17th Symposium IMEKO TC 4, 3rd Symposium IMEKO TC 19 and 15th IWADC Workshop
Instrumentation for the ICT Era
Sept. 8-10, 2010, Kosice, Slovakia

# In-flight Space Flow Cytometer Specification Requirements

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***Abstract***— In the paper the working context analysis and the required project hardware and software specifications of an innovative cytometry system able to operate in the space environment are presented. This new flow cytometer is the object of the "Space micro Environment Portable Active Cytometry Terminal" research project supported by the Italian Space Agency.

## I. Introduction

Flow cytometry (FCM) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. This technology has applications in a number of fields, including clinical science, cell biology, microbiology, plant and animal science, pharmaceutics, reproductive medicine [1]. FCM is also of essential importance in view of long duration space missions, for example for i) health monitoring of the crew (control of blood formula and therapy; diagnosis of diseases; etc.); ii) biological research (gravitational biology including plant research, biotechnology, radiobiology, etc.); iii) medical research (testing of countermeasures as for example physical training, radioprotective substances, supplemented food, etc.; immunology; hematology; etc.); iv) control of cell/tissue bioreactors; and v) environmental monitoring (microbial contamination of air, water, food) [2]. However, until recently, the limitations that have prevented the development of a spaceflight compatible flow cytometer (FC) have been largely mechanical. Standard commercially available FC are large, complex instruments using high-energy lasers and requiring significant training to operate [3]. These reasons have made impossible to perform in situ and real time analysis on space biological samples and specimens. The lack of a portable FC to accompany the astronauts on their shuttle missions has determined for example that blood samples from astronauts in space had to be brought back to evaluate what effects the microgravity of space and ionizing radiation have upon their immune systems and DNA. A large number of spaceflight research studies have been so performed post-flight on crew members utilizing FCM methodology and identifying physiologic changes associated with spaceflight [3]. It is, therefore, impossible to know if these changes occur during flight or they are a result of the stress of landing and re-adaptation to unit gravity. In general, in fact, the physiologic changes observed post-flight in crew members cannot necessarily be extrapolated to the in-flight condition without conducting in-flight analysis [3]. The FC versatility for general research (biological, microbial, environmental, and physiologic studies) and diagnostic medicine would be a major asset to future exploration-class space missions. From these reasons, the research project, supported by the Italian Space Agency, entitled "Space micro Environment Portable Active Cytometry Terminal" aimed at developing an innovative cytometry system able to operate in the space environment, arises. In this paper, the first project results concerning the analysis of the working context and the project hardware and software specifications are presented. In the following, space instrumentation general requirements are first discussed to focus on the FC typical architecture characteristics that prevent its use in the space environment. Then, some recent research proposals dealing with space FC prototypes are described to end with the "Space micro Environment Portable Active Cytometry Terminal" project description. A deep discussion about the project results will be presented in the full paper.

## II. Space instrumentation

The environment in which space instrument are operated is hostile, remote and limited in resource [4]. Therefore, the most obvious difference between other hardware and space flight hardware designs is the need to consider the effect of the space environment (vacuum, low gravity, radiation, etc.) [5]. Accelerations and vibrations at launch, continuous microgravity, fluctuations in ambient pressure, luck of effective heat exchange through convection, and relatively high levels of radiation, may damage the equipment or cause performance decrements, errors, and outages during its operation [6]. Constraints concerning the weight, volume and power arise directly and indirectly from the limitations imposed by the launch vehicle [5]. There is a limit on the amount of payload weight that a launch vehicle can place in orbit [5]. These severe weight restrictions necessitate extreme

measures to reduce weight. Volume is limited by the launch vehicle shroud while the limitation on power is directly connected to weight [5]. The power subsystem tends to be very heavy due to items like batteries, and power requirements are controlled carefully to keep the power subsystem weight down. Most spacecraft obtain electrical power from solar cell arrays, which are limited in output by weight and size constraints. They only produce power in sunlight and so must be augmented by batteries to provide power in eclipse. Thermal dissipation is also a consideration in power constraints [5]. The last but not the least constraint is the reliability, since there is little or no opportunity for servicing space hardware in the event of a failure. This means that the hardware must be designed and tested so that it either will not fail or will tolerate likely failures. Hardware or system redundancy is one method used, but the ever-present weight constraint must be considered. The costs of reliability coupled with the cost of launch are what make space flight hardware so expensive [5]. The longer the life, the more challenging the reliability goal because of the greater time available for a failure to occur.

## III. Flow Cytometer typical architecture space incompatibilities

The principle behind FC is to stain individual cells with fluorescent molecules (flurochromes) specific for various cell responses and to assess fluorescence levels emitted by stained cells after the passage through a laser beam (Fig.1) [7]. In particular, following staining with fluorescent molecules, cells are hydrodynamically aligned using a fluid sheath and pass through a laser beam one by one. Fluorescence signals and light scatterings are collected using a set of filters for different wavelengths, allowing the analysis of multiple parameters for each cell. Data are collected and integrated using specialized software. Increase or reduction in light emission correlates with the cellular response. Photomultiplier detectors equipped with filters allow the quantification of light emitted at different wavelengths, as well as the laser beam scattering that is a function of size and structure of the cells. There are several ways to label cells depending on the parameter targeted [7].

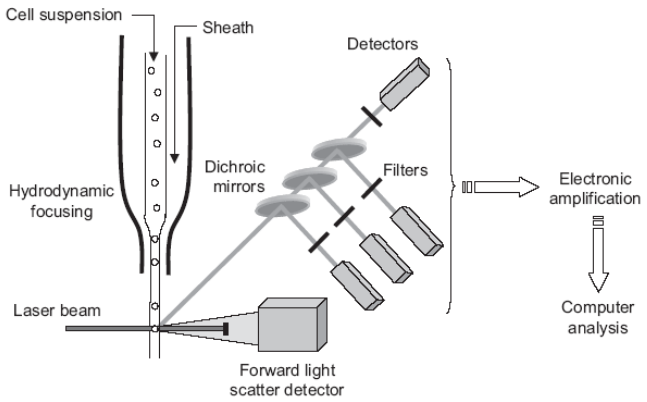


Figure 1 Schematic overview of a typical FC setup [7].

Typically, FCs are instruments that demand constant maintenance, high electrical power consumption and often a specialized operator. Their mass and volume is large and incompatible with space requirements [7].
Further, FCs use a sheath fluid to hydrodynamically focus the particles passing through the laser beam. This sheath fluid increases instrumental complexity and creates large volumes of biohazard material that must be handled in some manner [7]. Spaceflight experiments have demonstrated that fluid physics is dramatically altered in microgravity and previous studies have shown that sheath-fluid based hydrodynamic focusing may also be altered during microgravity [3]. For these reasons, any spaceflight compatible FC design should abandon the sheath fluid requirement. The elimination of sheath fluid, in fact, would remove both the problems of weight associated with large volumes of liquids, as well as the large volume of liquid waste generated. Obviously, it would also create the need for a method to create laminar particle flow distinct from the standard sheath-fluid based method [3].
FCs are also not designed for supporting launch conditions since bulk optics are often used whose alignment tolerances are tight and can be perturbed by vibrations [7].
The requirements for a spaceflight compatible FC are so distinct from those of a terrestrial cytometer. Consequently, spaceflight limitations require that the instrument meet the following design objectives [3]:

- Simple to operate, proven technology.
- Robust for launch and deployment vibrations.
- Minimal moving parts.
- Hard-mounted optical path to preserve alignment.
- Minimal fluid use or waste generation.
- Modular construction for ease of repair and part replacement.

- Low weight and low power consumption.

## IV. Space flight Flow Cytometer proposals

Recent advances in cytometer technology have enabled features that now make a spaceflight-compatible cytometer possible [3]. An example of a small, robust novel cytometer with design features that minimize or eliminate many incompatibilities with spaceflight has been presented in [3]. Purpose of this study was to configure a cytometer, developed by Guava Technologies Inc., for a brief reduced gravity evaluation to validate flow cell function, instrument precision, fluidics, and data acquisition ability during true reduced gravity. This cytometer, highly miniaturized and lightweight, does not require sheath fluid, and uses a low-energy diode laser but is also limited to three-parameter/two-colour capability, does not allow compensation between colours, does not allow linear analysis, and is operated by rather inflexible software with limited capabilities. However, the current limitations of this instrument could be overcome by modifying the software and adding additional lasers, color PMTs, side scatter capability, color compensation capability, and further miniaturization [3]. Before the evaluation, the cytometer was modified by the authors [3] so that stained liquid cell samples could be delivered during reduced gravity (Fig.2). The cytometer was, then, evaluated onboard the NASA KC-135 reduced gravity research aircraft. Bead-based FC precision, photomultiplier tube linearity, and leukocyte immunophenotype analysis were performed during reduced gravity. The flight data were then compared with ground-based control data and data generated using a reference FC (Beckman-Coulter XL) [3].

Figure 2. The Prototype Flight Cytometer bolted to the floor of the KC-135 aircraft for microgravity evaluation [3].

This FC functioned well during reduced gravity and produced data comparable to those of ground-based controls with only minor caveats. The reduced gravity cell immunophenotype data were indistinguishable from ground control data and reference cytometric data. Bead-based instrument precision (coefficient of variation) was slightly increased during reduced gravity operation, but not to a degree that would affect most common FC applications [3].
The achieved results, let the authors conclude that, the successful microgravity evaluation of the cytometer and the need to collect real-time experimental data onboard International Space Station (ISS) for both research and clinical diagnosis purposes warrant the continued development of a spaceflight prototype FC [3].
Another important example of innovative micro-FC meeting the constraints imposed by space travel is under development at the Institut National d'Optique (INO) [7]. It combines INO's fiber-based FC and its microoptical bench, resulting in a device that is on the order of several square centimetres in size.
INO's platform turns on a custom designed optical fiber through which a hole is transversally bored by laser micro-machining. INO's multifunctional micro-optical bench is a 3-D integration platform for passive (e.g. lens) and active (e.g. lasers) components in much the same way that an optical table is, but on the micron scale.
The first prototype bench consisted of two levels on each of which are assembled five optical fibers, a micro-lens and a micro-reflector.
Current development is directed towards integrating the FC into the micro-optical bench platform. The bench will then be integrated to the optical fiber via standard connectors external to the bench.
Finally, in a further developmental step, the source and detector will be integrated onto the bench [7].

## V. Space flight Flow Cytometer project

A portable image FC measurement (IFC) device, based on both hardware and software innovative architectures, optimized for diagnostic applications in the oncology field, has been presented in [8,9].
The hardware innovations of the IFC prototype concern with: (i) the pulses acquisition system, (ii) the optoelectronic single cell detection system, and (iii) the cells flow chamber.
The innovative software implementation involves the following sections: (i) digital filter, (ii) peak detector, (iii) Gaussian distribution plot, (iv) adjustable logarithmic amplification, and (v) Micro Nuclei (MN) detection.

This new IFC permits to overcome the disadvantages of the conventional cytometers, that are:

- the manual technique, based on the microscope scanning of all the bi-nucleated cells in order to estimate the MN frequency;
- the operator dependency of test;
- the length and the high cost of the method.

The realized IFC, compared with the traditional and actual commercial device, has shown better performances due to possibility of modifying acquisition parameters and post processing algorithms.
The IFC architecture proposed in [8,9] has been successively expanded into the new instrument called CYTOSAT satisfying the following new specifications: (i) reduced size, (ii) portability, (iii) satellite communication, and (iv) remote control [10]. CYTOSAT can be used to realize a distributed measurement system based on satellite communications for diagnosis and data collection campaigns on field. In particular, it can be useful (i) where medical units are far from patients, (ii) where the local personnel skills are inadequate, and (iii) for realizing a large database by taking information on site and analyzing it remotely, useful tool for researchers on blood-related illnesses, as AIDS. In Fig.3 the IFC proposed in [8,9] and the new portable FC prototype described in [10] are shown. It can be seen the compactness of the new architecture, since all the digital components are integrated in a box having a laptop size width and length, and the satellite modem (the white component at the bottom of the figure) is placed on the top, making this device suitable to be carried by a vehicle. CYTOSAT has been recently the starting point of a new research project, supported by the Italian Space Agency, to carry out the working context analysis and the required project hardware and software specifications of the innovative system called Space micro Environment Portable Active Cytometry Terminal (SmE_PACT). This system can arise from a redesign of CYTOSAT architecture to make it suitable to operate in the space environment. SmE_PACT will have to be able of automatically operating both in stand alone and remote desktop control panel condition, on board of a satellite to carry out FCM analysis concerning zero gravity, ionizing radiation and infertility effects on the life, reproductive and genetic mechanisms of cells and microorganisms. SmE_PACT will have also to be miniaturized and resistant to mechanical shock, vibration and environment condition variations. Further SmE_PACT characteristic will have to be its connectivity to the ground station by an embedded software able to communicate with a ground control centre and to allow the local and/or remote FC system control.

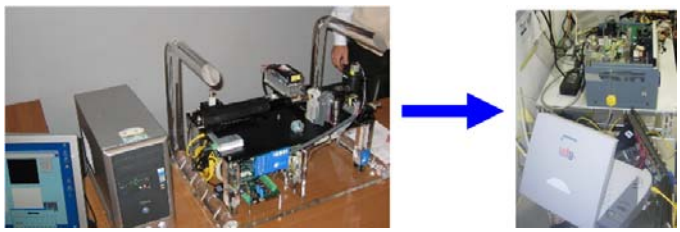

Figure 3. The IFC proposed in [8,9] (right side) and an open top compact FC with the satellite modem (left side)[10].

A preliminary list of the main SmE_PACT characteristics includes:

- system size (mm): 150 x 60 x 60 (with ±20% of tolerance)
- weight (gr): max 800
- chassis insulated from the vibration proof shell and resistant to acceleration
- single pneumo-fluidic-optic block built by using microelectromechanical systems (MEMS) technology with MEMS based gyroscope/accelerometer fluids embedded control
- electronics and power compliant with aerospace standards
- simultaneous detection up to 8 parameters (2 scatter + 6 fluorescent)
- full automation of data transmission

In the full paper, SmE_PACT project specifications will be deeply analyzed and discussed.

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