Cell Biology in Space:

Basic research and potential applications

Augusto Cogoli & Marcel Egli

The purpose of this paper is to present an overview of our work conducted in space in the past three decades. We describe the use of microscopic techniques such as phase contrast, fluorescence and electron microscopy, in experiments performed on the Space Shuttle/Spacelab system, on sounding rockets, on the International Space Station and on the ground in clinostats generating conditions similar to microgravity as well as in centrifuges at hypergravity levels of 10-20 g. Recently, a Digital Holographic Microscope has been adapted for the use on a three-dimensional clinostat (also called the Random Positioning Machine, RPM). This enables us to study morphological changes of cells under simulated microgravity conditions in real-time (Pache et *al.*, 2008).



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Most of our investigations have been dedicated to the study of the activation of human lymphocytes in microgravity. Our interest focused both on the immune system of humans in space and on signal transduction in differentiating cells. As a model we used the mitogenic activation *in-vitro* of T-lymphocytes.

In an earlier paper we reported the changes of the ultrastructure observed by transmission electron microscopy in human lymphocytes activated by concanavalin A and exposed to simulated microgravity in a fast rotating clinostat: the appearance of mitochondria-rich cells and other structural changes indicated that cells are sensitive to gravity changes, (Cogoli *et al.*, 1980). In another paper we described the movements of HeLa cells on a gold substrate by dark-field microscopy. While in the controls at 1 g the cells displayed normal motility, at 10 g the cells were not capable of any movement during a period of 48 h, (Tschopp and Cogoli, 1983).

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Our first experiment in space was performed in the summer of 1983 in the flight deck of the space shuttle Challenger. The objective was to establish whether human embryonic kidney cells which produce urokinase, an enzyme used in the treatment of thrombosis, are capable of adhering to microcarrier beads in zero gravity. As shown in Figure 1, the cells adhered quite strongly to the substrate. This result paved the way to bioprocesses based on adhesion-dependent cells that could take advantage of weightless conditions by significantly increasing the surface available, (Tschopp *et al.*, 1984).

In November 1983 the Spacelab module was flown in the payload bay of the shuttle Columbia, the Spacelab 1 mission. In an incubator manufactured in our workshop we determined the mitotic index of T cells activated and chemically fixed in flight. Activated cells were detected and measured by autoradiography of tritiated tyhmidine incorporated into DNA. There was a dramatic loss of activity (more than 95%) compared to identical samples kept on the ground (Figure 2), (Cogoli *et al.*, 1984). That discovery triggered the interest of the scientific community, and several experiments were performed by us and others in space and on the ground in order to clarify how gravity could interfere with the signal transduction and differentiation mechanisms in T cells.

In all the following studies in space we used a reference centrifuge generating artificial 1 g gravity.

The results from Spacelab 1 were confirmed in 1985 in the Spacelab D1 mission. Transmission electron microscopy clearly showed that the 1 g control in space displayed the typical ultrastructure of T blast cells (Figure 3A), whereas in the 0 g samples typical apoptotic features could be identified (Figure 3B). However, at that time the phenomenon of apoptosis had not yet been described, (Cogoli, 1993).

A new approach was adopted in a study performed in 1991 in Spacelab SLS1 (there had been a long interruption of the shuttle flights after the catastrophe of Challenger in 1986). T lymphocytes (known to be adhesion-independent cells) were coated on to microcarrier beads. To our great surprise the depression of mitogenic activation observed in resuspended cells was not observed in coated cells (Figure 4), (Bechler *et al.*, 1992, Cogoli *et al.*, 1993).

In the Spacelab IML 2 mission, flown in 1994, a centrifuge microscope developed by the German Space Agency, DARA GmbH, based on



Fig.1. Scanning electron microscopy of human embryonic kidney cells adhering to microcarrier beads in microgravity.

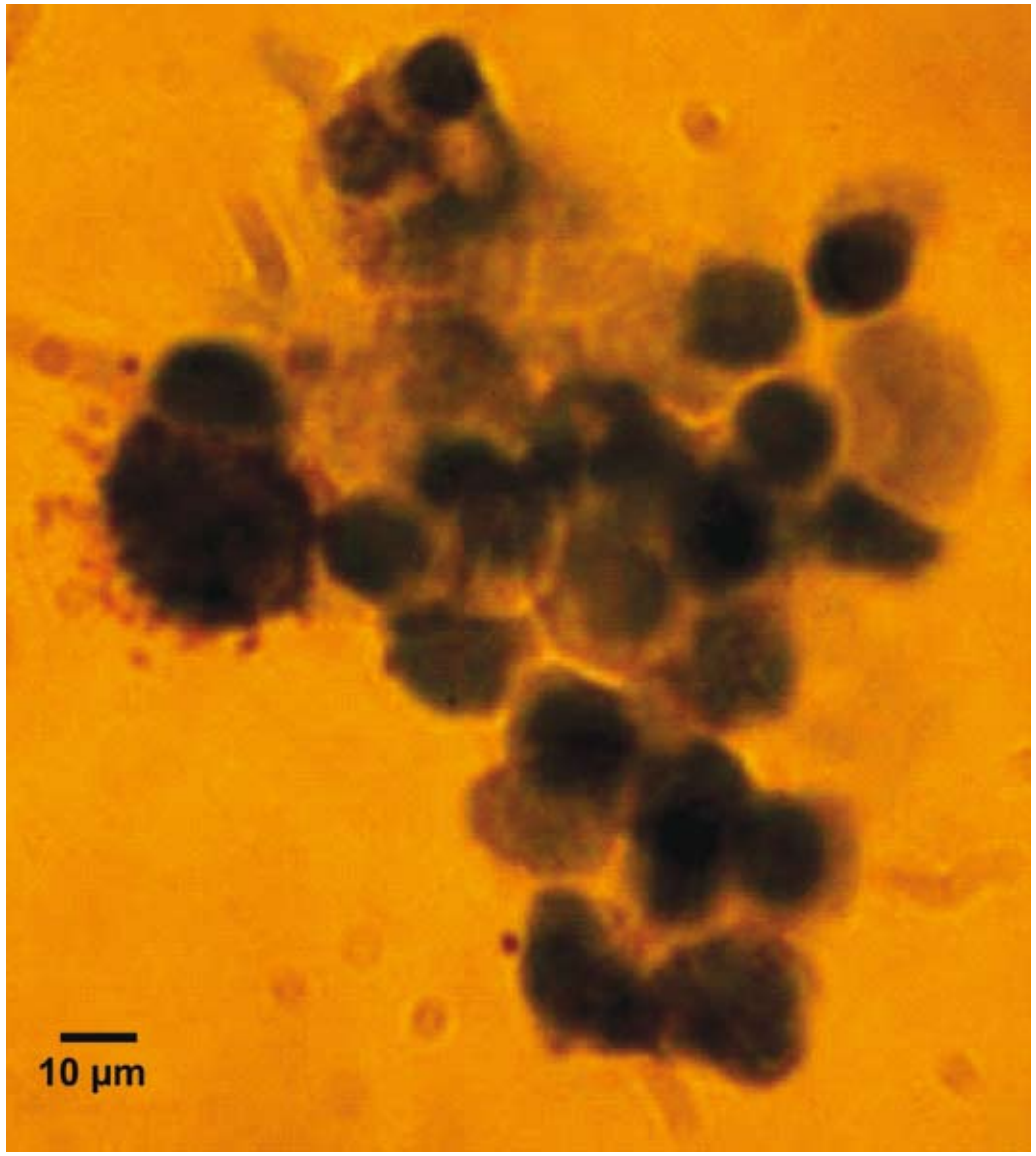


Fig.2. Light microscopic autoradiography of T lymphocytes activated in space with concanavalin A and labelled with tritiated thymidine.

plans proposed by Briegleb and Hemmersbach, Friedrich *et al*., (1996) allowed the recording of movements and interactions of lymphoycytes in microgravity. The experiment addressed a crucial question related to the loss of T cell activation in space, namely whether the cells are capable of autonomous movements and of establishing cell-cell contacts under 0 g conditions. In fact, T cell activation requires tight contacts between each other as well between T cells and monocytes as antigen-presenting cells. We were able to see that cells display autonomous movements and interactions in 0 g, (Cogoli-Greuter *et al*., 1996).

The interesting and somewhat unexpected behaviour of cells in space induced us to develop sophisticated instruments for biotechnological processes in the space laboratory. A first step was the development of a bioreactor that was flown on three space missions (Spacelab IML 2, STS-76 in 1996 and STS 107, that ended with the catastrophe of Columbia in 2003) with yeast cells

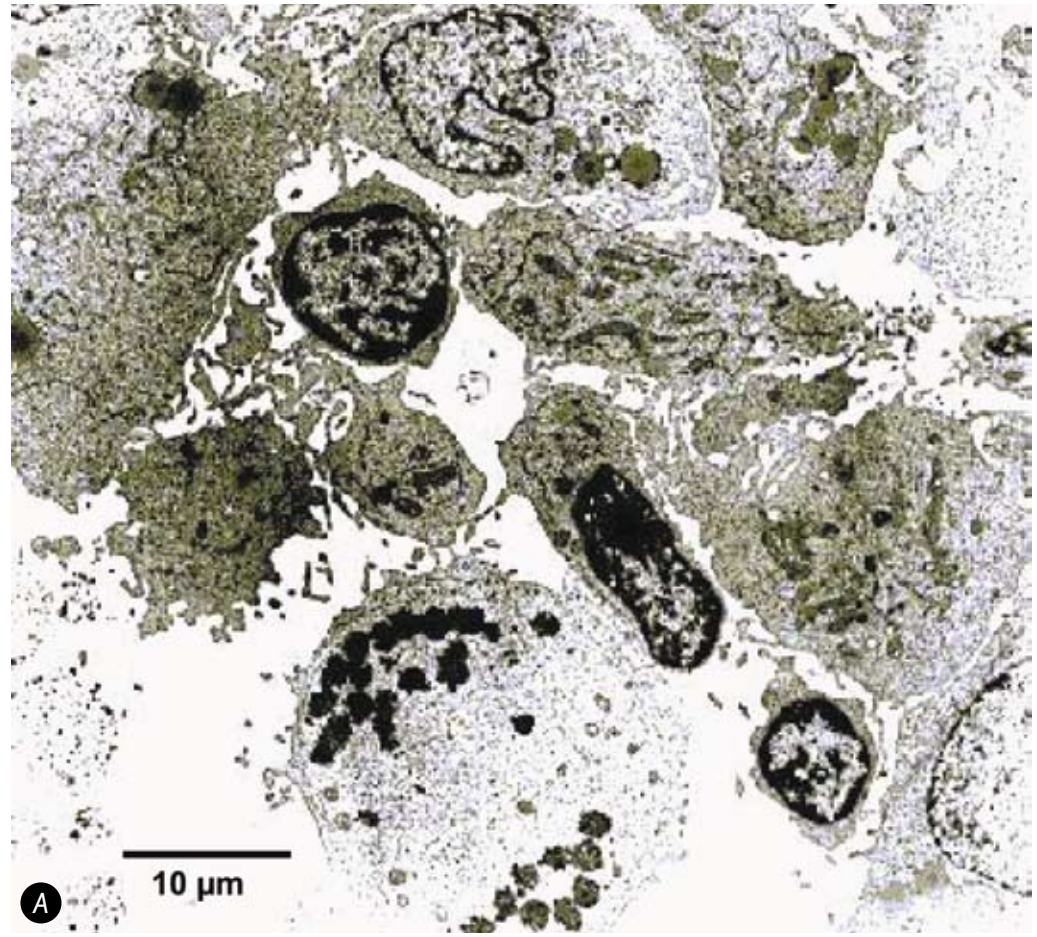
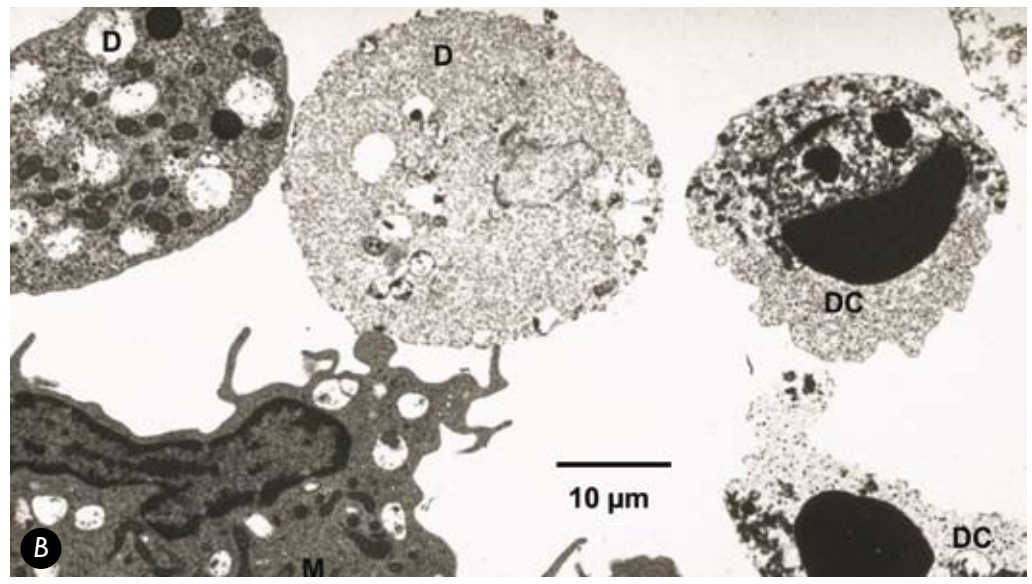
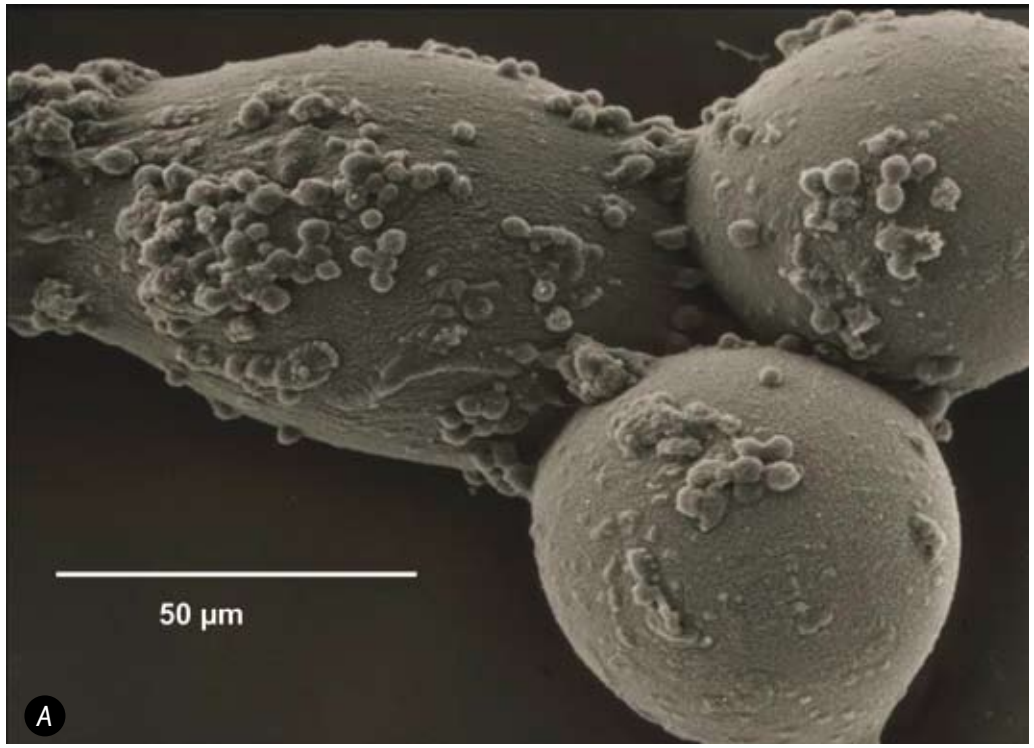


Fig.3. Electron micrographs of Lymphocytes cultured in space and activated with the mitogen concanavalin A. Fig.3A: Control samples kept in reference 1 g centrifuge showing typical aggregates of stimulated cells and a mitosis (Mit); Fig.3B: Samples kept at 0 g showing macrophages (M), apoptotic dead cells (DC) and cellular debris (D).





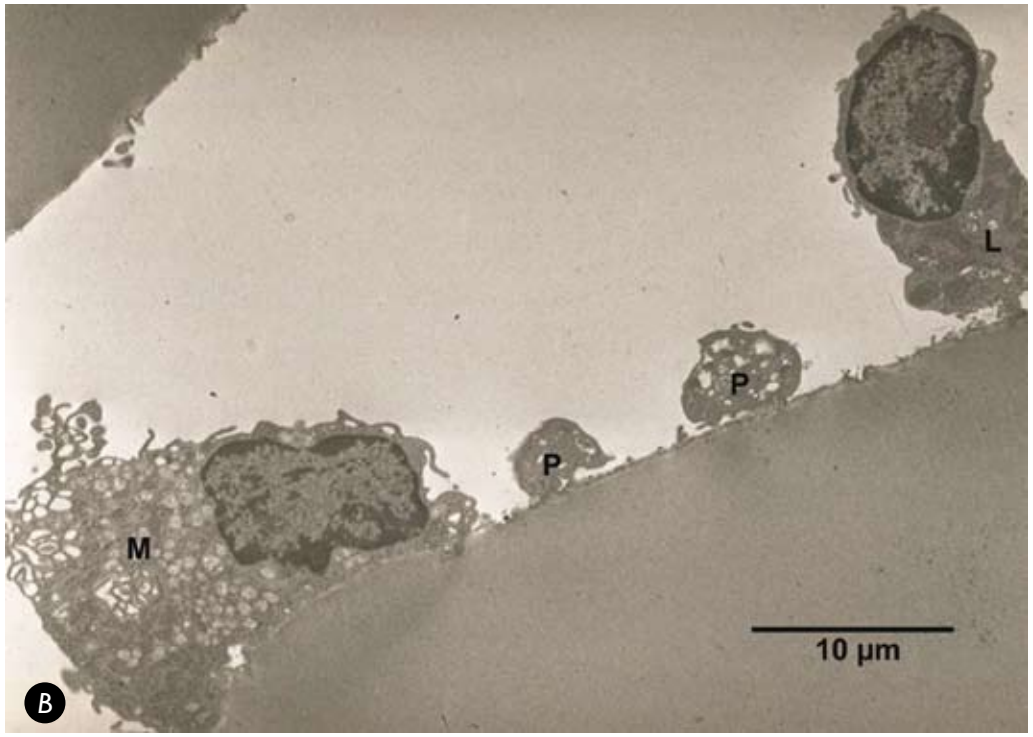


Fig.4. Lymphocytes, monocytes, and blood platelets attached to microcarrier beads in microgravity. Fig.4A: Scanning electron micrograph; Fig.4B: Transmission electron micrograph showing a monocyte (M), a lymphocyte (L) and a two platelets (P).

We studied the behaviour of epithelial cells in microgravity for two reasons

of *Saccharomyces cerevisiae*. The analysis of the electron micrograph from the experiment on IML 2 showed a significant difference in the distribution of the bud scars (remaining after the separation of a daughter cell) between 1 g in flight controls and 0 g samples: while a majority of the scars show bipolar distribution at 1 g, there was a significantly higher random distribution at 0 g, (Figure 5), (Walther *et al.*, 1996). These data suggest that there are remarked changes in the intracellular structure.

We investigated the structure of the cytoskeleton of the intermediate filaments of vimentin in Jurkat cells (a cell line derived from human T cells) by cytofluorography on the sounding rocket MAXUS 1B. There was a significant higher formation of large bundles of filaments after only 30s exposure to 0g thus showing that the cytoskeleton undergoes important and immediate changes in microgravity, (Cogoli-Greuter *et al.*, 1998).

The structure of F-actin filaments was investigated in 2006 in a facility called KUBIK on board of the International Space Station, ISS. There are again important differences between the distribution of actin between 1 g and 0g in the macrophage cell line JM-111, (Cogoli-Greuter *et al.*, (manuscript in preparation)).

In an effort to profit from microgravity for biomedical and biotechnological applications we started a series of investigations conducted in the three-dimensional clinostat, also called random positioning machine (RPM).

One project is dedicated to the study of osteoporosis by means of a newly developed bone resorption assay. The experimental model consisted of the human bone marrow derived FLG 29.1 cell line, previously characterized as an osteoclastic precursor model which adhered to bone slices on which a groove was scraped with a scalpel. The profile of the groove was measured after 3 days of incubation in the RPM. There was a clear and measurable resorption of the bone. No change was detected in the control samples, (not shown here), (Monici *et al.*, 2006). We plan to use such a model to test drugs and food supplements that could prevent osteoporosis.

We studied the behaviour of epithelial cells in microgravity for two reasons. One, is to better understand how wound healing in space proceeds. In fact, it has been known since the early days of human space flight that wound healing is retarded at 0g. The other reason is to investigate angiogenesis in microgravity in order to explore whether the production of artificial tissue (e.g. blood vessels) could be improved in zero g.

Autofluorescence proved to be a useful tool in studying metabolism and structural features of epithelial cells in simulated microgravity in the RPM, (Morbidelli *et al.*, 2005): Fluorescence images were directly digitized in the CCD controller with a 14 bit dynamic and transferred to the storage computer on a digital interface. In control cells (1g), autofluorescence emission arises from cytoplasmic organelles and the distribution of the blue (NAD(P)H) and green (flavines) components is very similar. In hypogravity-cultured cells, blue and green components have different distribution and a disruption of the organelles occurs.

In another study, the formation of a tube-like structure by epithelial cells was observed in the RPM (Infanger *et al.*, 2006): A tubular structure (like a lumen) with a length of 1.8 mm and an artificial endothelium layer along the extracellular matrix were formed after 72 h culture under simulated microgravity.

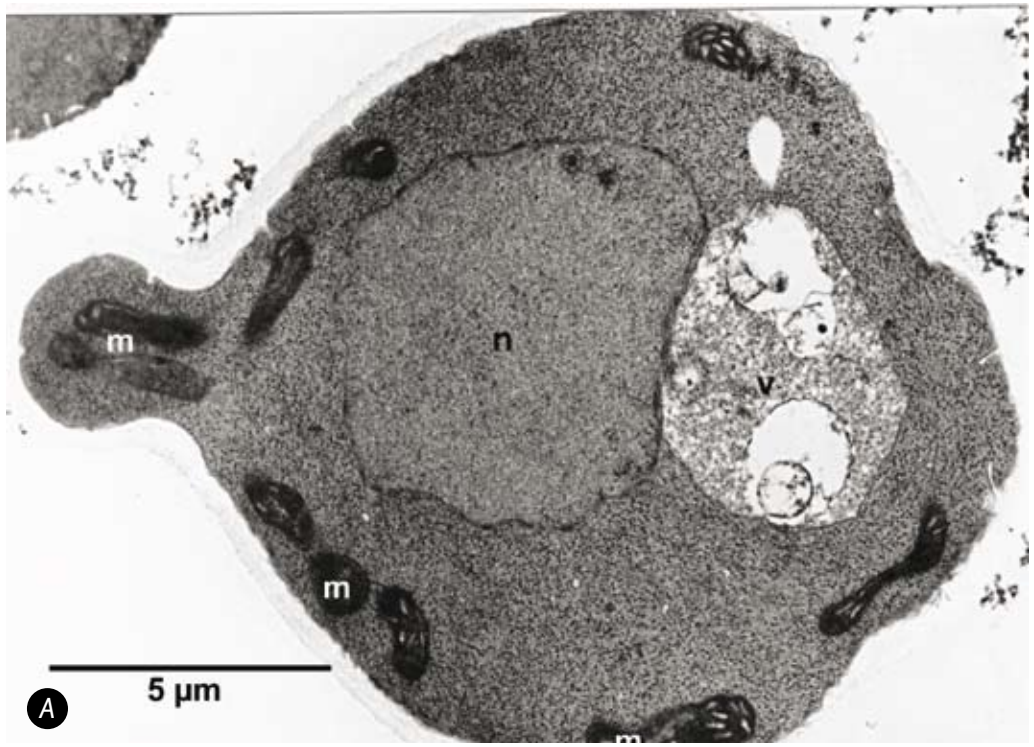
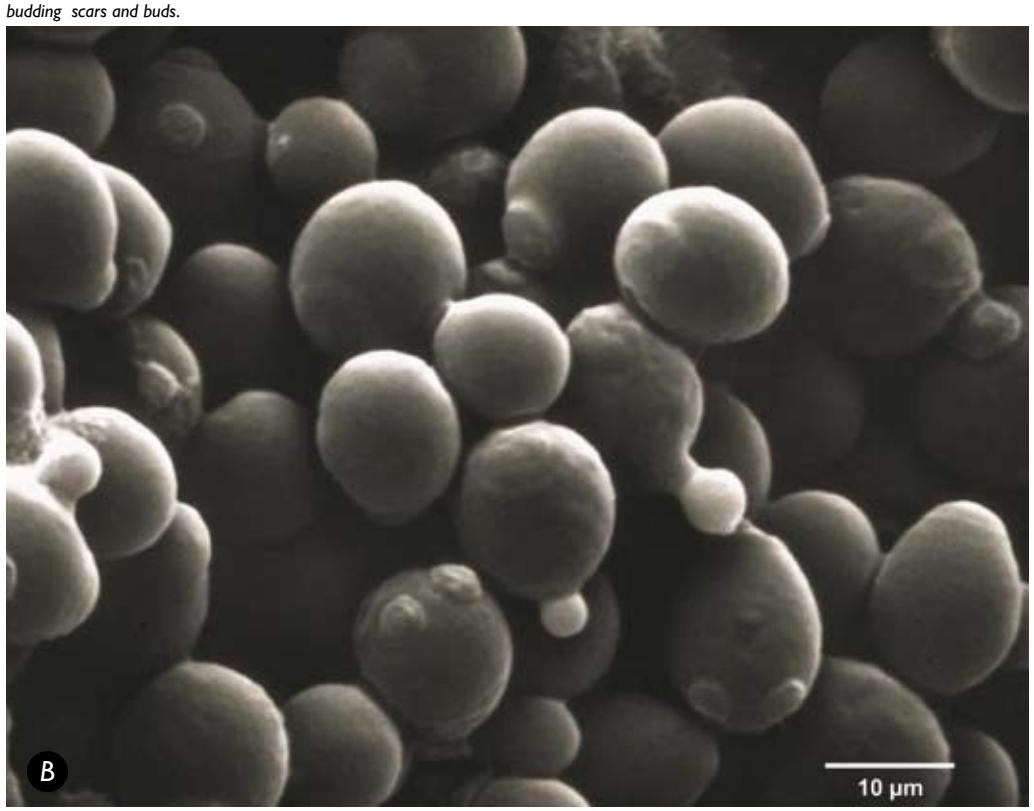


Fig.5. Electron micrographs of yeast cells cultured in a bioreactor in space. Fig.5A:Transmission electron micrograph: clearly visible are the nucleus (n), a vacuole (v) and several mitochondria (m), two of them are in the cell bud at the left; Fig.5B: Scanning electron micrograph of cells with budding scars and buds.



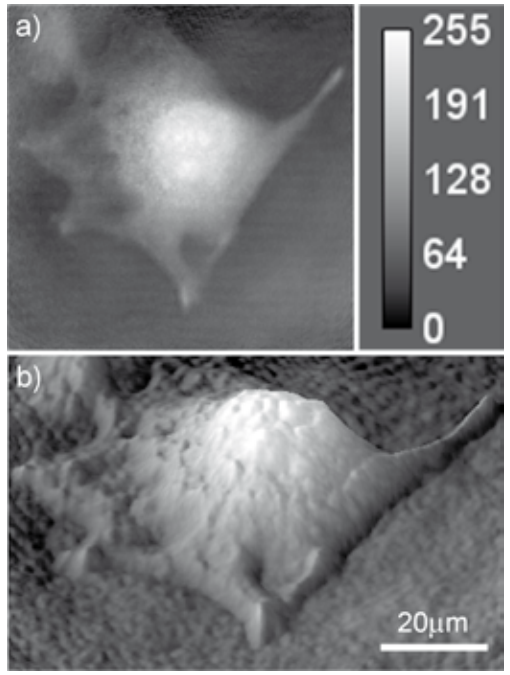


Fig.6. DHM images of a living cell under simulated microgravity condition on the RPM. a) Phase image (vertical scale in degrees [°]); b) Perspective image of a). FOV: 70 x 70 µm2

Spheroids of tumour cells are useful cell aggregates that can be regarded as a tumour model suitable for the study of anti-tumoral drugs. We have investigated the formation and properties of spheroids of thyroid carcinoma cells grown in the RPM (Grimm *et al.*, 2002).

Recently, Digital Holographic Microscopy (DHM) has been adapted to fit on a Random Positioning Machine for investigating morphological changes of cells under simulated microgravity in real-time, (Pache *et al.*, 2008). Preliminary investigations were carried out on mouse myoblasts (C2C12) and the results showed that besides the already described cellular modifications (by using conventional microscopy techniques), the cell responded with an increase of the phase height, which has not yet been reported. The DHM technique provides a new approach in the study of cellular adaptations to microgravity, providing real-time images of living cells. Furthermore, the DHM technique allows a detailed analysis of morphological aspects of living cells: their topography can be revealed as well as internal cell structures (Figure 6).

In conclusion, microscopic techniques are necessary to detect structural changes of the cytoskeleton and to interpret molecular and biochemical data from experiments conducted in microgravity. The new facilities presently installed on the International Space Station are equipped with light microscopes. Cytofluorimetry and electron microscopy will continue to be performed on the ground on samples fixed in space. A major and promising development is real time DHM on the RPM.

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Augusto Cogoli was born and grew up in Italy. In 1965 he obtained his Ph.D. in Organic Chemistry at the University of Pavia. In 1968 he moved to the University of Zurich and started to work on enzymology. In 1974 he obtained his doctorate in Biochemistry (Dr. Sc. Tec.) at the Swiss Federal Institute of Technology in Zurich. He specialized in immunology and cell biology as visiting scientist at the Weizmann Insitute of Science, Rehovot. He founded the Space Biology Group of the ETHZ in Zurich and started to work in space biology conducting several experiments in space laboratories (Spacelab, MIR station, International Space Station), sounding rockets and automated satellites. In collaboration with aerospace industries, he participated in the development of sophisticated space instrumentation, such as bioreactors and incubators. Augusto Cogoli is the author of over 170 publications in international journals and has served on several national and international committees on space activities.

In 2004 he founded, with his wife Marianne, Zero-g LifeTec GmbH, a spin-off company of the ETHZ. Augusto Cogoli was awarded the Medal of the ETHZ in 1975, the Nello Pace award of the International Society of Gravitational Physiology in 2004, and the medal of the European Low Gravity Association in 2005.

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Marcel Egli is head of the Space Biology Group, Swiss Federal Institute of Technology Zurich, Switzerland (ETH Zurich). He graduated from the University of Bern (MD-Ph.D.) before spending several years as Postdoctoral Fellow at the University of Melbourne (Howard Florey Institute, Australia) and at the Florida State University (Department of Biology). The appointment to lead the Space Biology Group brought him back to Switzerland. Marcel Egli has published various papers in the fields of neurobiology, neuro-endocrinology, and currently in space-biology. He is also the author of several book chapters. His current research interests include hypothalamic control of hormone secretion, and the influence of biological rhythms on immune function in humans. In particular, the question of how the internal biological clock is altered by microgravity. Furthermore, he fostered numerous collaborations with Universities of Applied Sciences, and the École Polytechnique Fédérale de Lausanne (EPFL) to develop new technologies for space applications. He is Principal Investigator of several experiments that were selected by the European Space Agency (ESA) for a space flight. Marcel Egli is a regular guest lecturer at the University of Bern and the ESA Summer School for Astrobiology.

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