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Red Blood Cell Dynamics: The Contribution of Microgravity in the BIOMICS Project

Thomas Podgorski, Gwennou Coupier and Christophe Minetti

Abstract

The complexity of blood flows is an intense subject of research since the pioneering works of Poiseuille and through studies over a wide range of scales, from the single red blood cell to dense suspensions in capillary networks, using a large variety of techniques. The subtle effects that are responsible for the structure of blood flows in vessels can benefit from the use of microgravity platforms in order to suppress sedimentation that prevents precise measurements of red blood cell dynamics. The BIOMICS experiment was performed in the MASER11 and MASER12 sounding rockets and was preceded and followed by several parabolic flight experiments in which two important phenomena were investigated using red blood cells and biomimetic model systems like lipid vesicles: the lift forces that push red blood cells away from walls and hydrodynamic interactions between cells that contribute to spreading, mixing, and segregation of different cell types. Parabolic flights played a crucial role in the definition of the scientific questions, preliminary experiments, hardware development and testing, as well as the definition of protocols, and were central in an experimental program combining ground and flight experiments on different platforms.

Keywords: blood cells, vesicles, suspensions, hydrodynamic interactions, holography

1. Introduction

The flows and rheology of suspensions of particles in a fluid are a longstanding topic in physics and mechanics, with a wide spectrum of applications from geophysics (sediment transport, mud flows), chemical engineering or biological fluids. Owing to the complexity of the physics involved in the hydrodynamics of these systems, which couple fluid and solid mechanics, chemical physics or biology and are spread over several scales from microscopic to macroscopic, they are an intense subject of research in different fields. Blood is a particular example of these complex fluids that has attracted a renewed interest in the past 15–20 years thanks to the rapid development of numerical simulation and new opportunities for experiments that appeared with the rise of microfluidics and new optical techniques of investigation, both in vivo and in vitro.

Blood is indeed a suspension composed of around 40–50% by volume of red blood cells and 1% of leukocytes and platelets in plasma, an aqueous solution of salts and proteins which behaves as a Newtonian liquid under normal conditions.

The main function of red blood cells, which are deformable cells made of a membrane encapsulating a haemoglobin solution, is the transport of oxygen. Their complex dynamics [1–4] and the hydrodynamic and mechanical interactions between cells and with the vascular walls have decisive consequences on the rheology of blood, the circulation of which covers a large range of vessel sizes and flow rates. Pathologies associated with blood flow and cardiovascular function, most of which linked to modifications of red blood cell mechanical properties and flow conditions, are a primary cause of mortality worldwide, and specific disorders have also been identified among astronauts in relation to long spaceflight and exposure to microgravity. Besides heart rate alteration and changes in plasma volume which lead to modification of the shear stress experienced by vessel walls and possible endothelial dysfunction, biochemical perturbations have also been reported, such as an increase of amylase activity [5] as well as with variations in red blood cell membrane phospholipid composition [6]. These phenomena have a possible impact on red blood cell mechanics and interactions and are a motivation in the space domain and more generally at the physics-medicine interface for a better understanding of the influence of mechanical properties of red blood cells on their dynamics. While blood dynamics has always been a topic of interest for physicists since the pioneering work of Poiseuille[1] who studied haemodynamics in veins and capillaries in the 1830s, there has been a new surge of interest for interdisciplinary research on blood in recent years thanks to the development of new experimental techniques and the increasing performance of numerical simulation which allow to tackle the difficulty of studying red blood cell dynamics at the microscale.

Blood flows through a complex system of vessels with dimensions that span over several orders of magnitude from the main arteries (several cm in diameter) down to capillaries with diameters as small as 5 μm that are comparable to the size of cells. At the smallest scales (arterioles, capillaries and venules), blood can no longer be considered a homogeneous fluid, and the complex mechanics of red blood cells lead to the formation of structures, inhomogeneous cell concentration and variations of the local effective viscosity of blood and heterogeneous distribution of red blood cells in capillary networks. These phenomena are directly related to mechanical and hydrodynamic interactions between flowing cells and with vessel walls. In the experiments that we report here, we focused on two basic mechanisms that are fundamental for red blood cell dynamics in small vessels: the lift and migration phenomena that move cells away from vessel walls and the hydrodynamic interactions between flowing cells that give rise to a shear-induced diffusion phenomenon in vessels (see **Figure 1**): when deformable objects such as red blood cells or giant lipid vesicles (which we used as a model for red blood cells) flow near a solid surface or in a channel, hydrodynamic forces tend to push them away from walls (**Figure 1a** and **b**), leading to the formation of a cell-free layer that lubricates the flow and decreases the overall flow resistance. Similarly, when cells meet in the flow, they experience a cross-streamwise displacement (**Figure 1c** and **d**), which balances the migration towards the centreline and leads to an equilibrium cell concentration profile in the section of the channel.

These phenomena, although they are ubiquitous in blood flows in vivo where they have direct consequences in pathological situations, and in vitro where they can be exploited for lab-on-chip applications such as cell sorting and analysis, had seldom been quantified experimentally when the biomimetic and cellular systems (BIOMICS) project started.

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[1] Jean Léonard Marie Poiseuille (1797–1869) who was a physicist and a physician actually derived the more general Hagen-Poiseuille law (which describes the laminar flow of viscous liquids in tubes) from his studies on blood flow.
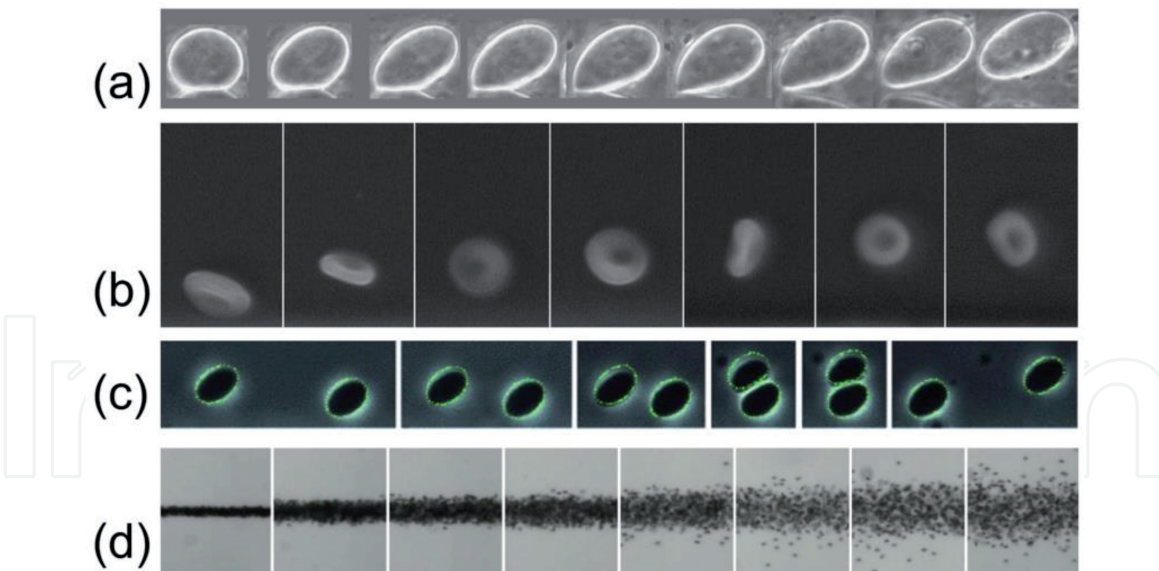
Figure 1.
Basic phenomena of vesicle and red blood cell dynamics in confined flows. (a) Unbinding and lift of a vesicle in shear flow, (b) migration of a red blood cell in channel flow (from [7]), (c) repulsive hydrodynamic interaction in a pair of vesicles in shear flow (from [8]) and (d) shear-induced diffusion of red blood cells in a channel (from [9]).

An experimental difficulty faced by scientists when trying to make a quantitative evaluation of migration forces and velocities at the single cell level is the rapid sedimentation of cells. Indeed, the density difference between red blood cells and plasma (or any suspending buffer with physiological osmolarity and viscosity) is about 0.1 g/cm^3, which leads to sedimentation velocities of several μm/s for cells with a diameter of 7–8 μm. This sedimentation velocity is indeed of the same order of magnitude as lift velocities, which prevents from making precise and quantitative measurements of these subtle hydrodynamical effects under gravity. Thanks to the access to microgravity platforms granted by the *Centre National d'Etudes Spatiales* (CNES, French Space Agency) and the European Space Agency (ESA) since 2004, we have been able to perform several reference experiments which constitute a solid basis for the understanding and modelling of red blood cell dynamics in flow and the mechanics of the microcirculation.

In the following, we will review the preparatory steps that led to the BIOMICS experiments on the dynamics of vesicle suspensions in shear flow that took place during the MASER 11 (2008) and MASER 12 (2012) sounding rocket flights as well as different experiments that were performed in parabolic flights for the preparation of the sounding rocket experiments, equipment validation and preliminary results. Additional and complementary experiments that gave a significant amount of additional results using the equipment that was developed for these opportunities will also be summarized. Besides the exciting challenges that are inherent to microgravity and space-related projects, the development of equipment and experimental techniques for these missions was a formidable opportunity for spinoffs in the form of many other experiments, both on the ground and in microgravity.

2. The BIOMICS experiment

The experimental collaboration between the teams of the Laboratory for Interdisciplinary Physics (LIPhy) in Grenoble, France, and the Microgravity Research Center (MRC) in Brussels, Belgium, was initiated in the framework of the BIOMICS Topical Team organised by ESA. After the initial meetings in 2004 and

2005, the teams gathered during the 2005 biennial symposium of the European Low Gravity Research Association (ELGRA) in Santorini, Greece. The team of Grenoble had started working on the dynamics of giant lipid vesicles in flow a few years earlier. Giant vesicles, which are lipid bilayer membranes enclosing a fluid with a characteristic size between 5 and 50 μm, are a convenient tool for physicists to study the dynamics of soft objects in flow and can be considered as a simple model for red blood cells. Interests raised in developing experimental systems that would allow precise characterization of the structure and dynamics of suspensions in confined flows while at the same time the team of Prof. Frank Dubois at MRC had developed holographic microscopy techniques that had great potential for the study of the dynamics and blood cell suspensions. In addition, the technique, which we will briefly describe later, had already been implemented in space experiments, notably the protein crystallization experiment PromISS [10] aboard the International Space Station, which was an indication of its robustness in microgravity and flight environment.

From these discussions, a project emerged aiming at a quantitative characterization of the structure (concentration profile) of a vesicle suspension in simple shear flow between plates. This configuration was chosen as one in which the two fundamental mechanisms (lift force and shear-induced diffusion) compete, providing means to measure the parameters of these processes and test theoretical models. Besides, the 3D reconstruction capabilities of digital holographic microscopy (DHM) [11] developed by MRC appeared to be a powerful tool to study dynamical phenomena in suspensions with statistical significance thanks to the large quantity of data generated by the processing of DHM images. Although there was at that time little available data on vesicles or red blood cell migration velocities or on shear-induced diffusion coefficients, a rough estimation of time scales in the involved phenomena was made based on preliminary experiments and related literature, from which it was suspected that at reasonable shear rates (50–100 s^{-1}), a time of tens of seconds or minutes would be required to achieve a steady state in a shear flow between plates with a gap of tens or hundreds of micrometres. As these experiments were to be performed in microgravity to avoid sedimentation over these time scales, sounding rockets were quickly identified as the appropriate platform.

3. Preparatory steps and preliminary experiments

3.1 Designing the experiment

The first draft of the sounding rocket experiment proposal was written quickly after the ELGRA symposium and the first meeting between the scientific team, ESA and the Swedish Space Corporation (SSC) took place on November 7, 2005 in Paris, France. During this meeting, the general objectives of the experiment were defined as well as a general schedule and a preliminary design was presented.

While the main optical instrument, the digital holographic microscopy, was already available at MRC, the first step in the project was to design a prototype of shear flow chamber for several purposes which include (i) the dimensioning of the final system to obtain proper flow conditions, (ii) testing and tuning the optical system and the 3D-reconstruction capabilities of the DHM with vesicle samples and (iii) testing the experiment principle on ground and in parabolic flights in order to progress in the definition of experiment parameters such as vesicle sample characteristics, flow conditions and time scales.

The requirements for this shear flow chamber were rather strict and constraining: we needed to produce a constant shear flow between plates with shear rates in the range of 1–100 s^{-1} during a period of up to several minutes. The gap between plates had to be in the range of 100–200 µm in order to be large compared to vesicle sizes in our samples (typically 5–30 µm) but still sufficiently small to allow reaching a steady state of the vesicle suspension distribution at the time scale of the experiment. There were constraints on the parallelism of the plates to ensure constant and uniform shear rate in the chamber, on water tightness of the system and the possibility to evacuate air bubbles when filling. Finally, for compatibility with the optical technique and the configuration of the DHM, plates had to be transparent and of optical quality, and the bottom one needed to be thin compared to the working distance of objective lenses.

Thanks to the skills of engineers at LIPhy, the shear flow chamber of **Figure 2a** and **b** was designed. In this design, a transparent plexiglass disc (diameter 12 cm, thickness 1 cm) rotates about the central axis inside a chamber made of aluminium for the top part (except for a small glass window allowing the passage of the incident laser light) and closed at the bottom by a 2-mm-thick glass disc. This produces a shear flow between the bottom glass plate and the rotating disc. The rotating axis, which is rather long for mechanical stability and alignment, is entrained by a cog wheel linked to a stepping motor and gear box. This flow chamber was built in early 2006 and tested on ground and in parabolic flights (2006) in order to set up filling procedures, check optical compatibility and obtain the first images that would help refine the experiment plan, especially regarding time scales.

As for all prototypes, this first design had a number of flaws that were highly instructive for the design of the final experiment by SSC, who were in charge of developing the experimental module for MASER. These included difficulties to fill the chamber without entrapping air bubbles, insufficient mechanical alignment of the rotating disc, some loss of optical quality due to the materials (plexiglass), and a relative fragility of the large and thin bottom glass disc. Nevertheless, it provided the first digital holographic images of vesicles in shear flow, which allowed to start developing sophisticated software for their treatment. On the scientific side, a first rough curve of the evolution of the distance between sheared vesicles and the bottom plate was obtained.

In parallel, during the development phase of the cell, a first draft of the experiment requirements document was produced, and the final version of the flow chamber was designed by SSC, as shown in **Figure 2c** and **d**. Gaining from the experience of the prototype, significant improvements were made: an excellent mechanical precision ensuring parallelism of the shearing surfaces, mechanical stability and a compact design, optical quality thanks to the use of glass only in the optical path; a clear view of the interior from the top that allows to monitor the proper filling and bubble removal and a much smaller dead fluid volume of the chamber that allows to optimize sample use.

3.2 Testing sample compatibility and limits

Space-related experiments that are performed or launched on sites that are far from the research laboratory require to think about details that usually do not come up to the scientist mind when entering the domain of gravity-related research. Sounding rocket experiments have specific constraints that have to be taken into account, especially when dealing with biological or biomimetic samples. Indeed, doing the experiment requires preparing samples in advance, travelling with them to Esrange (Kiruna, Sweden) for the flight campaign, staying there for pre-flight
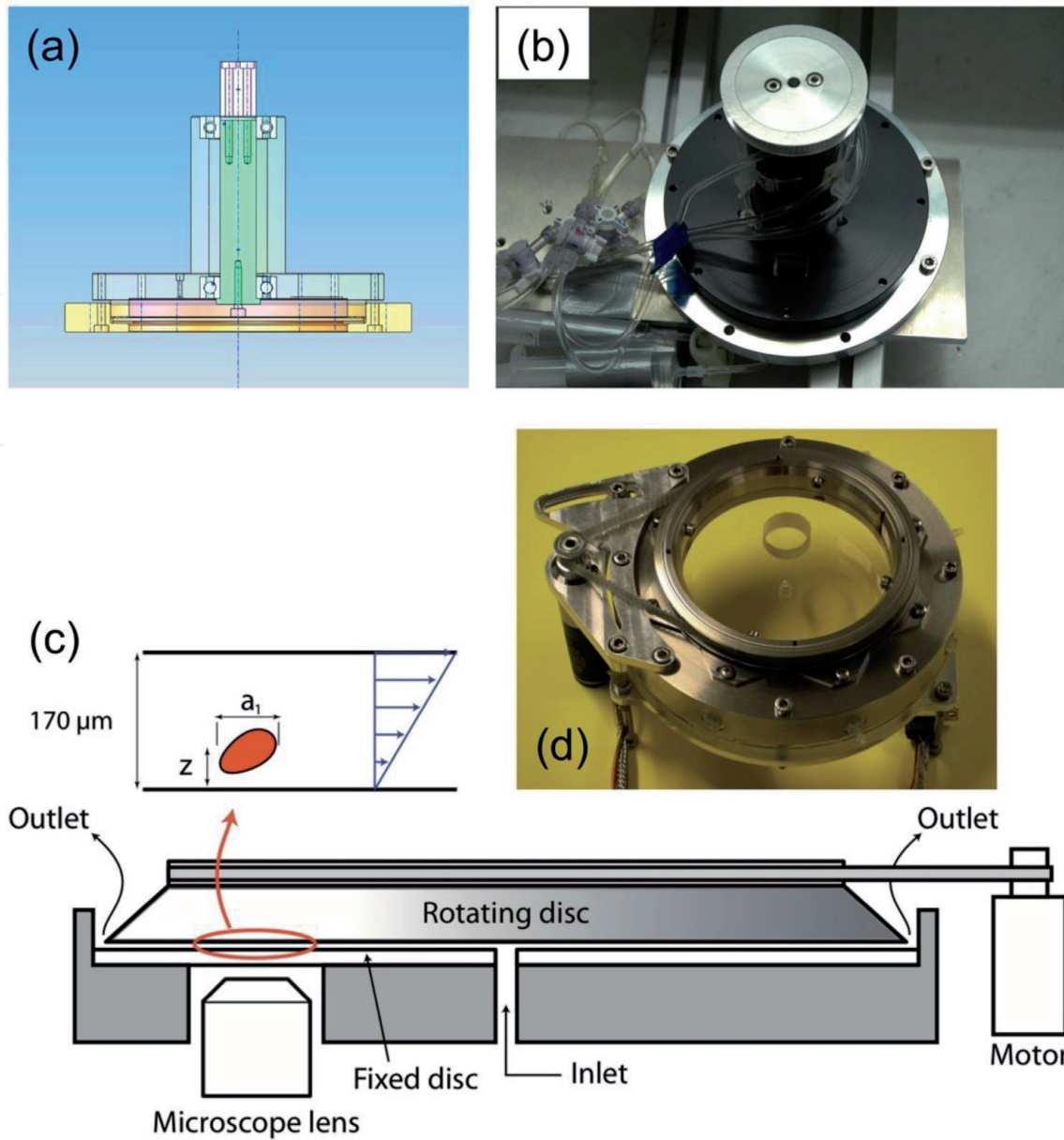
Figure 2.
Two versions of the shear flow chamber developed for microgravity experiments. (a) and (b) Sketch and picture of the prototype designed at LIPhy and tested in the parabolic flights campaigns of spring 2006 (ESA) and September 2006 (CNES); (c) and (d) sketch and picture of the shear flow chamber designed and developed by SSC and used in the MASER 11 (2008) and MASER 12 (2012) sounding rocket flights and in ESA and CNES parabolic flight campaigns since 2007 for various experiments on vesicles and red blood cell dynamics (from [12]).

tests and wait until weather conditions are favourable to start a countdown, which may take up to several weeks on site. Then samples need to withstand a waiting time of up to several hours in the experimental module before launch and resist the strong acceleration levels experienced when the motor ignites and in the ascending phase of the rocket (up to 12 g).

These conditions and constraints are of course extremely different from what happens in the home laboratory where the scientist can prepare samples and experiment with them within hours in ideal environmental conditions. While doing experiments in parabolic flights, which we had done previously, is already a challenge (samples have to be transported to Bordeaux 1 week before the first flight, wait for about an hour in the experimental rack before the first parabola comes and withstand vibrations and accelerations of the plane's take-off and of hyper gravity phases (1.8 g) before and after each parabola), sounding rocket campaigns represent a significant step forward in complexity. The requirements for the MASER 11

campaign, the first one for the BIOMICS experiment, included then to qualify our samples through a series of environmental tests, namely:

- Centrifugation tests that would ensure that vesicle samples resist the acceleration levels of the rocket. They were also aimed at confirming that a stirring system would be necessary to prevent sedimentation of the samples once they are installed in the module and before the microgravity phase.

- Stirring tests: samples need to resist magnetic stirring over several minutes, the stirring (by a magnetic bar inside a syringe) should sufficiently homogenize the samples, and degradation of the samples had to be quantified in order to tune the needed initial concentration.

- Lifetime tests to quantify the degradation of samples over times of several weeks during the campaign.

- Travel tests to check the possible degradation of samples during travels that may last a day or two, during which samples are no longer at 4°C and their containers may be shaken. Some samples would also need to be sent to SSC in Solna, Sweden, by mail for various tests during the preparation.

As trivial as they may seem, these simple but time-consuming and sometimes long tests were indeed extremely critical for the feasibility of the project. They were all successful, an important step to move on to the final development phase.

3.3 Developing a full prototype for parabolic flight experiments: a decisive technical validation

As mentioned above, a parabolic flight experiment was developed for several ESA and CNES campaigns that took place in 2006 and 2007 before the MASER 11 campaign, with the purposes of testing equipment and procedures as well as obtain scientific results that would serve defining the optimal parameters for the sounding rocket campaign. The setup, which was developed in close collaboration between MRC and LIPhy, is shown in **Figure 3**. The experiment consists in injecting a vesicle suspension in the shear flow chamber. A constant shear flow is created, and the dynamics of the suspension is recorded with the digital holographic microscope. Vesicles initially sedimented on the bottom wall are lifted up thanks to a hydrodynamic lift force and drift towards the middle plane of the shear flow chamber.

The experiment setup is made of a shear flow chamber, mounted on a digital holographic microscope. The instrument allows, after processing the recorded holographic information, to obtain information on the 3D position and shape of vesicles in the chamber during the flow. An injection system (**Figure 4**) consisting in several syringes containing samples and filling and rinsing fluids (vesicle suspensions and glucose-sucrose solutions) allows to sequentially inject samples in the flow chamber through the bottom glass plate. The first two campaigns (spring and fall 2006) were performed with the prototype shear flow chamber, and a final preparation parabolic flight campaign took place in September 2007 with the final flow chamber designed and built by SSC, with the presence of a SSC representative during the campaign to build know-how and procedures that would be useful for the sounding rocket campaign.

From a technical viewpoint, these three campaigns were decisive for the team as they provided important information for hardware development and procedures. As the project emerged from a new collaboration that started only 1 year earlier
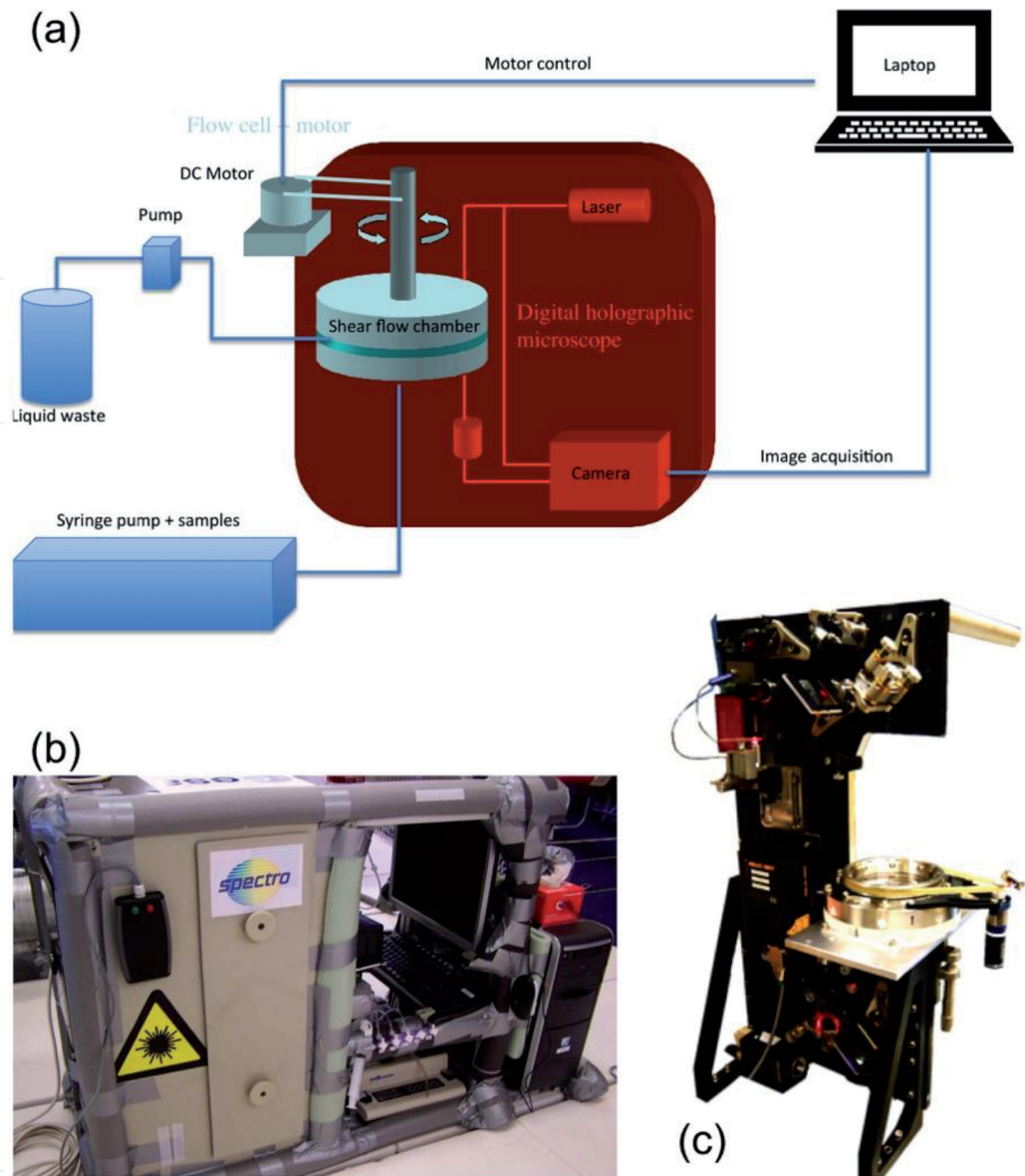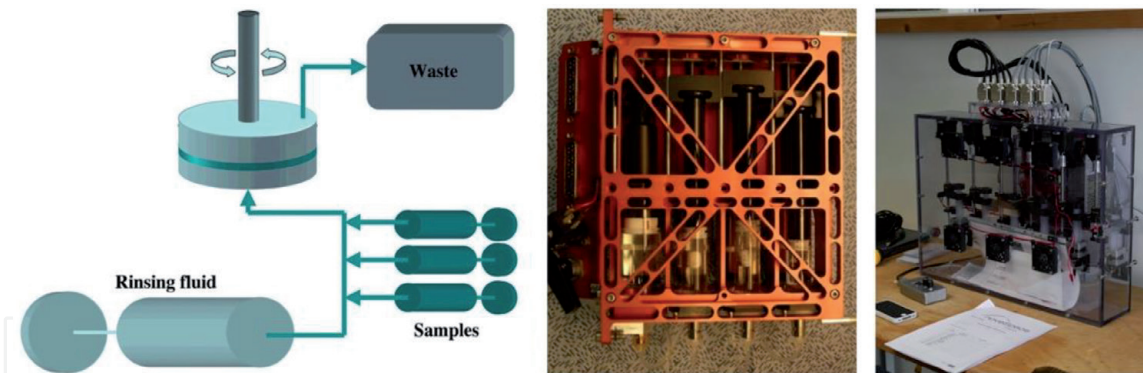
Figure 3.
(a) Design principle of the parabolic flight setup used for BIOMICS experiments. The shear flow chamber is installed on a digital holographic microscope that allows 3D reconstruction of vesicle and red blood cell suspensions in flow. Samples are injected thanks to a multi-channel syringe pump; (b) parabolic flight experimental rack, 2006 version; (c) digital holographic microscope designed and developed at MRC, ULB.

thanks to the networking opportunities offered by ESA Topical Teams, it was also a precious time for the scientific team to know each other's skills, strengths and weaknesses as well as human and social qualities. A good team spirit is an asset in long-term projects in which groups of collaborators spend long periods of intense work time in different environments that are far from home. The 2006 and 2007 campaigns allowed to forge what was called the "Moutchic Spirit" and allowed to maintain a continuous collaboration that is still alive today.

Technical definition of the MASER experiment moved on during this period, especially with the development of two important sub-systems: a sample storage and injection device inspired by the rudimentary initial parabolic flight system and a sounding rocket version of the digital holographic microscope to suit the geometrical and mechanical constraints of MASER rockets. The injection system
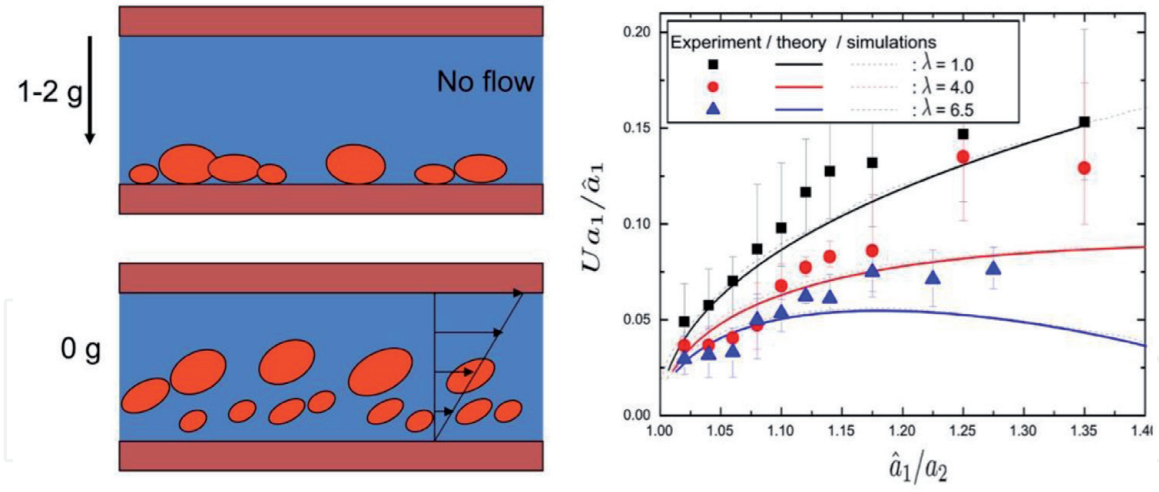
Figure 4.
Multichannel injection system. Left, sketch of the system; middle, multichannel syringe pump with embedded stirring system designed by SSC for the BIOMICS module in MASER rockets; right, similar system developed at LIPhy for recent parabolic flight campaigns (2017–2019).

developed by SSC (**Figure 4**, middle) needed to fulfil several requirements such as having three samples in separate syringes as well as a bigger filling/rinsing fluid syringe and an internal stirring system to homogenize the samples and prevent sedimentation. On the practical aspect, ergonomic considerations required the system to be easily filled before late access with an easy removal of air bubbles. Unlike parabolic flight systems that used disposable plastic syringes, the sounding rocket system includes more rigid and precise glass syringes enclosing a small magnetic stirring bar controlled by external electromagnets. This device also required several sessions of tests at SSC premises in Solna, Sweden, in order to have well-defined filling and handling procedures. A noticeable achievement of the whole BIOMICS project is that the technology of this device that was built on experience gained in laboratory and parabolic flight experiments became itself a source of inspiration a few years later for the development of a new injection device needed for the study of the aggregation of red blood cells in a parabolic flight experiment started in 2017 (**Figure 4**, right).

3.4 Scientific insight gained from parabolic flights: original and important results on the hydrodynamic lift of vesicles and cells

On the scientific side, it was necessary to gain a better insight on one of the main mechanisms of structuration of vesicle suspensions under shear: the hydrodynamic lift of vesicles due to their interaction with walls. This phenomenon, in which vesicles are pushed away from walls in shear flow towards the centre of the flow, sets the time scale of the establishment of a steady spatial distribution in conjunction with hydrodynamic interactions between vesicles or cells. The first parabolic flight campaigns in 2006–2007 allowed to estimate this time scale for the MASER experiment timeline at the same time that equipment and procedures were tested, and the study that had fundamental interests in itself was pursued during additional parabolic flight campaigns in 2009. The results of the pre-MASER 11 parabolic flight campaigns led to the publication of a first research article on the hydrodynamic lift of vesicles [12] that has received a strong attention from the community and has become a reference article. It provided the first experimental validation of earlier theoretical predictions of scaling laws governing this phenomenon [3].

The experimental principle and sample results are shown in **Figure 5**. The experimental procedure is a good example of the way the particular succession of different g-levels in parabolic flights can be exploited to give original results: during the normal and hyper-gravity phases, the flow is stopped, allowing sedimentation of

Figure 5.
Experiments on the lift of vesicles in shear flow near a wall performed in parabolic flights for the determination of lift coefficients. The motor of the shear flow chamber is stopped during normal and hyper-gravity phases (1–2 g) to allow sedimentation of vesicles on the bottom wall, and the fluid is sheared during microgravity phases only to study the resuspension of vesicles. Results show a strong dependency of the dimensionless lift velocity U on vesicle parameters such as viscosity ratio λ and elongation a_1/a_2 (from [13]).

vesicles on the bottom wall of the shear flow chamber, providing a reproducible initial condition for all parabolas. Then, when the microgravity phase starts, the shear flow starts, leading to progressive lift of vesicles. The dynamics is recorded by digital holographic microscopy, which allows after reconstruction to analyse the evolution of the distance between vesicles and walls as a function of time. This phenomenon is strongly dependent on the size of vesicles, their geometric characteristics (surface to volume ratio or equivalently, their reduced volume, a dimensionless parameter that compares the internal volume to the volume of a sphere having the same membrane area) and mechanical properties (viscosity ratio λ between internal and external fluids). We showed that the lift velocity follows a scaling law in which the velocity is proportional to shear rate, vesicle diameter and $1/z^2$ where z is the distance to the wall. The proportionality constant (a dimensionless lift velocity) could be extracted as a function of vesicle parameters and numerical simulations performed later quantitatively matched experimental results: the dimensionless lift velocity U is stronger when the vesicles are more deformable and thus more asymmetric with respect to the flow direction. This asymmetry is favoured by a low viscosity ratio λ between the internal and external fluids and by a higher surface to volume ratio of the vesicles that gives them more flexibility. This is reflected by a higher apparent aspect ratio a_1/a_2 of the long and short axis of the quasi-elliptic recorded shape of vesicles (see **Figure 5**). From the theoretical and numerical perspective, this experiment was a benchmark that allowed a quantitative validation of numerical models [13].

Results from parabolic flight campaigns were also the opportunity to establish reliable image processing methods for the DHM images [11, 14]. A powerful reconstruction and segmentation technique were indeed needed to process the large quantity of data generated by these experiments (up to thousands of flowing objects for a typical parabola). An interesting outcome was also that this technique could be used to measure 3D shapes of vesicles and concentration profiles in other types of flow [15], capabilities that could be useful for other applications.

3.5 Developing new sample preparation techniques thanks to microfluidics

The second BIOMICS sounding rocket flight (MASER 12) required the development of new sample preparation techniques in order to study the dynamics of
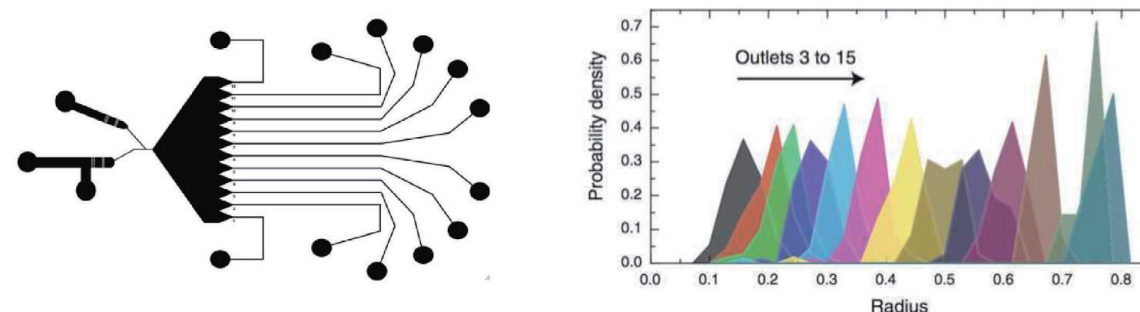
Figure 6.
Sketch of the microfluidic sorting device developed at LIPhy for the production of monodisperse vesicle samples for the MASER 12 experiment (overall size 50 × 30 mm²) and example of vesicle size distributions at the different outlets of the system [17].

well-controlled monodisperse or bidisperse vesicle samples. The aim was to be able to control the size distribution of vesicles in samples, in order to study more specifically the interaction between the lift- and shear-induced diffusion phenomena in monodisperse samples, as a function of vesicle size, and to get a quantitative understanding of the role of heterogeneous pair interactions in suspensions (i.e. collisions or hydrodynamic interactions between objects of different sizes). This was motivated by segregation or margination phenomena that had been reported in blood flow (platelets or white cells tend to be closer to vessel walls while red blood cells flow in the centre) [16] and whose mechanisms still deserved quantitative description and modelling.

The classic production method for giant lipid vesicles, electroformation, produces samples that are highly polydisperse in size. While other methods (e.g. using micro-emulsions as an intermediate) had been proposed for a better control of vesicle size, they were not suitable for the production of rather large samples (>10 mL) with significant vesicle concentration. We therefore decided to keep the efficient electroformation method and developed a microfluidic sorting device to produce monodisperse samples (**Figure 6**).

The system is based on the principle of pinch-flow fractionation, and its performance is described in [17]: the initial vesicle suspension is introduced through one inlet, while pure suspending fluid is introduced at through the other inlet with a much higher flow rate. This effectively squeezes vesicles against one wall after the converging bifurcation, leading to a configuration in which the distance between the centre of mass of vesicles and the wall is equal to their radius. A diverging chamber then amplifies this ordering and distributes vesicles in several outlets. As shown in **Figure 6**, we were able to separate vesicles in to up to 16 different fractions with a size dispersity that is of order 10% only in each fraction, which met the requirements of the experiment we wanted to implement in MASER 12. This system which was developed in the framework of microgravity experiments has been used many times since then for various experiments on different types of samples at LIPhy and other laboratories.

4. Sounding rocket flights

4.1 Experimental module

The BIOMICS module that was developed by SSC for the sounding rocket flights was directly inspired from the experimental setup that we had developed and tested in parabolic flights, by adapting it to the geometrical, mechanical, electrical and environmental constraints of MASER rockets.

The module, approximately 60 cm in height, is built around a central optical breadboard on which the holographic microscope is assembled (**Figure 7**). This version of the digital holographic microscope was developed by the Lambda-X company from Belgium in order to meet specifications (compact design, robustness of the alignment of elements and insensitivity to strong accelerations and vibrations). Compared to the parabolic flight version, the sounding rocket module also includes thermal control, an overview camera that allows the team to check that correct filling of the chamber is achieved and that rotation takes place as scheduled during the experiment and of course specific electronics for experiment management, data storage and transmission. The module housing is equipped with a hatch for late access before the flight: to avoid sample degradation, the injection unit (red part in **Figure 7**) and its syringes are filled with samples after countdown has started and installed in the module only minutes before take-off.

After a series of environmental and functional tests in Solna, Sweden, as well as the first flight simulation tests which included setting up an efficient protocol for the filling of the syringe unit without entrapping bubbles, the module was ready and approved for the flight.

4.2 The campaign: beyond the far north to outer space

The weeks before the MASER 11 and 12 campaigns were hectic. After months of hardware testing, protocol definition and parabolic flight experiments, the team's attention was largely devoted to the preparation of samples. As we were aware of the significant risk of countdown delays and interruptions, and the need to perform several flight simulation tests on site before the launch, a large quantity of samples was needed to deal with the expected and unexpected. It took several days and nights in the weeks preceding the campaigns to prepare the necessary volume of vesicle samples by electroformation, followed by the time required to pass them through the microfluidic sorting device (for MASER 12) and finally check their quality.

This intense, almost monastic, activity was a premise of the polar environment in which we were going to be immersed for several weeks. Although scientists do not have the chance to go and conduct their experiment in space themselves, all the more so when it comes to automated and remotely controlled sounding rockets, the journey to the Esrange launch site near Kiruna in Swedish Lapland is in itself an immersion in a scenic, unusual and dramatic environment. And although we do not experience the degree of confinement that astronauts do in spacecrafts and orbital
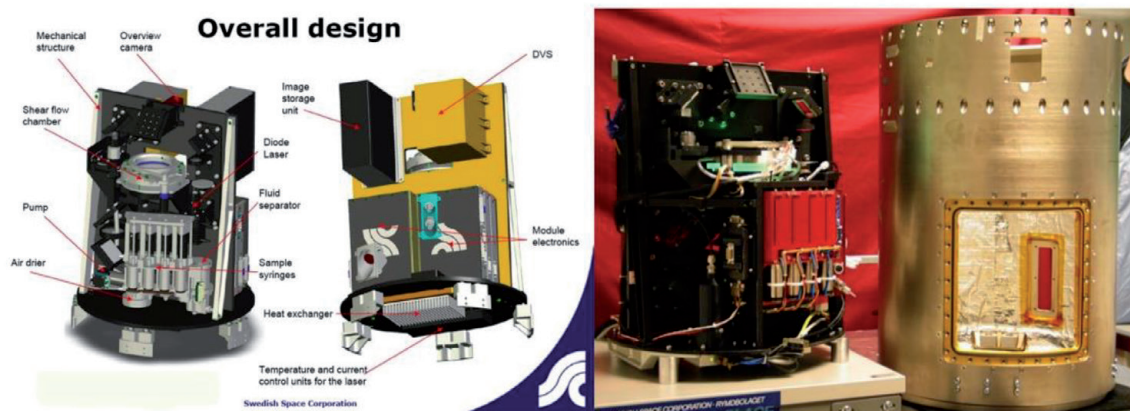
Figure 7.
The BIOMICS module developed by SSC with a contribution of lambda-X for the holographic microscope. Left, overall design. Right, picture of the module and its payload housing with the late access hatch open.

platforms, the serene wide open spaces of Lapland and its harsh climate give a small taste of what it is to be confined at the edge of the common world. The succession of days and nights was indeed confusing—the MASER 11 campaign took place in May 2008, with almost no dark night, and MASER 12 in February 2012, with rather short days and temperatures that reached −40°C—and on countdown days, our schedules were not punctuated by the usual beacons of the clock but by launch opportunities and the organisation around foreseen launch times that were dictated by weather conditions. The MASER 11 campaign was especially long due to almost permanent windy conditions that led to an unusual number of interrupted countdowns. A strange feeling, which work and social activities actually did not make unpleasant, was the impression of being in a universe halfway between the village of the old TV series "The Prisoner" and the classic film "Groundhog day", doomed to repeat the same procedure that lasts several hours until the wind sends its white bouncing ball that sends everyone to bed. Despite the large quantity of samples that has been prepared, these repeated countdown stops had seriously eroded the stock when finally a successful launch took place.

4.3 Results

From a technical viewpoint, the two campaigns were a success for the BIOMICS experiment: the whole flight sequence took place nominally, with an excellent image quality that would ensure ideal conditions for data processing. Experience gained from the first flight (MASER 11) also leads to hardware improvement (modifications of the laser diffuser in the holographic microscope) that ensured even better interferometric contrast in MASER 12.

From a scientific viewpoint, we have been able to show that the suspension reaches a steady and symmetric distribution centred between plates in shear flow, even at low volume fraction, a configuration that is impossible to reach under normal gravity (**Figure 8**). As this distribution is the result of the balance between the lift force (already well characterized in parabolic flights) and shear-induced diffusion, it allows us to determine a dimensionless diffusion coefficient f_2 that only depends on vesicle mechanical properties, from the height and width of the distribution (see, e.g., **Figure 9**, left panel). From different sets of data and independently of vesicle size, we found $f_2 = 0.063 \pm 0.024$, a value that is consistent with theoretical predictions, suggesting that it can be indirectly derived from the analysis of individual pair interactions [8, 13, 18].

For the MASER 12 campaign, we considered three samples of vesicles of different sizes and a viscosity contrast of $\lambda = 1$. Suspensions of large, medium and small vesicles were successively injected. The radii of these populations lie, respectively, in the ranges 22–35 μm, 15–20 μm and 5–15 μm. The flight sequence included the study of the three separate populations, which clearly showed that large vesicles produce sharp, centred concentration peaks while small ones produce broader distributions. While it was known from studies on the lift phenomenon in parabolic flights that the lift force increases with vesicle size and deflation, the influence of these parameters on shear-induced diffusion coefficients as well as the interplay between lift and diffusion was still unclear at this point, especially since shear-induced diffusion also increases with vesicle size and deformability.

Finally, when injecting a mixture of two populations, as in **Figure 9** (right panel), we found that the small vesicles are expelled from the centre, as a result of both the weaker lift forces they experience and the asymmetry in their interaction with large ones: small particles are indeed pushed away from the centre zone by large ones. This segregation phenomenon is similar to platelet or leucocyte margination in blood flow and has strong implications for the structure of blood flow,
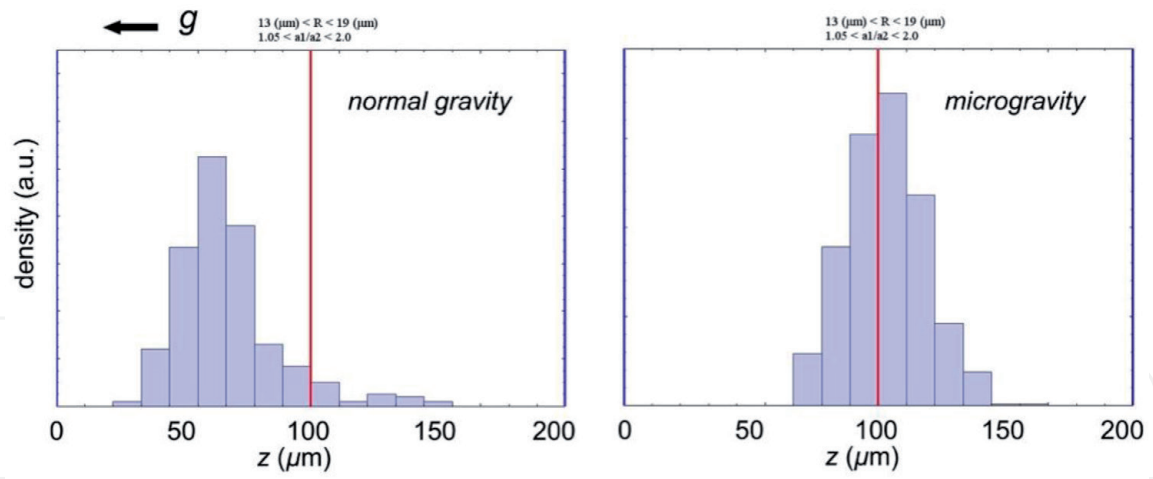
Figure 8.
Comparison of vesicle distributions in the gap of the shear flow chamber on the ground (normal gravity) and during the MASER 11 flight. The red line corresponds to the centre of the gap. In microgravity, the distribution is centred and symmetric, allowing an evaluation of vesicles dynamic parameters (diffusion coefficients and lift forces), while the distribution is off-centred and asymmetric under normal gravity.
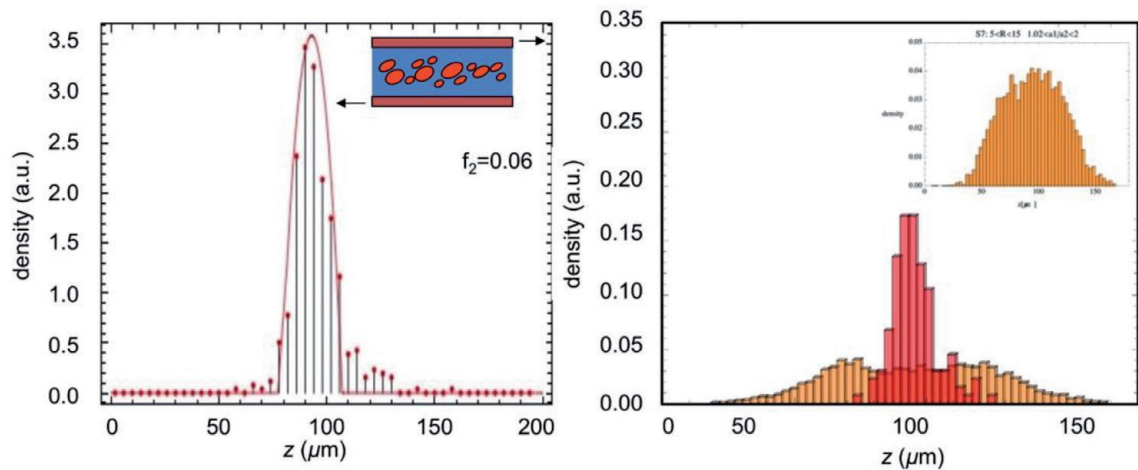
Figure 9.
Some results from MASER 11 and MASER 12 [13, 18]. Left, the steady distribution of a monodisperse vesicle sample allows the determination of shear-induced diffusion coefficients (inset, sketch of the experiment). Right, steady distribution for a bidisperse suspension of vesicles in MASER 12 (yellow, small vesicles with average radius 10 μm; red, big vesicles with average radius 20 μm). The double peak for small vesicles (to be compared to the unique, smooth peak when they are alone as shown in the insert) reveals segregation phenomena in the flow of polydisperse cellular suspensions.

blood rheology in small vessels and the immune response. As such, it is a topical issue in the blood flow community which has received renewed interest over the past decade through advances in theoretical modelling and numerical simulation. Our experimental results provide quantitative data for the fine-tuning of models which are still a topic of interest today.

5. Experience and follow-up

Like all space-related projects, the preparation and realization of these sounding rocket experiments have had multiple repercussions in terms of experience, technical developments and inspiration for a large number of works that have taken place since then in the research carried out in the laboratory and during many subsequent parabolic flight campaigns.

The parabolic flight setup and the shear flow chamber that were developed in this framework were subsequently used for several other experiments which include the lift of red blood cells [9], the complex dynamics of red blood cells in shear flow [19] or more recently the dynamics of aggregation of red blood cells. More experiments are indeed still planned today with different samples such as polymeric capsules. In addition to large quantities of experimental results on different systems, the collaboration that was initiated in this project also led to significant improvements in the processing of holographic data for suspension flows, which can be used for a wide variety of purposes [11, 14, 15].

The results also raised many questions on various aspects of vesicle and red blood cell flows that triggered many studies in the lab. Notably, for a better understanding of the phenomenon of shear-induced diffusion, a study of the elementary mechanism behind it was performed in microfluidics, namely, the hydrodynamic repulsion in a pair of interacting vesicles in a shear flow [8], while shear-induced diffusion was characterized in channel flows of red blood cells [9]. The lift phenomenon that we fully characterized for vesicles [12, 13] and red blood cells [9] in simple shear flow thanks to parabolic flight experiments led to the question of validity of the established scaling laws in channel flows where the velocity profile is parabolic instead of linear. In that case, we proposed empirically modified scaling laws for both vesicles [20] and red blood cells [7] that are now a reference for the development of cell-sorting applications and the refinement and benchmarking of numerical simulation codes.

These projects have had a strongly structuring effect on our collaborative network at the European scale, beyond the central collaboration between MRC and LIPhy, with ramifications of the topic with German and Italian collaborators, for instance. A strong expertise in the preparation and manipulation of samples was gained, which led to the establishment and sharing of reference protocols with several other partners.

On management aspects, dealing with space-related projects as all team members were young researchers when the collaboration started was a decisive opportunity for building experience and know-how on the management of large-scale projects in a complex environment, combining the characteristics of experiments on large instruments with the specificities of the space domain (technical constraints, security, interactions with many different actors between space agencies, contractors and technical staff).

6. Conclusions

Developing a space experiment is usually a long process with specificities that are often unknown to the average scientist. Besides specific procedures and constraints related to security and quality control, these experiments require an unsuspected number of tests and validation as well as extremely precise protocols that have to be defined in advance and do not withstand improvisation. As strange or caricatural as they may seem to the experimentalist who usually works on his laboratory bench, this collection of many different tests is indeed a precious key to a successful experiment by ensuring that no detail, which may result in experiment failure, is overlooked.

In many cases, parabolic flights are a recommended or even compulsory step for the precise definition of the scientific question, tuning of parameters and protocols and testing of hardware. For the BIOMICS project, it was the opportunity to develop a full prototype of the experiment and directly interact with it, in microgravity conditions (see **Figure 10**), before the development of an automated and

Figure 10.
Team at work and group picture in parabolic flights (photos, CNES).

remotely controlled module for the sounding rocket flights. A decisive advantage of parabolic flights is that, despite rather short microgravity periods (22 s), the number of repetitions is large and the interval between campaigns is short, of order weeks or months only. In addition, the possibility to get important scientific results during this preparation phase should not simply be viewed as a side product of the final space experiment but rather as a part of a multi-faceted project that involves several experimental platforms.

The road to space is long but intense and extremely well-paced by the organising agencies and contractors. Beyond the fact that successful launches felt like a relief and achievement in themselves, the whole process of a well-conducted preparation and campaign itself is a source of scientific, technical and humane benefits and spin-offs that go far beyond the sole sounding rocket experiment.

Acknowledgements

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