

Facilities for Simulation of Microgravity in the ESA Ground-Based Facility Programme

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Received: 12 June 2015 / Accepted: 26 October 2015 / Published online: 14 January 2016
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Abstract Knowledge of the role of gravity in fundamental biological processes and, consequently, the impact of exposure to microgravity conditions provide insight into the basics of the development of life as well as enabling long-term space exploration missions. However, experimentation in real microgravity is expensive and scarcely available; thus, a variety of platforms have been developed to provide, on Earth, an experimental condition comparable to real microgravity. With the aim of simulating microgravity conditions, different ground-based facilities (GBF) have

been constructed such as clinostats and random positioning machines as well as magnets for magnetic levitation. Here, we give an overview of ground-based facilities for the simulation of microgravity which were used in the frame of an ESA ground-based research programme dedicated to providing scientists access to these experimental capabilities in order to prepare their space experiments.

Keywords Levitation · Clinostat · Random Positioning Machine · Simulated microgravity

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Introduction

Limited access to space flight and high costs have promoted developments to achieve – at least to some extent – microgravity conditions on the ground. Altering the influence of the fundamental force of gravity, which has been constantly and permanently acting during the evolution of life, is quite an old idea. An early description of a clinostat for nullifying the influence of gravity was given in 1879 by von Sachs, and was further developed by electrical powering by Newcombe (1904), who also critically discussed its limitations. The idea of a 3D rotation, which means rotation around two axes, with the aim of simulating microgravity conditions especially for larger samples, emerged around half a century ago (Scano 1963) and was further developed and intensively studied, with an initial focus on plant research (Hoson et al. 1992, 1997). These kinds of rotation devices are based on the assumption that biological systems need to be exposed to the gravity vector for a minimal period of time to allow them to adjust to it. If the gravity vector constantly changes its orientation, however, the object loses its sense of direc-

tion and thus shows a behavior similar to the one seen under real microgravity conditions. It is therefore crucial to set the rotation speed of the frames to be faster than the biological process studied (Mesland 1996a), but not so fast that other forces are introduced (Van Loon 2007).

Magnetic levitation is a further approach to modifying the gravitational force with the aim of simulating microgravity conditions. The principle is that a magnetic force counterbalances the gravitational force, meaning that a superconducting or resistive electromagnet is used to levitate diamagnetic materials, such as liquids, biological cells and tissues, and even small plants or animals.

Ground-based studies have great importance in gravitational and space biology and human physiology (Beysens and van Loon 2015; Van Loon et al. 1999). They contribute to our understanding of how biological systems (from cells to humans) sense gravity and to studies of the consequences if the influence of this fundamental force is lacking, the impact on health and signaling cascades, and also the mechanisms of adaptations to this new environmental condition. Herranz et al. (2013a) have pointed out that the ideal device has to be found for each biological sample and that the quality of the device as well as nomenclature in this field of research should be carefully considered.

Here, we give an overview of available ground-based microgravity simulators, which have been in use in the frame of ESA's Ground-Based Facility Programme, explain the underlying principles, and describe the devices and their experimental possibilities. We focus on facilities simulating microgravity conditions, thus the drop tower as a ground-based facility is excluded as it provides real microgravity conditions.

Ground-Based Facilities – The Principles

Clinostat Principle

A clinostat is defined as a device that rotates a sample in order to compensate for the influence of gravity. Clinostats have either one single horizontal axis of rotation (2D-clinostat) or two axes of rotation (3D-clinostat). In this chapter we focus on 2D-clinostats.

A 2D-clinostat is a device in which samples are located in the center of rotation and are continuously rotated around one axis perpendicular to the direction of the gravity vector. Consequently, the question arises of whether this results in an omnilateral gravistimulation or whether the system no longer perceives the gravity stimulus and thus experiences “microgravity”. Although slow rotation already leads to neutralization of clearly and easily observable gravireponses such as gravitropism of plants or gravitaxis of

free-moving organisms, resulting in random growth direction and random swimming directions, the gravity sensing systems may still be continuously mechanically stressed (Briegleb 1992; Häder et al. 2005; Hensel and Sievers 1980; Herranz et al. 2013a) if an adaptation to this new situation does not take place.

Taking thresholds and response times into account, clinostat speeds have been set up to fast rotations of 50–100 rpm (fast-rotating clinostat) (Briegleb 1992). This fast rotation results in a neutralization of sedimentation, as shown in Fig. 1. In a small rotating compartment, like a sample cuvette with a diameter of only a few millimeters, a cell is forced onto a circular path whose radius is defined by the speed of rotation. Fast rotation results in fewer relative movements compared to slow rotation (Herranz et al. 2013a) and an excessively high speed in centrifugal accelerations, which is also a reason to keep the effective radius at a minimum. The resulting centrifugal accelerations can be calculated by:

$$a = \omega^2 r,$$

with r = radius, ω = angular velocity, and a = acceleration

Studies with mammalian cells have shown that the speed of rotation has a direct effect on the cellular response. Horn et al. (2011) clinorotated macrophages at 60 and 2 rpm and performed online kinetic measurements of the oxidative burst reaction, an indicator of the physiological status of the cells. In other studies with cells of invertebrates (hemocytes of mussels) it was confirmed that cells responded with a decrease in reactive oxygen species (ROS) production under clinorotation at 60 rpm compared to 1 g , a result which was later verified in real microgravity during parabolic flight (Adrian et al. 2013; Unruh et al. 2015). However, slow rotation (2 rpm) led to abolishment of the signal, which was assumed to be a result of a constant stress due to the slow change in the direction of the gravity vector (Horn et al. 2011).

In general, it can be stated that the quality of the simulation is dependent on the speed of rotation, the effective diameter, and the graviresponse-time of the chosen test system. This has to be defined for each organism and requires the adaptation of the facilities to the demands of each experimental approach (Herranz et al. 2013a).

Random Positioning Machine (RPM) – Principle

The working principle of the Random Positioning Machine (RPM) is based on random rotation of the biological samples around two axes (Mesland et al. 1996b). Such movement causes a continuous reorientation of the gravity vector that is acting on the samples. A microgravity-like environment is then created by directing the rotation of the samples

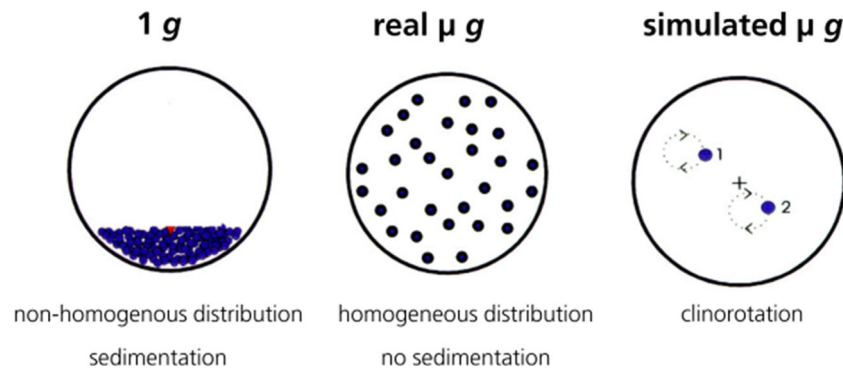


Fig. 1 Scheme of the 2D-clinostat principle. Objects will sediment under normal 1 *g* conditions due to density differences between the object and the surrounding medium. In free fall (real microgravity) objects are evenly distributed and sedimentation does not occur.

Clinorotation forces the objects onto circular paths whose geometries are defined by speed and distance from the center of rotation. Adapted from Häder et al. (2005)

such that, over time, the trajectory of the gravity vector points in all directions (from the samples' point of view). Averaged over time, the gravity vector converges to zero mathematically (Fig. 2). It is important that the movement of the samples is randomly generated in order to avoid a repetitive pattern to which the test samples may adapt.

To allow rotation of the samples in any direction, RPM systems usually employ two gimbal-mounted frames that can be turned independently (Figs. 9, 10 and 11). Dedicated algorithms designed to drive the frames ensure the rotation of the biological samples as described above. Because two axes are involved in the rotation of the samples, the RPM can be seen as a two-axis version of the clinostat and thus it is also called a 3D-clinostat.

The RPM has been shown to mimic microgravity responses of biological systems for several, but not all, experimental conditions. It seems, however, that certain cellular responses obtained by the RPM exposure are under- or overestimated when comparing them to the results gathered in real microgravity in space (reviewed in Wuest et al. 2015). Therefore, results obtained from the RPM have to be interpreted carefully and compared to experiments in real microgravity to fully assess their relevance.

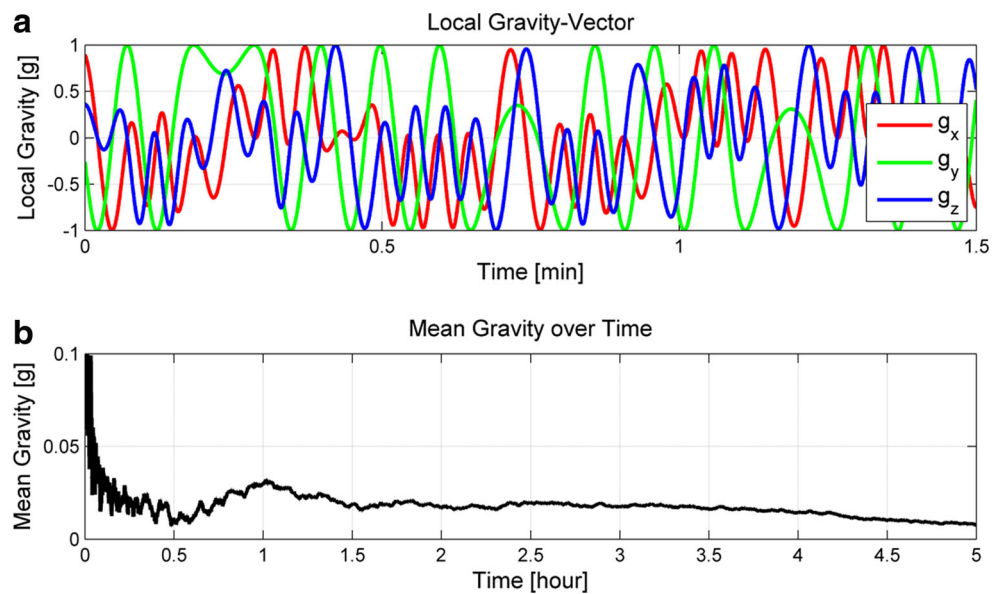
Magnetic Levitation Principle

Magnetic levitation is now well established as an attractive method of simulating microgravity conditions and is therefore one of the Earth-based alternatives to experiments in space. The basic idea is that a magnetic force F_m is used to counterbalance the gravitational force F_g (see Fig. 3) (Beaugnon and Tournier 1991a, b; Berry and Geim 1997; Valles et al. 1997). The magnetic force on a given material depends on the properties of the material (the so-called magnetic susceptibility χ) and the magnet used (the

field strength B and the field gradient B'), whereas the gravitational force depends on the density ρ of the material and the gravitational acceleration g . The magnetic levitation condition ($F_m = F_g$) does not depend on the volume of the object (Fig. 3) and is present down to the molecular level. A levitated droplet of a homogeneous liquid or a well-mixed solution behaves, therefore, similarly to a droplet in real microgravity. Magnetic levitation has been demonstrated for a large variety of substances, such as water, organic solvents (Beaugnon and Tournier 1991a, b), and cryogenic fluids (Weilert et al. 1996), but also for strawberries, nuts, and even animals, such as grasshoppers and frogs (Berry and Geim 1997). Over the years this has led to the worldwide installation of many superconducting and water-cooled electro-magnet systems dedicated to achieving magnetic levitation in reasonably large volumes for extended periods of time. An important design target for such magnets is the capability to, at least, levitate water (requiring a BB' value of 1360 T²/m, which can be reached in 15–17 T class magnets), which is important in view of applications in fluid dynamics (Beaugnon et al. 2001; Hill and Eaves 2008; Pacheco-Martinez et al. 2013; Lorin et al. 2015) and biology (Herranz et al. 2013b; Hill et al. 2012; Manzano et al. 2012; Moes et al. 2011; Valles et al. 1997).

Since the magnetic levitation depends on the strength (B) and spatial profile of the magnetic field (B'), this technique can also be used to tune the effective gravity that is experienced by the material. This unique feature has been used to modify gravity (Guevorkian and Valles 2006; Micali et al. 2012), realizing enhanced gravity and reduced gravity (including Lunar and Martian gravity, Valles Jr. et al. 2005). It is even possible to change the direction of the effective gravitational force to pointing upwards (inverted gravity) when the upward magnetic force is larger than the downward gravitational force (see also Fig. 3). Inverted gravity has proven useful in studying damping of convection during crystal growth (Poodt et al. 2005; Heijna et al. 2007)

Fig. 2 Illustration of the gravity values generated by the RPM. **a** Evolution of the actual gravity exposure of samples on the running RPM during a period of 1.5 minutes, drawn separately for each axis (x: red, y: green, z: blue). **b** Mean gravity value calculated from the values obtained under **a**. After about 30 minutes, the mean gravity level has converged quickly towards zero



and the selection of supramolecular chirality (Micali et al. 2012).

Despite the power of the magnetic levitation technique, it also shows some limitations. The levitation condition depends on the BB' product and the ρ/χ ratio (Fig. 3). The BB' product depends on the field strength and the position within a magnet, leading to spatial variations of the effective gravity. In practice, milligravity conditions can be realized in volumes of the order of approximately centimeters cubed. In mixed systems, such as objects in a solution, suspensions, or biological cells, the variation in the ρ/χ ratio might give rise to inhomogeneity in the effective gravity environment. Typically, the variation in ρ/χ amongst the

different biological systems can be as large as 10 %. The sensitivity to the ρ/χ ratio gives the opportunity to facilitate levitation by using a paramagnetic medium (Catherall et al. 2003; Guevorkian and Valles 2006; Ikezoe et al. 1998). Finally, the effects of the magnetic field itself should also be considered, such as magnetic field alignment and magnetic deformation of anisotropic materials (Denegre et al. 1998; Hemmersbach et al. 2014; Maret and Dransfeld 1985; Rikken et al. 2014). As with all ground-based microgravity simulation techniques, one should be aware of possible artifacts and perform sufficient control experiments (Herranz et al. 2013a).

Description of the Ground-Based Facilities

Clinostat Devices

Pipette/Cuvette Clinostat This type of clinostat accommodates sample containers such as cuvettes, tubes, or pipettes containing the organisms of interest. Configurations differ in terms of the number and diameter of the containers. At the German Aerospace Center (DLR) in Cologne (Germany), a pipette clinostat allows fixation of the samples during rotation (Fig. 4). Up to 10 pipettes (1 ml volume, \varnothing 3.5 mm) can be rotated in parallel with a speed between 0 and 90 rpm. The pipettes can be tilted during rotation to transfer the material directly into a fixative in order to avoid the effects of stopping the clinostat, thus preventing termination of the experiment under 1 g conditions. Several investigators have used this type of clinostat to expose suspended (mammalian) cells to simulated microgravity conditions and have gained knowledge on, for example, how immune cells respond to altered gravity conditions (Adrian et al. 2013; Brungs et al. 2015a; Paulsen et al. 2010).

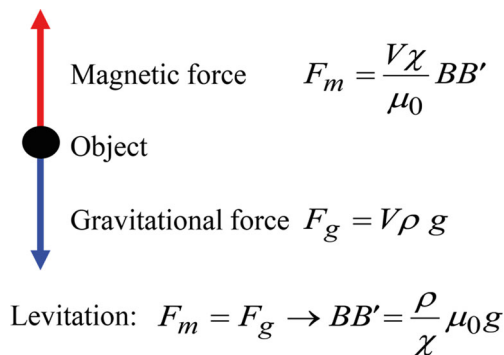
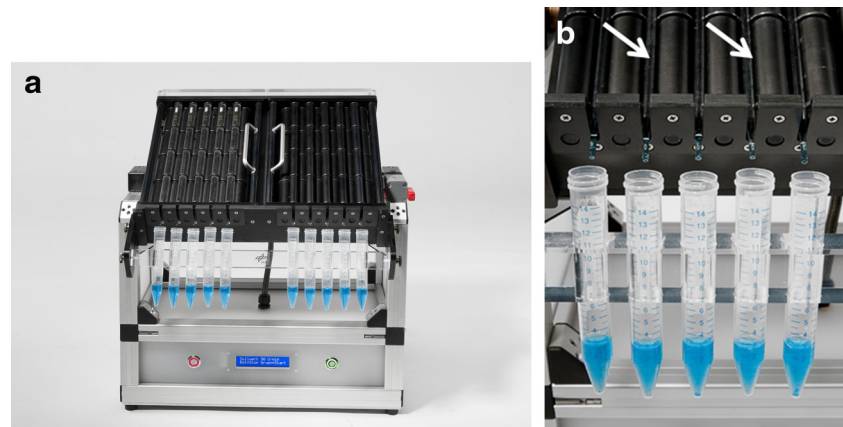


Fig. 3 Principle of magnetic levitation: a magnetic force F_m balances the gravitational force F_g . V : volume of the object; χ : magnetic susceptibility; μ_0 : magnetic constant; B : magnetic field strength; B' : magnetic field gradient; ρ : density of the object; g : gravitational acceleration. The levitation condition ($BB' = \rho\mu_0g/\chi$) is independent of the volume of the object, which means that gravity is compensated for down to the molecular level

Fig. 4 Pipette clinostat. The pipette holder can be tilted during rotation to transfer the sample immediately into the prepared fixative in order to avoid stopping and fixing under 1 g conditions. Arrows point exemplarily to two of the ten pipettes (design: Jens Hauslage, DLR, Cologne)

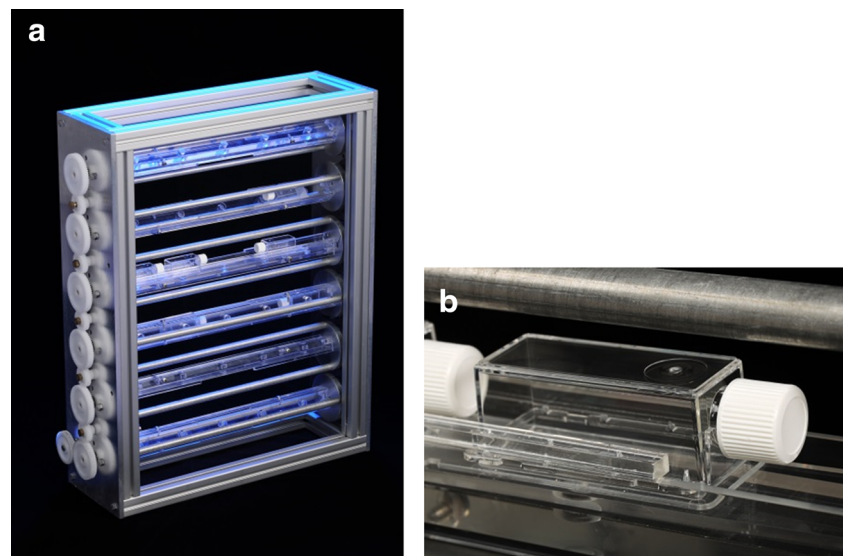


Slide-flask Clinostat This kind of clinostat is dedicated to studies with adherent cells. Slide flasks are widely used to cultivate adherent cells under standard laboratory conditions. After removal of the flask, the remaining slide with the grown and exposed cell layer can then undergo a fixation and staining protocol. Due to the small size of the slideflask and the ability to completely fill the flask with degassed medium, this container was adapted for use in a 2D-clinostat (Eiermann et al. 2013) (Fig. 5). Cells are grown in the flasks, which are completely filled with degassed medium and then mounted onto the device and rotated at a speed of 60 rpm. The slide of the flask is in the center of the rotation axis for optimal simulation conditions. Afterwards, the flask is removed and the cells on the slide can be used directly for microscopy or scraped off the slide to undergo any cell lysate protocol (e.g., for real-time PCR - Polymerase Chain Reaction). Clinorotation of human 1F6 melanoma cell line in slide flasks, which was thereby exposed in the ranges of

≤ 0.012 , ≤ 0.024 , or ≤ 0.036 g for 24 h, showed that the down-regulation of GC-A mRNA in cells depends on the residual acceleration values, with a maximal reduction at ≤ 0.012 g. Eiermann et al. (2013) clearly demonstrated that the radius of exposure is important and has to be carefully considered. Cells located off-center also experience inertial shear forces (Van Loon et al. 2003).

Photomultiplier Clinostat So far, clinorotation experiments have been performed in many cases as end-point measurements. The photomultiplier clinostat (Horn et al. 2011; Fig. 6) was invented in preparation for the TRIPLE LUX experiments in the BIOLAB of the Columbus module on ISS (Adrian et al. 2013; Hansen and Unruh 2005). In this experiment, a luminol-based reaction is used to visualize the production of ROS during host defense of mammalian and mussel immune cells. The photomultiplier clinostat enables on-line luminescence measurement during clinorotation and

Fig. 5 Slide-flask clinostat consisting of six rotating axes, each expected to sustain up to four slide-flasks (a). Detail showing a slide flask (b)



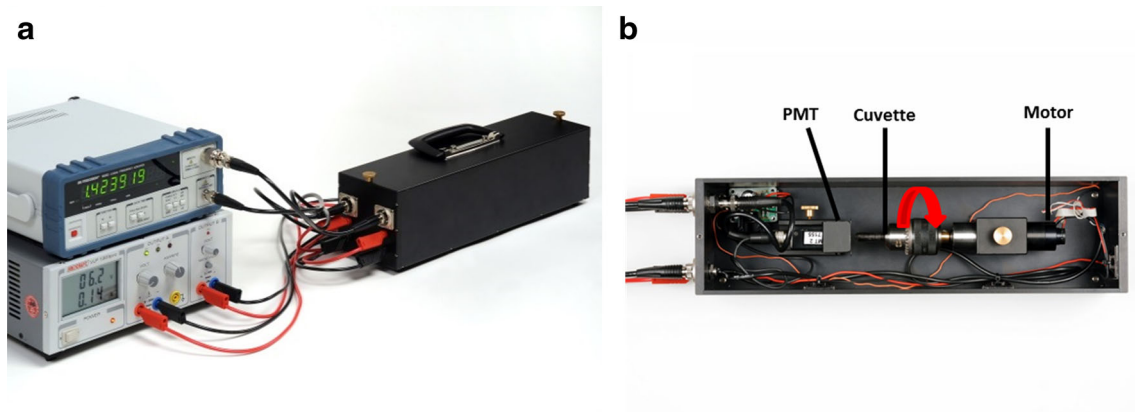


Fig. 6 Photomultiplier clinostat (*black box*) connected to a power supply and frequency counter (upper left) (**a**). Clinostat box consisting of a photomultiplier (PMT), sample cuvette, and motor (**b**)

under 1 *g*. This kind of clinostat is equipped with a photomultiplier tube to amplify and measure the photons emitted by the biological system (Fig. 6). Comparable studies in real microgravity during parabolic flights verified this kind of simulation approach. In both cases a significant reduction in ROS production was observed, while hypergravity revealed the opposite response (Adrian et al. 2013; Brungs et al. 2015a; Horn et al. 2011; Unruh et al. 2015).

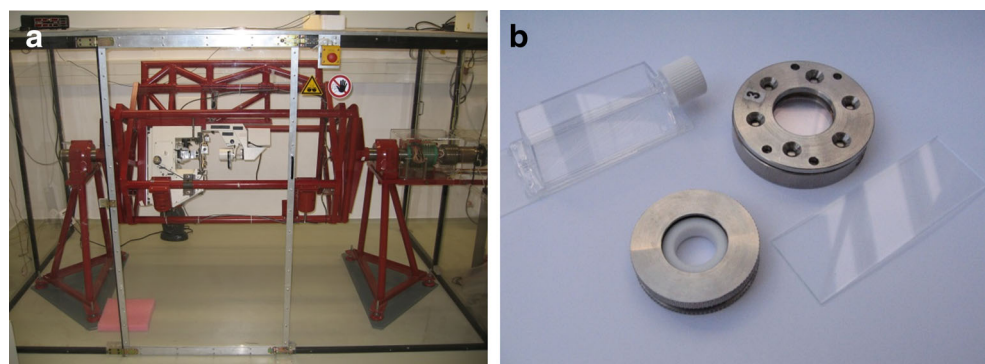
Clinostat Microscope On-line observation during clinorotation is achieved by the combination of a clinostat with a microscope (Fig. 7). The basis is a horizontally positioned microscope (Zeiss Axiovert) equipped with objectives providing from 5- to 63-fold magnification, a digital camera, phase-contrast unit, and video registration. The rotation speed can be selected from 2 to 90 rpm. Several investigators used this clinostat microscope to observe the behavior of their samples during clinorotation. As an example, unicellular organisms (ciliates, *Paramecium*) lost their gravitaxis in a time frame of 1–2 minutes during clinorotation

as well as in real microgravity, while the high linearity of their swimming paths was maintained (Hemmersbach et al. 1996). In contrast, exposure on the RPM showed an increase in directional turns and course corrections, indicating mechanical stimulation of the cells induced by this kind of experimental approach (Herranz et al. 2013a).

Submersed Clinostat Submersed (underwater) clinostats allow studies on aquatic organisms subjected to conditions of simulated microgravity. Six perforated tubes are mounted in an underwater cassette and rotated in parallel (Fig. 8). Each of the tubes has a diameter of 4 mm and can accommodate, for example, larval fish (Brungs et al. 2011) or water fleas (Fischer et al. 2015). The tubes have been modified (increased diameter) to accommodate mussels (Unruh et al. 2015). Each of the tubes is perforated and connected to a fresh water stream to guarantee oxygen supply.

Anken et al. (2015) used the submersed clinostat to study the adaptation of inner ear otoliths to simulated microgravity in the larvae of cichlid fish. They showed in

Fig. 7 Microscope mounted into the clinostat frame. The optical path is identical to the horizontal rotation axis (**a**). Different chambers, slide flasks, and microscope slides are in use for observation of the samples during clinorotation (**b**)



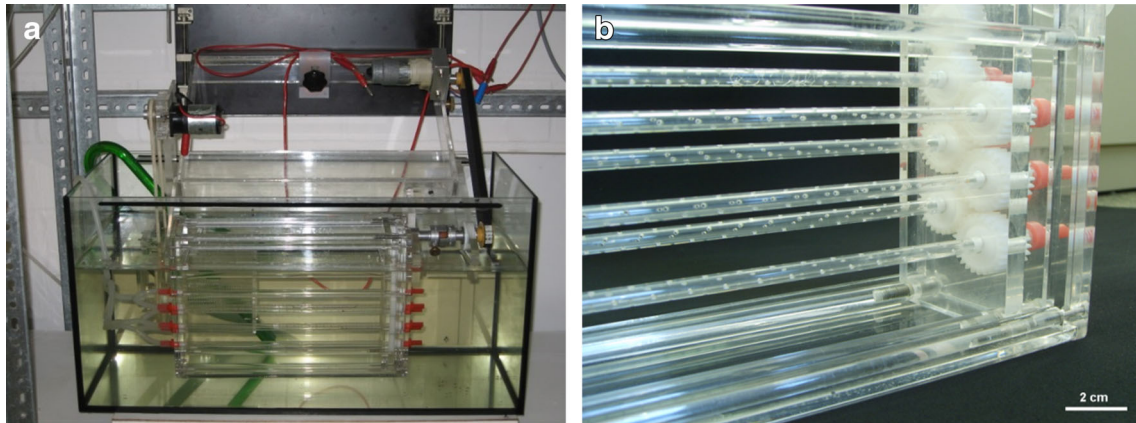


Fig. 8 Submersed clinostat in an aquarium (a) and detailed view of the tubes (b)

their study that clinorotation produced significantly larger otoliths compared to the 1 *g* control but identical to a FOTON satellite mission (Anken et al. 2015).

Random Positioning Machine (RPM) Devices

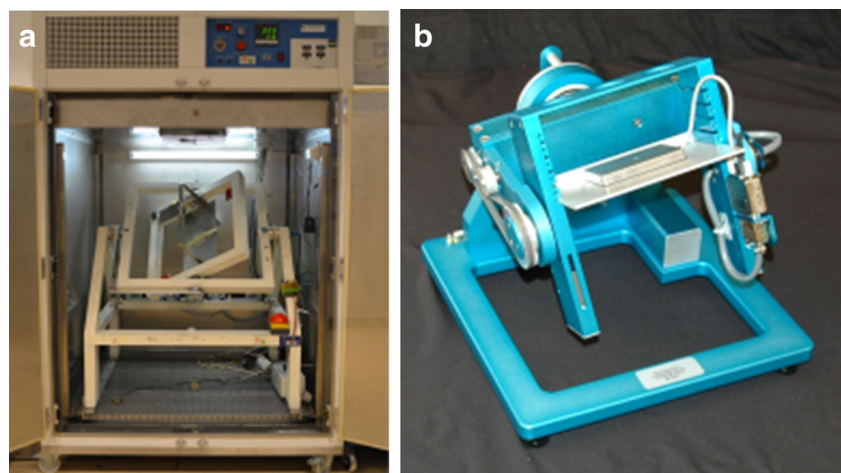
The use of the RPM principle to simulate microgravity was already mentioned in the early 1960s (Scano 1963). Its application, however, became popular after the former Fokker Space introduced a commercial product in 1997 (as shown in Fig. 9a). This model offered basically a simulated microgravity environment for experimental hardware, culture flasks, and so on up to a weight of 20 kg. Furthermore, power as well as communication to the rotating platform was made available by slip rings. The rotation speed of the frames can be adjusted between 30 and 360°/s for both frames (3D random walk) or for one frame only (2D-clinostat mode) (Borst and van Loon 2009).

It is assumed that the size of the sample flasks within an RPM is less critical compared to other kind of simulators. The quality of the simulated microgravity stays high and only small residual forces are introduced when moderate rotational velocities are selected (typically 60°/s) and the samples are placed within a radius of about 10 cm around the center of rotation (Van Loon 2007; Wuest et al. 2014, 2015). Comparative studies and the choice of appropriately sensitive parameters will also validate this kind of simulation approach.

RPM Adaptation for Mammalian Cell Cultures

Biomedical studies are often conducted on mammalian cell culture samples. In order to conduct such cell culture experiments, the RPM machine has to be operated in a climate-controlled room. For this purpose, Fokker Space introduced a small desktop RPM (Fig. 9) that can fit into a standard

Fig. 9 The very first full-scale RPM fitted in a custom fully controlled incubator located at the Life & Physical Science, Instrumentation and Life Support Laboratory (ESA-ESTEC, TEC-MMG, Noordwijk, the Netherlands) (a). Novel benchtop RPM for microgravity and partial gravity simulation (Dutch Space, Leiden, the Netherlands) (b)



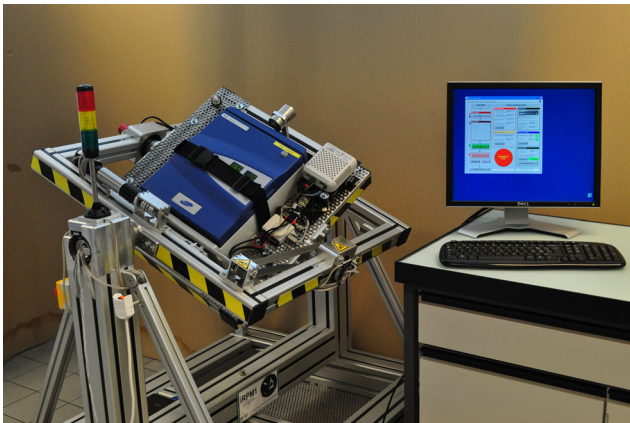


Fig. 10 Random Positioning Incubator (RPI) built by the University of Applied Sciences Northwestern Switzerland in collaboration with the Center of Competence in Aerospace Biomedical Science and Technology of the Lucerne University of Applied Sciences and Arts

laboratory incubator (max. size $50 \times 50 \times 50$ cm) (Fig. 9b). The Competence Center Aerospace Biomedical Science and Technology followed another approach by installing a commercial CO_2 incubator onto the rotating frames of a custom made RPM (Fig. 10). This RPM, called a “Random Positioning Incubator” (RPI) (Benavides Damm et al. 2014; Wuest et al. 2014) has the advantage of having a larger sample-holding capacity than the desktop RPM of Fokker Space and is independent of laboratory incubators.

In order to further optimize the system by combining the advantages of having a compact RPM running in a laboratory incubator and running numerous samples in parallel under simulated microgravity conditions, another type of RPM, called the “Microgravity Incubator” (MGI) (Fig. 11), was developed. This type of RPM is unique in having

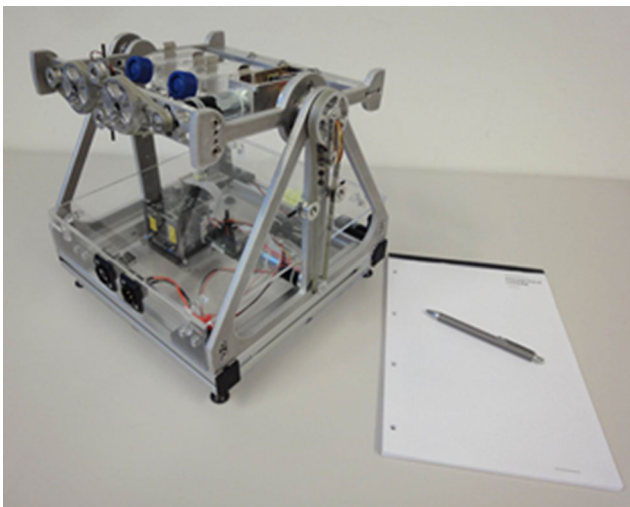


Fig. 11 Microgravity incubator (MGI) built by Lucerne University of applied sciences and arts



Fig. 12 A picture of a 33 T poly-Bitter magnet of the High Field Magnet Laboratory, Nijmegen. The magnet consists of four electrical coils mounted inside a 1m-diameter, 1m-high cylindrical housing. At maximum field, the electrical current is about 37000 A using a power of 17 MW. The magnet is cooled by cold water (12°C) at a flow rate of 145 l/s. Access to samples and *in situ* measurement equipment is possible from above and below the magnet, inside a 32 or 50 mm-diameter vertical bore

multiple inner rotating axes in parallel. This design ensures that all cell culture flasks are placed at the center of rotation and are thus treated in exactly the same way. Because of the very compact concept, the MGIs can be operated inside incubators as well and thus provide ideal environmental culture conditions for the living cells, comparable to the Dutch Space desktop RPM.

RPM Adaptation for Live Cell Imaging

Applying optical techniques and following the changes in real-time is very useful in detecting cellular responses to microgravity or simulated microgravity exposure. The RPM, however, provides unfortunate conditions for taking high-magnification images while running. The Competence Center Aerospace Biomedical Science and Technology thus combined the technique of Digital Holographic Microscopy (DHM) with an epifluorescent microscope to allow optical analysis of cells while on the operating RPM. The DHM is an innovative interferometric microscope that is less sensitive to vibrations due to its fast and continuous digital autofocusing system. This enables refocusing of the samples

Table 1 Examples and main outcomes from projects in the frame of the ESA Ground-Based Facility Programme

Cell type	Ground-Based Facility	Main outcome	Reference
Human chondrocytes	RPM 60°/s, real random mode 2-D clinostat 60 rpm	Scaffold-free cultivation of chondrocytes for 7 d on the RPM and the clinostat resulted in spheroid formation, accompanied by changes in the cytoskeleton.	Aleshcheva et al. 2015
Human thyroid carcinoma cells (FTC-133)	RPM 60°/s, real random mode 2-D clinostat 60 rpm	Speroid formation Increased cytokine release on the RPM and a decreased one on the clinostat.	Warnke et al. 2015
Mouse embryonic stem cells (CGR8)	2-D clinostat 60 rpm	Hardware development: Pipette-based method suited to study embryoid body formation in pluripotent stem cells under clinorotation.	Shinde et al. 2015
Microcrustaceans (<i>D. magna</i> ; <i>D. cucullata</i> , <i>H. incongruens</i>)	Submersed 2-D clinostat 60 rpm	Clinorotation does not induce acceleration in form of (predator resembling) small scale turbulence, which can be perceived by <i>Daphnia</i> . Normal embryonic development (<i>D. magna</i> , <i>H. incongruens</i>).	Fischer et al. 2015
Larval cichlid fish <i>O. mossambicus</i>	Submersed 2-D clinostat 60 rpm	Real microgravity FOTON M-3 enhanced growth of inner ear otoliths, whereas clinorotation did not fully mimic the conditions from spaceflight.	Anken et al. 2015
Blue mussel <i>M. edulis</i> cryo-conserved primary cells	Submersed 2-D clinostat 60 rpm Centrifuge 1.8 g	Reactive oxygen species production during immune response is reduced during clinorotation, centrifugation and parabolic flight.	Unruh et al. 2015
Human thyroid cells (Nthyori 3-1)	Centrifuge 1.8 g	Hypergravity alters gene expression. Control experiments for space studies.	Wehland et al. 2015
Wt and YC3.6 mutant (cell suspension) <i>A. thaliana</i>	Centrifuge 3 g	Decrease in Ca ²⁺ levels at 3 g compared to 1 g	Neef et al. 2015
<i>A. thaliana</i> (cell suspension)	Diamagnetic levitation	Magnetic levitation in suspension cultures is not possible	Kamal et al. 2015
<i>A. thaliana</i> (7d old)	RPM used as 2-D clinostat	Newly developed plant “ARADISH” hardware	Schüler et al. 2015
Rat macrophages (NR8383) <i>R. norvegicus</i>	Centrifuge 1.8 g 2-D clinostat 60 rpm	The consecutive use of clinostat and centrifuge is technically feasible but only suited for slow responding organisms to simulate parabolic flight g-profiles.	Brungs et al. 2015b

Table 1 (continued)

Cell type	Ground-Based Facility	Main outcome	Reference
<i>A. thaliana</i> (roots)	2-D clinostat 60 rpm	Compensation of statolith sedimentation and subsequent auxin redistribution	Wang et al. 2015
<i>A. thaliana</i> (cell suspension)	Real (parabolic flight) and simulated (clinostat) partial gravity (0.38 g, 0.16 g, 0 g)	Molecular responses to partial-g are in part different from μg ; clinorotation can induce changes in levels of transcripts similar to μg .	Fengler et al. 2015

for high-resolution imaging taken by the conventional epifluorescent microscope (Pache et al. 2010; Toy et al. 2010, 2012a, b).

RPM Adaptation for Partial Gravity Experiments

Instead of averaging the gravity vector to zero (Fig. 2b), the RPM rotation algorithm (as explained above) can be adjusted to average the vector to any value between 0 and 1 g (Benavides Damm et al. 2014). Various algorithms have been developed to simulate partial gravity (0 to 1 g) and thus the situation which is for example present on the Moon or Mars can be minimized (Benavides Damm et al. 2014; Manzano et al. 2014).

Partial gravity is achieved basically by altering the random walk of the RPM in such a way that the Earth's gravity vector is no longer completely randomized and points (from the sample's point of view) in a specific direction for a prolonged period of time. In the paper by Benavides Damm et al. (2014), three algorithms were tested on suspended human T cells and adherent mice myoblasts. The results revealed a decreased response of the cells that correlated strongly with the decreasing simulated mean gravity values (Benavides Damm et al. 2014).

Usability of RPM Devices

It can easily be demonstrated mathematically that the mean gravity value converges to zero over time when distributing it in all directions (Fig. 2a and b). The accelerations caused by the RPM's continuous rotation of the samples can be mathematically described as well (Hasenstein and van Loon 2015; Van Loon 2007; Wuest et al. 2014, 2015). As a rule of thumb, artifacts can be avoided by keeping the rotational velocity, the sample's distance from the center of rotation, and the rotational acceleration (during velocity transitions) minimal (Van Loon 2007; Wuest et al. 2015). Besides the residual accelerations, the rotation of the samples on the

RPM introduces fluid motion in the culture flask, which leads to shear forces and enhanced convection inside the flasks. Therefore the nutrition supply of cells on the RPM is enhanced as compared to static control experiments or experiments conducted in real microgravity. To reduce the influence of additional mechanical stimulation such as shear stress, a moderate rotational velocity needs to be chosen and the velocity transitions have to be smooth (Leguy et al. 2011).

Magnetic Levitation

Within the ESA GBF Programme, magnetic levitation experiments have been performed using a 17 T superconducting magnet at the University of Nottingham (United Kingdom) and several 30 T class water-cooled Bitter Magnets at the High Field Magnetic Laboratory of the Radboud University (Nijmegen, the Netherlands, see Fig. 12, Perenboom et al. 2013). These magnets have a bore size of 30–50 mm and offer a thermostatic sample environment, equipped with several *in situ* measurement techniques, such as video imaging (Hill and Eaves 2008; Hill et al. 2012) and different types of optical microscopy (Heijna et al. 2007; Hemmersbach et al. 2014; Herranz et al. 2013a; Poodt et al. 2005).

Conclusion

The European Programme for Life and Physical Sciences (ELIPS) of the European Space Agency provides a broad spectrum of unique mission opportunities for Life and Physical Sciences. Since 2009 a relatively small but increasingly important part of ELIPS is the special Ground-Based Facility (GBF) element which has been providing the scientific user community with the ability to access a large variety of European research laboratories and institutions that simulate some of the conditions that can be found in the space environment. The wide range of research capabilities offered by

the GBF programme does not only improve the preparation of space experiments, but can also yield further scientific knowledge of the basic influence of gravity or other space or planetary conditions on life-, physical-, and interdisciplinary processes in general. An elaborate ground-based research programme is of vital importance for building up more detailed knowledge about a system in preparation for an actual space flight as well as for standalone basic research.

The ESA Ground-Based Facility Programm gave scientists the possibility to prepare their space experiments by using facilities aiming to provide functional weightlessness (simulated microgravity) - magnetic levitation, fast 2-D clinorotation and 3-D random positioning were the methods chosen Table 1. Major differences between these approaches are the underlying physical principles and hypotheses – compensation of the gravity field by a magnetic field or randomization of the influence of gravity by either constant unidirectional fast clinorotation versus random positioning by means of changing speed and direction (RPM). Verification and final validation needs comparable experiments in true (real) microgravity, which is still lacking in most of the experiments. Whether microgravity is simulated in an appropriate manner can finally be answered, if a corresponding experiment is performed in real microgravity. Examples in the frame of GBF projects showing this direct comparisons are the reduced ROS production of hemocytes of the Blue mussel *M. edulis* (Unruh et al. 2015) and of macrophages (NR8383) of *R. norvegicus* (Brungs et al. 2015a; Adrian et al. 2013) revealing a decreased immune response under clinorotation and in real microgravity.

The programme provided further experiences in using microgravity simulators and initiated new hardware developments for the adaptation of the specific experiments (Schüler et al. 2015; Shinde et al. 2015) and applications, such as attempts to simulate partial gravity (Fengler et al. 2015) and of parabolic flight profiles (Brungs et al. 2015b). While clinostat experiments were performed by exposing samples in a small radius around the rotation axis, e.g. in pipettes with a radius of 3.5 mm (Shinde et al. 2015), the size of the sample containers within an RPM is considered to be less critical according to the developers that even within a radius of 10 cm and moderate rotational velocities (typically 60°/s) only small residual forces are induced (van Loon 2007; Wuest et al. 2014, 2015). Sample size determines experimental time as exchange of medium or life support systems are necessary for prolonged cultivation, which limits ground-based approaches. Even new fields of applications such as tissue engineering (Aleshcheva et al. 2015) have been promoted by the ESA ground-based programme.

The advantage of using ground-based facilities has considerably increased the potential for experimenting under

simulated space conditions as compared to experiments under real space and microgravity mission opportunities, which at present are still limited. Sometimes also complementary parameter studies or further post-flight experiments are performed which increase the yield of the flight experiments. The projects performed in the frame of the ESA GBF programme demonstrate its high efficiency providing the basis for excellent space experiments.

References

- Adrian, A., Schoppmann, K., Sromicki, J., Brungs, S., von der Wiesche, M., Hock, B., Kolanus, W., Hemmersbach, R., Ullrich, O.: The oxidative burst reaction in mammalian cells depends on gravity. *Cell. Commun. Signal* **11**, 98 (2013)
- Aleshcheva, G., Bauer, J., Hemmersbach, R., Egli, M., Grimm, D.: Tissue Engineering of cartilage on ground-based facilities. *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9479-0
- Anken, R., Bauer, U., Hilbig, R.: Clinorotation increases the growth of utricular otoliths of developing cichlid fish. *Microgravity Sci. Technol.* **22**(2), 151–154 (2015)
- Anken, R., Brungs, S., Grimm, D., Knie, M., Hilbig, R., Fish inner otolith growth under real microgravity (space-flight) and clinorotation: *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9459-4
- Beaugnon, E., Tournier, R.: Levitation of water and organic substances in high static magnetic fields. *J. Phys. III France* **1**, 1423–1428 (1991a)
- Beaugnon, E., Tournier, R.: Levitation of organic materials. *Nature* **349**, 6309 (1991b)
- Beaugnon, E., Fabregue, D., Billy, D., Nappa, J., Tournier, R.: Dynamics of magnetically levitated droplets. *Physica B* **294**, 715–720 (2001)
- Benavides Damm, T., Walther, I., Wüest, S.L., Sekler, J., Egli, M.: Cell cultivation under different gravitational loads using a novel random positioning incubator. *Biotechnol. Bioeng.* **111**(6), 1180–1190 (2014)
- Berry, M.V., Geim, A.K.: Of flying frogs and levitrons. *Eur. J. Phys.* **18**, 307–313 (1997)
- Beysens, D.A., van Loon, J.J.W.A. (eds.): Generation and applications of extra-terrestrial environments on earth. River Publishers, Aalborg (2015). ISBN: 978-87-93237-53-7
- Borst, A., van Loon, J.J.W.A.: Technology and developments for the random positioning machine, RPM. *Microgravity Sci. Technol.* **21**(4), 287–292 (2009)
- Briegleb, W.: Some qualitative and quantitative aspects of the fast-rotating clinostat as a research tool. *ASGSB Bull* **5**, 23–30 (1992)
- Brungs, S., Hauslage, J., Hilbig, R., Hemmersbach, R., Anken, R.: Effects of simulated weightlessness on fish otolith growth: clinostat versus rotating-wall vessel. *Adv. Space Res.* **48**, 792–798 (2011)
- Brungs, S., Kolanus, W., Hemmersbach, R.: Syk phosphorylation – a gravisensitive step in macrophage signaling. *Cell Commun. Signal* **13**(1), 9 (2015a)
- Brungs, S., Petrat, G., von der Wiesche, M., Anken, R., Kolanus, W., Hemmersbach, R.: Simulating parabolic flight like g-profiles on ground - a combination of centrifuge and clinostat. *Microgravity Sci. Technol.* (2015b). doi:10.1007/s12217-015-9458-5
- Catherall, A.T., Eaves, L., King, P.J., Booth, R.: Floating gold in cryogenic oxygen. *Nature* **422**, 579 (2003)

- Denegre, J.M., Valles Jr., J.M., Lin, K., Jordan, W.B., Mowry, K.L.: Cleavage planes in frog eggs are altered by strong magnetic fields. *Proc. Natl. Acad. Sci. USA* **95**, 14729–14732 (1998)
- Eiermann, P., Kopp, S., Hauslage, J., Hemmersbach, R., Gerzer, R., Ivanova, K.: Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells. *Microgravity Sci. Technol.* **25**, 153–159 (2013)
- Fengler, S., Spirer, I., Neef, M., Ecke, M., Hauslage, J., Hampp, R.: changes in gene expression of *Arabidopsis thaliana* cell cultures upon exposure to real and simulated partial-g forces. *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9452-y
- Fischer, J., Schoppmann, K., Knie, M., Laforsch, C.: Responses of microcrustaceans to simulated microgravity (2D-clinorotation) - preliminary assessments for the development of Bioregenerative Life Support Systems (BLSS). *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9470-9
- Guevorkian, K., Valles, J.M.: Swimming *Paramecium* in magnetically simulated enhanced, reduced, and inverted gravity environments. *PNAS* **103**, 13051–13056 (2006)
- Häder, D.P., Hemmersbach, R., Lebert, M.: Gravity and the Behavior of Unicellular Organisms. Cambridge University Press, Cambridge (2005)
- Hansen, P.-D., Unruh, E.: TRIPLE LUX – B: Phagocytosis in mussel hemocytes. Proc. 9th Eur. Symp. Life Sciences Research in Space. 26th Annu. Int. Gravitational Physiology Meeting. Cologne, Germany, ESA SP – 585 (2005)
- Hasenstein, K.H., van Loon, J.J.W.A.: Clinostats and other rotating systems—Design, function, and limitations. In: Beysens, D.A., van Loon, J.J.W.A. (eds.) Generation and Applications of Extra-Terrestrial Environments on Earth. River Publishers, Aalborg (2015)
- Heijna, M.C.R., Poodt, P.W.G., Tsukamoto, K., de Grip, W.J., Christianen, P.C.M., Maan, J.C., Hendrix, J.L.A., van Enckevort W.J.P., Vlieg, E.: Magnetically controlled gravity for protein crystal growth. *Appl. Phys. Lett.* **90**, 264105 (2007)
- Hemmersbach, R., Voormans, R., Häder, D.P.: Graviresponses in *Paramecium bicaurelia* under different accelerations: studies on the ground and in space. *J. Exp. Biol.* **199**, 2199–2205 (1996)
- Hemmersbach, R., Simon, A., Waßer, K., Hauslage, J., Christianen, P.C.M., Albers, P.W., Lebert, M., Richter, P., Alt, W., Anken, R.: Impact of a high magnetic field on the orientation of gravitactic unicellular organisms – A critical consideration about the application of magnetic fields to mimic functional weightlessness. *Astrobiology* **14**, 205–215 (2014)
- Hensel, W., Sievers, A.: Effects of prolonged omnilateral gravistimulation on the ultrastructure of statocytes and on the graviresponseroots. *Planta* **150**, 338–346 (1980)
- Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C., Geest, M., Hauslage, J., Hilbig, R., Hill, R., Lebert, M., Medina, F., Vagt, N., Ullrich, O., van Loon, J., Hemmersbach, R.: Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. *Astrobiology* **13**(1), 1–17 (2013a)
- Herranz, R., Manzano, A.I., van Loon, J.J.W.A., Christianen, P.C.M., Medina, J.F.: Proteomic signature of *Arabidopsis* cell cultures exposed to magnetically induced hyper- and microgravity environments. *Astrobiology* **13**, 217–224 (2013b)
- Hill, R.J.A., Eaves, L.: Nonaxisymmetric shapes of a magnetically levitated spinning water droplet. *Phys. Rev. Lett.* **101**, 234501 (2008)
- Hill, R.J.A., Larkin, O.J., Dijkstra, C.E., Manzano, A.I., de Juan, E., Davey, M.R., Anthony, P., Eaves, L., Medina, J.F., Marco, R., Herranz, R.: Effect of magnetically simulated zero-gravity and enhanced gravity on the walk of the common fruit fly. *J. R. Soc. Interface* **9**, 1438–1449 (2012)
- Horn, A., Ullrich, O., Huber, K., Hemmersbach, R.: PMT (photomultiplier) clinostat. *Microgravity Sci. Technol.* **23**, 67–71 (2011)
- Hoson, T., Seiichiro, K., Masuda, Y., Yamashita, M.: Changes in plant growth processes under microgravity conditions simulated by a three-dimensional clinostat. *Bot. Mag. Tokyo* **105**(1), 53–70 (1992)
- Hoson, T., Kamisaka, S., Masuda, Y., Yamashita, M., Buchen, B.: Evaluation of the three-dimensional clinostat as a simulator of weightlessness. *Planta* **203**(1), 187–197 (1997)
- Ikezoe, Y., Hirota, N., Nakagawa, J., Kitazawa, K.: Making water levitate. *Nature* **393**, 749–750 (1998)
- Kamal, K.Y., Herranz, R., van Loon, J.J.W.A., Christianen, P.C.M., Medina, J.F.: Evaluation of simulated microgravity environments induced by diamagnetic levitation of plant cell suspension cultures. *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9472-7
- Leguy, C.A., Delfos, R., Mathieu, J.B.M., Pourquie, Ch.P., Krooneman, J., Westerweel, van Loon, J.J.W.A.: Fluid motion for microgravity simulations in a random positioning machine. *Gravit. Space Biol. Bull.* **25**(1), 36–39 (2011)
- Lorin, C., Hill, R.J.A., Mailfert, A.: Magnetic levitation. In: Beysens, D.A., van Loon, J.J.W.A. (eds.) Generation and Applications of Extra-Terrestrial Environments on Earth. River Publishers, Aalborg (2015)
- Manzano, A.I., van Loon, J.J.W.A., Christianen, P.C.M., Gonzalez-Rubio, J.M., Medina, J.F., Herranz, R.: Gravitational and magnetic field variations synergize to reveal subtle variations in the global transcriptional state of *Arabidopsis* in vitro callus cultures. *BMC Genom* **13**, 105 (2012)
- Manzano, A., den Toom, A., Dowson, A., Valbuena, M.A., Medina, F.J., Herranz, R., van Loon, J.J.W.A.: Progressive effects from simulated microgravity to hypergravity on cell growth and proliferation and on gene expression in the Brassicaceae family. In: 30th Annu. American Society for Gravitational and Space Research (ASGSR) Conf., Pasadena, CA, USA (2014)
- Maret, G., Dransfeld, K.: Biomolecules and polymers in high steady magnetic fields. In: Herlach, F. (ed.) Topics in Applied Physics, vol. 57: Strong and Ultrastrong Magnetic Fields and their Applications, pp. 143–204. Springer, NY (1985)
- Mesland, D.: Novel ground-based facilities for research in the effects of weight. *ESA Microgravity News* **9**, 5–10 (1996a)
- Mesland, D., Anton, A., Willemsen, H., van den Ende, H.: The Free Fall Machine—a ground-based facility for microgravity research in life sciences. *Microgravity Sci. Technol.* **9**(1), 10–14 (1996b)
- Micali, N., Engelkamp, H., van Rhee, P.G., Christianen, P.C.M., Monsù Scolaro, L., Maan, J.C.: Selection of supramolecular chirality by application of rotational and magnetic forces. *Nat. Chem.* **4**, 201–207 (2012)
- Moes, M.J.A., Gielen, J.C., Bleichrodt, R., van Loon, J.J.W.A., Christianen, P.C.M., Boonstra, J.: Simulation of microgravity by magnetic levitation and random positioning: effect on human A431 cell morphology. *Microgravity Sci. Technol.* **23**, 249–261 (2011)
- Neef, M., Denn, T., Ecke, M., Hampp, R.: Intracellular calcium decrease upon hyper gravity-treatment of *Arabidopsis thaliana* cell cultures. *Microgravity Sci. Technol.* (2015). doi:10.1007/s12217-015-9457-6
- Newcombe, F.C.: Limitations of the clinostat as an instrument for scientific research. *Science* **20**, 376–379 (1904)
- Pache, C., Kühn, J., Westphal, K., Fatih Toy, M., Parent, J., Büchi, O., Franco-Obregón, A., Depeursinge, C., Egli, M.: Digital holographic microscopy real-time monitoring of cytoarchitectural alterations during simulated microgravity. *J. Biomed. Opt.* **15**(2), 026021 (2010)

- Pacheco-Martinez, H.A., Liao, L., Hill, R.J.A., Swift, M.R., Bowley, R.M.: Spontaneous orbiting of two spheres levitated in a vibrated liquid. *Phys. Rev. Lett.* **110**, 154501 (2013)
- Paulsen, K., Thiel, C., Timm, J., Schmidt, P., Huber, K., Tauber, S., Hemmersbach, R., et al.: Microgravity-induced alterations in signal transduction in cells of the immune system. *Acta Astronaut* **67**, 1116–1125 (2010)
- Perenboom, J.A.A.J., Maan, J.C., van Breukelen, M.R., Wiegers, S.A.J., den Ouden, A., Wulffers, C.A., van der Zande, W.J., Jongma, R.T., van der Meer, A.F.G., Redlich, B.: Developments at the high field magnet laboratory in Nijmegen. *J. Low. Temp. Phys.* **170**, 520–530 (2013)
- Poodt, P.W.G., Heijna, M.C.R., Tsukamoto, K., de Grip, W.J., Christianen, P.C.M., Maan, J.C., van Enkevort, W.J.P., Vlieg, E.: Suppression of convection using gradient magnetic fields during crystal growth of NiSO₄·6H₂O. *Appl. Phys. Lett.* **87**, 214105 (2005)
- Rikken, R.S.M., Nolte, R.J.M., Maan, J.C., van Hest, J.C.M., Wilson, D.A., Christianen, P.C.M.: Manipulation of micro- and nanostructure motion with magnetic fields. *Soft Matter* **10**, 1295–1308 (2014)
- Scano, A.: Effetti di una variazione continua del campo gravitazionale sullo sviluppo ed accrescimento di *Lathyrus Odoratus*. Communication presented at 6th Int. and 12th Eur. Congr. Aeronautical and Space Medicine, Rome (1963)
- Schüler, O., Krause, L., Görög, M., Hauslage, J., Kessler, L., Böhmer, M., Hemmersbach, R.: ARADISH – Development of a standardized plant growth chamber for experiments in gravitational biology using ground-based facilities. *Microgravity Sci. Technol.* (2015). doi:[10.1007/s12217-015-9454-9](https://doi.org/10.1007/s12217-015-9454-9)
- Shinde, V., Brungs, S., Hescheler, J., Hemmersbach, R., Sachinidis, A.: Pipette-based method to study embryoid body formation derived from mouse and human pluripotent stem cells partially recapitulating early embryonic development under simulated microgravity conditions. *Microgravity Sci. Technol.* (2015). doi:[10.1007/s12217-015-9469-2](https://doi.org/10.1007/s12217-015-9469-2)
- Toy, M.F., Parent, J., Kühn, J., Egli, M., Depeursinge, C.: Dual-mode digital holographic and fluorescence microscopy for the study of morphological changes in cells under simulated microgravity. *Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XVII*, 7570–7573 (2010)
- Toy, M.F., Kühn, J., Richard, S., Parent, J., Egli, M., Depeursinge, C.: Accelerated autofocusing of off-axis holograms using critical sampling. *Opt. Lett.* **37**(24), 5094–5096 (2012a)
- Toy, M.F., Richard, S., Kühn, J., Franco-Obregón, A., Egli, M., Depeursinge, C.: Enhanced robustness digital holographic microscopy for demanding environment of space biology. *Biomed. Opt. Express* **3**(2), 313–326 (2012b)
- Unruh, E., Brungs, S., Langer, S., Bornemann, G., Frett, T., Hansen, P.-D.: Comprehensive study of the influence of altered gravity on the oxidative burst of mussel (*Mytilus edulis*) hemocytes. *Microgravity Sci. Technol.* (2015). doi:[10.1007/s12217-015-9438-9](https://doi.org/10.1007/s12217-015-9438-9)
- Valles, J.M., Lin, K., Denegre, J.M., Mowry, K.L.: Stable magnetic field gradient levitation of *Xenopus laevis*: Toward low-gravity simulation. *Biophys. J.* **73**, 1130–1133 (1997)
- Valles Jr., J.M., Maris, H.J., Seidel, G.M., Tang, J., Yao, W.: Magnetic levitation-based Martian and Lunar gravity simulator. *Adv. Space Res.* **36**, 114–118 (2005)
- Van Loon, J.J.W.A., Veldhuijzen, J.P., Kiss, J., Wood, C., van de Ende, H., Guntemann, A., Jones, D., de Jong, H., Wubbels, R.: Microgravity research starts on the ground! Apparatus for long term ground based hypo- and hypergravity studies. In: Wilson, A. (ed.) *ESA SP-433*, pp. 415–419. ESTEC Noordwijk, the Netherlands (1999)
- Van Loon, J.J.W.A., Folgering, E.H.T.E., Bouten, C.V.C., Veldhuijzen, J.P., Smit, T.H.: Inertial shear forces and the use of centrifuges in gravity research. What is the proper control? *ASME J. Biomech. Eng.* **125**(3), 342–346 (2003)
- Van Loon, J.J.W.A.: Some history and use of the random positioning machine, RPM, in gravity related research. *Adv. Space Res.* **39**(7), 1161–1165 (2007)
- von Sachs, F.G.J.R.: Über Ausschliessung der geotropischen und heliotropischen Krümmungen während des Wachstums. *Würzburger Arbeiten* **2**, 209–225 (1879)
- Wang, H., Li, X., Krause, L., Görög, M., Schüler, O., Hauslage, J., Hemmersbach, R., Kircher, A., Lasok, H., Haser, T., Rapp, K., Schmidt, J., Yu, X., Pasternak, T., Ausbry-Hivet, D., Tietz, O., Dovzhenko, A., Palme, L., Ditengou, F.A.: 2-D clinostat for simulated microgravity experiments with *Arabidopsis* seedlings. *Micrograv. Sci. Technol.* (2015). doi:[10.1007/s12217-015-9478-1](https://doi.org/10.1007/s12217-015-9478-1)
- Warnke, E., Kopp, S., Wehland, M., Hemmersbach, R., Bauer, J., Pietsch, J., Infanger, M., Grimm, D.: Thyroid cells exposed to simulated microgravity conditions – comparison of the fast rotating clinostat and the Random Positioning Machine. *Microgravity Sci. Technol.* (2015). doi:[10.1007/s12217-015-9456-7](https://doi.org/10.1007/s12217-015-9456-7)
- Wehland, M., Warnke, E., Frett, T., Hemmersbach, R., Hauslage, J., Ma, X., Aleshcheva, G., Pietsch, J., Bauer, J., Grimm, D.: The impact of hypergravity and vibration on gene and protein expression of thyroid cells. *Microgravity Sci. Technol.* (2015). doi:[10.1007/s12217-015-9474-5](https://doi.org/10.1007/s12217-015-9474-5)
- Weilert, M.A., Whitaker, D.L., Maris, H.J., Seidel, G.M.: Magnetic levitation and noncoalescence of liquid helium. *Phys. Rev. Lett.* **77**, 4840–4843 (1996)
- Wuest, S., Richard, S., Walther, I., Furrer, R., Anderegg, R., Sekler, J., Egli, M.: A novel microgravity simulator applicable for three-dimensional cell culturing. *Microgravity Sci. Technol.* **26**(2), 1–12 (2014)
- Wuest, S.L., Richard, S., Kopp, S., Grimm, D., Egli, M.: Simulated microgravity: critical review on the use of random positioning machines for mammalian cell culture. *BioMed. Res. Int.* (2015). doi:[10.1155/2015/971474](https://doi.org/10.1155/2015/971474)