the capillary counterdiffusion experiments few of the crystals moved.

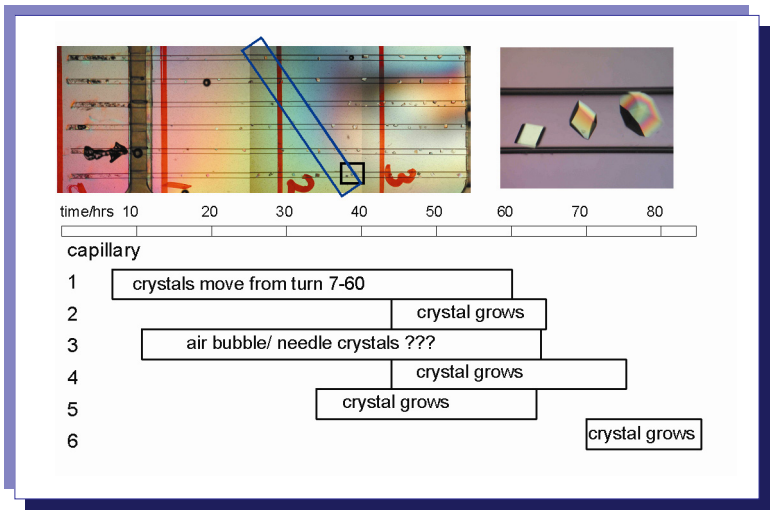

Figure 2-28: Results of an experiment with TIM in capillary geometry performed during PromISS I

Counterdiffusion crystallisation is used to effectively screen for optimal conditions of crystal growth. It requires a convection-free environment (gelled or in microgravity) that makes it possible for a precipitant wave to travel through a protein volume. It was found that for the three model proteins that were used in the PromISS experiments the use of counterdiffusion experiments resulted in crystals of different crystal forms. The difference in the outcome of the experiments can be explained on the basis of differences in the rate at which supersaturation is achieved.

2.3.2.3.1.3 Conclusions and Recommendations

The results show that counterdiffusion experiments can be useful not only for producing crystals of higher quality, but also in cases where one wants to obtain a different crystal form with improved (diffraction) properties.

The effect of diffusive conditions was very extensively investigated for the proteins TIM and cablys3*lysozyme. For TIM more than 100 datasets were collected from crystals grown either in non-convective environments (in microgravity or in gels on the ground) or by conventional techniques prone to convection. The results show that there is a clear effect of diffusion, and the crystal perfection is higher for crystals grown in a non-convective environment. Analysis of crystal growth rates and mass transport show that the depletion zone model can not explain this, as TIM crystals essentially grew in a regime controlled by surface growth rates. Diffusion is not rate-limiting and no depletion zone was formed. For other proteins like cablys3*lysozyme the influence of a diffusive environment was negligible.

Results from the PromISS experiments have shown that protein crystallisation is one of the processes found in the very complex landscape of phase behaviour of protein at high concentrations. To better understand the effect of crystal growth conditions on crystal quality requires the continued ground and microgravity research on the thermodynamics and kinetics of these processes.

2.3.2.3.1.4 Publications

I. Zegers, L. Carotenuto, C. Evrard, J.M. Garcia-Ruiz, P. De Gieter, L. Gonzales-Ramires, J.C. Legros, J. Martial, C. Minetti, F. Otalora, P. Queeckers, C. Schockaert, C. VandeWeerdt, R. Willaert, L. Wyns, C. Yourassowsky, F. Dubois, (2006), "Counterdiffusion protein crystallisation in microgravity and its observation with PromISS (Protein Microscope for the International Space Station)", *Microgravity Science and Technology, Vol. 18, Issue 3-4*, pp. 165-169

2.3.2.3.2 Granada Crystallisation Facility (GCF)

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2.3.2.3.2.1 Background, Objectives and Procedures

The experiment concerns the crystallisation of proteins in space by the counter-diffusion technique. The major objective of the present experiment was to produce a detailed analysis and quantitative interpretation of the relationship between the quality of the crystals and the environment in which they were produced. Two parallel experiments in space have been carried out. The first concerned the flight of the Granada Crystallisation Facility (GCF), in which a large number of capillary experiments can be accommodated, but no diagnostics are possible. In parallel an experiment with a digital holography instrument (PromISS) was performed on a limited number of crystallisation reactions. The experiments in the digital holography instrument were tuned to those in the Granada crystallisation facility.

The Granada Crystallisation Facility (GCF) is a metallic box that houses 23 Granada Crystallisation Boxes (GCB) carefully conditioned in individual plastic bags and wrapped in a high absorbency cloth, and a temperature data logger. The GCF is a completely passive instrument that does not require any activation or crew intervention beyond installing it as soon as possible at a place in the ISS where it is not exposed to inadvertent manipulations. It does not require any external supply. One GCB simply consists of 3 elements made of polystyrene:

1. A reservoir to introduce the gel
2. A guide holding the capillaries
3. A cover

The GCB principles are schematically depicted in Figure 2-29. After inserting the guiding element, a layer of buffer solution is formed at the bottom of the reservoir and gelled. The capillaries entirely filled with the protein solution are then inserted through the holes of the guide and punctured to a given depth into the gelled buffer layer. Finally the salt solution is poured onto the buffer layer and the box is hermetically sealed. The time at which the actual crystallisation experiments start is defined by the thickness of gelled buffer the precipitant has to diffuse through before it reaches the protein solution. The fact that the buffer is gelled makes this lag time insensitive to either manipulation or launch accelerations or vibrations. This lag time can be tuned to up to 3-4 days.

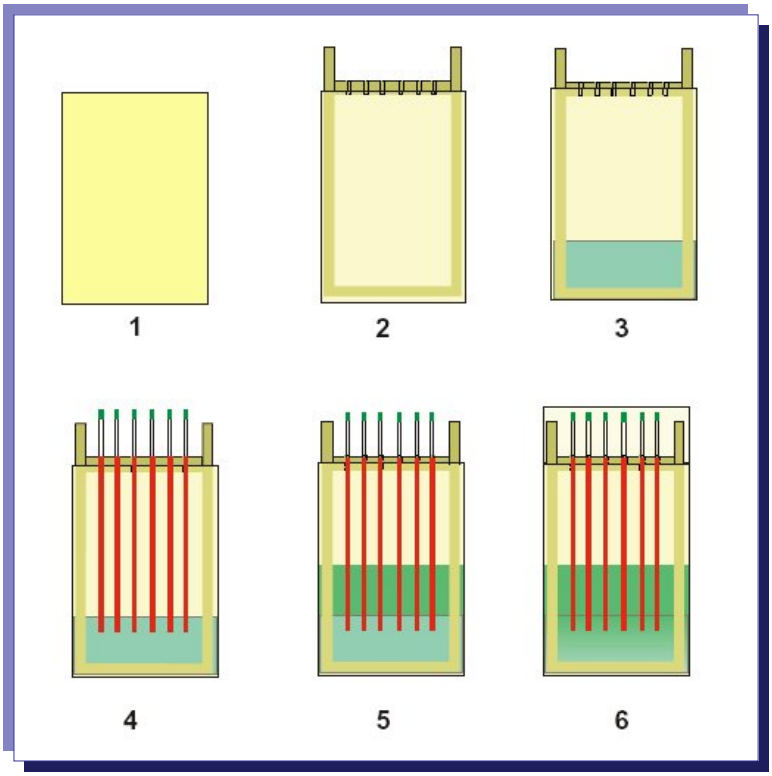

Figure 2-29: Principle of operation of a GCB

Each GCB accommodates up to six capillaries with a maximum diameter of 1 mm. The external dimensions of a GCB are 36 x 101 x 7 mm^3. The total weight of one GCB filled with six capillaries and the chemical solutions is 24 grams. No moving parts, or electrically powered systems are required to activate the experiments.
Once filled and hermetically sealed, the GCF occupies a volume of 13.4 x 13.4 x 8.4 cm^3 for a mass of about 1 kilogram (solutions and gels included). It is then packed in a 14 x 14 x 9 cm nomex bag designed for storage in ISS and return in the Soyuz vehicle. It is then fitted into a 30 x 30 x 30 cm foam insert designed for transport in Soyuz, also packed in a nomex bag. This ensemble of 2.4 kg is delivered for integration in the launch vehicle only a few hours before launch.
After docking, the GCF was extracted from its Soyuz transport container and transferred to the Russian segment of the ISS and stored in a quiet location (with stable microgravity and temperature, and no vibrations), where it remained undisturbed until the end of the mission.

2.3.2.3.2.2 Results

Twenty three GCB boxes were accommodated in the GCF, with fifteen different proteins being selected for this mission. These proteins belong to different European and Japanese research laboratories. For comparative studies, one additional GCF was prepared in parallel on-ground. The unique difference between the space and on-ground experiments is that for the on-ground experiment the viscosity of the protein solution was increased by adding agarose at a concentration of 0.1%.

In order to record the temperature profile during the experiment, a small temperature sensor cube from Meilhaus was located inside the GCF. After the flight all the GCBs were carefully inspected. It was confirmed that none of the 23 GCBs suffered any damage. The crystallisation patterns inside the capillaries were photographed with the help of a binocular microscope. Figure 2-30 shows crystals of different proteins grown in the space experiment and Figure 2-31 shows crystals obtained in the ground experiment for the same proteins.

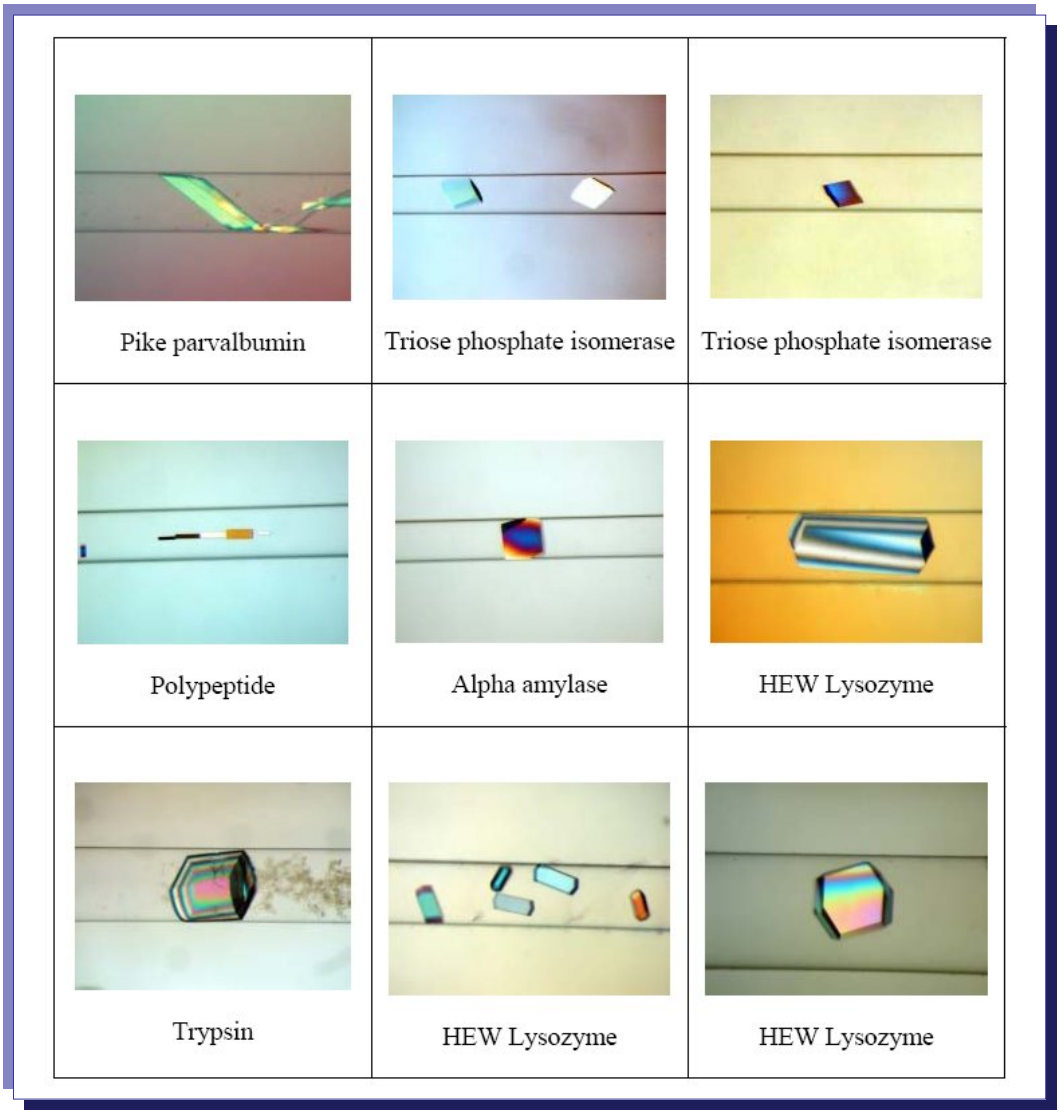

Figure 2-30: Example of proteins crystallised in the GCF in the ISS

The temperature profile recorded in-situ in the GCF (both space and gound control) with the Meilhaus temperature sensor is shown in Figure 2-32 . It is clear that the temperature on-ground was more stable than in the ISS. In fact, the temperature values in the location of the GCF at the ISS had many fluctuations during the mission. Temperatures gaps of up to eight degrees Celsius (14 to 22 ºC) were recorded.

Figure 2-31: Example of proteins crystallised in the GCF on-ground

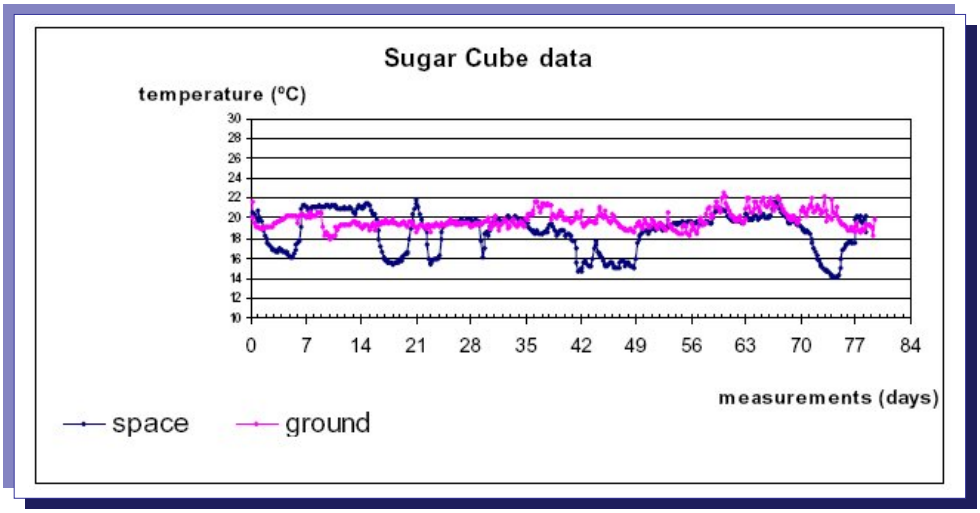

Figure 2-32: Temperature profile of both ground and space experiments

2.3.2.3.2.3 Conclusions and Recommendations

One of the objectives of the experiment was the comparison of the crystallisation process on ground and in space in terms of crystal quality and crystal morphology and pattern of precipitation. Because of the very different temperature profiles between the space and on-ground variation, it was impossible to properly perform these studies.

Unfortunately, an additional problem occurred during the mission. The GCF located in the ISS returned to Earth about two weeks later than the scheduled date on the Shuttle rather than the Soyuz vehicle. This extra time and the handling of the GCF inside the ISS from Russian to American modules and accommodation in the Shuttle could have affected the experimental results.
The experiments performed during the Belgian "Odissea" Mission confirmed that the Granada Crystallisation Facility and the Granada Crystallisation Box work properly for protein crystallisation in space implementing the counter-diffusion method. However, a problem was encountered, namely, the existence of large thermal fluctuations inside the ISS. Finally, it has been reported by the Japanese team using the GCB that the GCB can not be used with alcohol and that some leakage has been observed. Therefore, the following is strongly recommended:

- The use of unmanned flights of 15 days duration, which can provide the right gravitational scenario to grow crystals in space in a pure mass transport diffusion regime.
- The use of an inexpensive fully autonomous crystallisation facility such as GCF to be used in unmanned flight with a thermal control range accuracy of 1 degree Celsius for the duration of the experiment, i.e. from the preparation of the experiment to the reception in a synchrotron facility.
- To use a new capillary device made of glass to allow the use of any solvent of additives for protein crystallisation.
- To redesign the GCB for the duration of the experiment, reducing the number of capillaries per box (i.e. reduction of the GCB size) and identifying a better cover seal.

2.3.2.3.2.4 Publications

1. J.D. Ng, J.A. Gavira, J.M. García-Ruíz, (2003), "Protein crystallization by capillary counterdiffusion for applied crystallographic structure determination", *Journal of Structural Biology, Vol. 142*, pp. 218-231
2. J.M. García-Ruiz , L.A. González-Ramírez, J.A. Gavira, F. Otálora, (2002), "Granada Crystallisation Box: a new device for protein crystallisation by counter-diffusion techniques", *Acta Crystallographica D, Vol. 58*, pp. 1638-1642
3. H. Tanaka, K. Inaka, S. Sugiyama, S. Takahashi, S. Sano, M. Sato, S. Yoshitomi, (2004), "A simplified counter diffusion method combined with a 1D simulation program for optimizing crystallization conditions", *J. Synchrotron Rad., Vol. 11*, pp. 45-48

2.3.2.3.3 Study of aggregation mechanism and kinetics of ZSM-5 and Silicalite-1 nanoslabs into ZSM-5/Silicalite-1 hybrid phases under microgravity conditions (NANOSLAB)

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2.3.2.3.3.1 Background, Objectives and Procedures

Zeolites are microporous crystalline silicate materials. The applications of zeolites in catalysis, molecular separation and ion exchange processes are based on the reversible uptake of molecules and ions from the surrounding medium into the microchannels of the zeolite. The molecular mechanisms responsible for formation of zeolite materials out of monomeric or amorphous polymeric precursor materials are poorly understood. For many potential applications, optimum zeolite structures and compositions can be designed, but the knowledge is lacking on how to synthesise such tailor-made zeolites. The hypothesis of this project is that experimentation under microgravity conditions should enable considerable progress in the understanding of the formation processes of zeolite particles.

The Silicate-1 zeolite is a silicon dioxide polymorph that can be synthesised by self-organization of Silicalite-1 nanoslabs in suspension. Nanoslabs are discrete particles, measuring typically 4 x 4 x 1.3 nm, and having already the framework connectivity of the Silicalite-1 zeolite. Nanoslabs were discovered in the Team Members' laboratory, and the mechanisms and kinetics of their aggregation into Silicalite-1 crystals upon heating determined and modelled. The formation of Silicalite-1 crystals occurs via a sequence of aggregation steps of specific nano-species. Convection upon heating was observed to have a marked influence on the kinetics. Stirring of the nanoslab suspension resulted in a significant retardation of the aggregation process. Thus it was expected that microgravity would have a strong impact on the kinetics of zeolite formation.

The influence of microgravity conditions on the kinetic parameters has been studied in detail by sounding rocket experiments before missions aboard the ISS. The sounding rocket experiments were based on the post flight analysis of a series of samples of nanoslab suspensions heated for different time-spans before quenching. This way the complete aggregation sequence from nanoslabs to crystalline material could be studied. To ascertain the observed effects due to microgravity, on ground reference experiments under identical conditions using the same hardware were performed. A strong retardation of the aggregation rate of the nanoscopic entities was observed under microgravity as compared to gravity. This unexpected result led to the discovery of a liquid-crystal-like phase of the nanoslabs, which seems to be essential to enable aggregation.

The objectives of the NANOSLAB experiment were threefold:

1. Confirmation of the microgravity effect previously observed during the sounding rocket missions: During the sounding rocket missions a retardation of aggregation as a function of anisotropy of the particles and absence of gravity was observed. To confirm the accuracy of these findings additional microgravity experiments were called for.
2. Study of the temperature dependency of the microgravity effect: Due to the short duration of sounding rocket experiments (minutes) the experiment temperatures needed to be very high (T>150°C) to assure completion of the crystallization. A microgravity experiment at lower temperature with longer duration was necessary to find out if the microgravity effect is temperature dependent and, if so, in what manner.

3. Study of the effect of charge on the ordering of nanoslabs under microgravity conditions: By variation of the nanoslabs' composition, extra charge can be incorporated into the particles. It was expected that this extra charge not only has an impact on the ordering kinetics in general but also affects the microgravity effect. Comparison of the aggregation kinetics of the modified nanoslabs with the set of kinetic data of the native nanoslab aggregation was considered to be a suitable strategy to study the effect of extra charge on nano-particle ordering.

The NANOSLAB hardware consisted of a sample unit and an electronic box. The sample unit served to accommodate 30 cells, assembled in 10 cartridges of 3 cells with 1.5cc volume contained by 3 levels of containment each. The unit also provided a frame wherein the 10 cartridges were mounted, together with a top cover and bottom cover plate. The electronic box contained 3 Printed Circuit Boards (PCB). This hardware was designed to be installed and operated within the Microgravity Science Glovebox (MSG) onboard the International Space Station (ISS).

After filling the cartridges, launch and transfer to the ISS, Nanoslab was planned to be installed inside the MSG, running autonomously once switched on. The samples were to be grouped in ten cartridges of three cells each. Cartridges were to be heated actively and sequentially to the process temperature (95°C) at intervals of 4.8 hrs. Quenching was foreseen to occur for all 10 cartridges at the same time by pressing them down onto the cold-plate available in the MSG. The samples then had to be returned to ground for analysis. No data-downlink was necessary, readouts of the temperature sensors of the samples' environments was saved and stored. An independent temperature logger in the experiment was necessary to assure samples always were within acceptable temperature ranges during transport and stowage (below 28°C).

2.3.2.3.3.2 Results

During the first activation during the Belgian Soyuz mission, "Odissea" in October-November 2002 the LED "ON" on the electronic box did not operate. The foreseen contingency measure of re-starting took no effect. Checking if the heating sequence had started (possible LED failure only) resulted in the observation that the experiment had failed.
This caused the decision to return the electronic box instead of the sample unit to pinpoint the reason for the malfunction. It turned out that an electronic component was damaged during launch/transport preventing the electronic box from functioning.
A successful re-flight of the experiment took place in October 2003 during the Spanish Soyuz mission "Cervantes". These results will be reported in the Increment 8 summary review.

2.3.2.3.3.3 Conclusions and Recommendations

Not applicable.

2.3.2.3.3.4 Publications

Not applicable.

3 ACRONYMS

BAND	Banding
BDC	Baseline data collection
CFQ	Combustion front quenching
CHROMOSOME-1	Chromosomal aberrations in blood lymphocytes of astronauts
CNS	Central nervous system
COSMIC	Combustion synthesis under microgravity conditions
CSA	Canadian Space Agency
CTA-B	Aquarius B Transport/Ascent Incubator
CTR-B	Aquarius B Transport/Return Incubator
DCCO	Diffusion coefficients in crude oils
DNA	Deoxyribonucleic acid
ECU	Electronic control unit
EEA	Erasmus Experiment Archive
EEG	Electroencephalogram
ESA	European Space Agency
ESF	European Science Foundation
EVA	Extra Vehicular Activity
EVP	Event related potential
FISH	Fluorescence in-situ hybridisation
FO	Flight operations; Functional objective
GCB	Granada crystallisation boxes
GCF	Granada Crystallisation Facility
HZ	Hertz
HZE	Highly ionizing radiation
IFOC	ISS Flight Order Contract
IMC	Intermetallic matrix composite
ISS	International Space Station
JAXA	Japan Aerospace Exploration Agency
LED	Light-emitting diode
MASER	MAterial Science Experiment Rocket
MSG	Microgravity Science Glovebox
M-VEP	Motion-onset related visual evoked potential
NASA	National Aeronautics and Space Administration
NeuroCOG	Directed attention brain potentials in virtual 3-D space in weightlessness
PBU	Plunger box unit
PCB	Printed circuit board
PromISS	Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the ISS
RIA	Radioimmunoassay
RAMIROS	Cosmic radiation and microgravity related oxidative stress
ROI	Region of interest
Roscosmos	Russian Space Agency
SHS	Self-propagating high-temperature synthesis
TEXUS	Technologische EXperimente Unter Schwerelosigkeit
TIM	Thermotoga marirtima triose phosphate isomerase
US	United States
VEP	Visual evoked potential
VITAMIN D	Characterisation of the effects of microgravity on the mechanism of action of Vitamin D in osteoblasts
ZARM	Zentrum für Angewandte Raumfahrt Microgravitation