Simulator of a Flow Cytometer: A Prototype

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***Resumo*– A dificuldade em aprender a utilizar a citometria de fluxo, o elevado custo dos equipamentos bem como a dispersão geográfica dos utilizadores e dos centros de treino tornam a aprendizagem nesta área muito dispendiosa. Por estas razões a existência de um simulador para citómetros de fluxo pode ser muito interessante. Neste artigo apresenta-se um protótipo de um simulador para este tipo de equipamento presentemente em desenvolvimento para plataformas Windows. Este simulador deverá vir a ser utilizado por formandos e formadores quer directamente quer em cursos hipermédia que integrem simulações. Deve também vir a ser possível usar o simulador através da Internet.**

***Abstract*- The difficulty in learning how to use flow cytometry, the high costs of flow cytometers and the geographical dispersion of users and training centres, make training in this area very expensive. Thus, a simulator for training in flow cytometry would be very interesting. In this paper we present a prototype of such a tool, which is under development for Windows platforms and it is meant to be used either directly by the trainees or by developers of hypermedia courseware for producing simulations which can be integrated in their courses. It should also be possible to use this simulator through the Internet.**

I. INTRODUCTION

Flow cytometry, a technique which allows the measurement of physical and/or chemical characteristics of cells, began in the seventies and is currently used in a great number of areas in medicine (immunology, pathology, etc.) and biology. Its use has been increasing in the last years and this trend will probably continue in the near future [1,2].

Training people to use flow cytometry is a hard and time consuming task, since it is a difficult technique to use in its full extent and benefit. This imply great commitment of the experts that act as trainers. These reasons, the high costs of flow cytometers and the fact that users usually are distributed geographically and sometimes far from the main training centres, make this training very expensive. As in other areas where simulators of biological equipment are currently being developed and used with interesting results [3,4], the existence of simulators, as well as self study courses in which simulators can be integrated, with the possibility of being used by all the trainees, could decrease significantly the institutional effort needed to train flow cytometry users. This would be possible due to the decrease in the time trainees need to use the equipment, the help they need from their trainers and the time they have to be dislocated at a training centre.

A further advantage of using a simulator in training people is the fact that it is easy to create testing simulations, which can be very interesting either to self or formal evaluation of the trainees.

In this work we will present a prototype of a flow cytometer simulator, currently under development for Windows platforms. It was designed to be used not only directly by traineess or trainers in self-study or guided training sessions, but also to produce simulations which can be integrated in hypermedia courseware. The possibility of making this simulator available over the Internet is also under consideration since it would make it more accessible for geographically dispersed traineess.

II. THE FLOW CYTOMETER

Cytometry refers to the measurement of physical and/or chemical characteristics of cells (or by extension, of other biological particles). Flow cytometry is a process in which such measurements are made while the cells or particles pass in a fluid stream through the measurement apparatus [1,2].

There are many physical and chemical characteristics (or parameters) of cells or other biological particles which can now be measured by flow cytometry (cell volume and realtive size and complexity, DNA content, etc.), most commonly through optoelectronic measurements.

In a typical flow cytometer, unstained or stained cells in a suspension are made to flow in a laminar manner by a suitable hydrodynamic system, such that they line up in single file moving through a beam of illuminating light, usually from a laser. Once a cell has intersected a laser beam, the light which is subsequently transmitted is composed of two types, scattered light and fluorescent light. The resulting fluorescence and/or scattered light signals are collected by lenses, separated by optical filters and focused onto photomultiplier detectors. The electrical pulses generated by the photomultipliers are then amplified, measured and undergo a analog to digital conversion The pulses are processed by multichannel

analyzers to provide frequency distributions for each measured parameter.

Current commercial flow cytometers can perform five to ten simultaneous measurements. Whether measuring a single parameter or multiple parameters, appropriate optical filters must be carefully selected such that only light of specified characteristics (within a defined bandwidth) reaches each of the various detectors [1,2].

Figure 1 depicts important aspects of the internal organization of a flow cytometer.

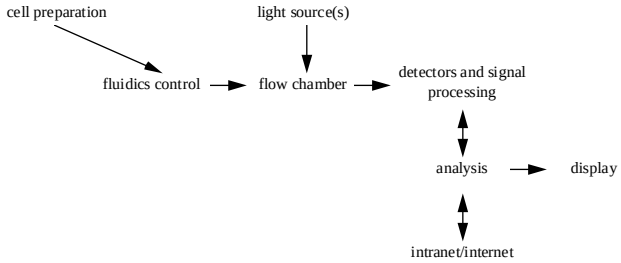

Figure 1- Flow cytometer internal organization (adapted from Phillip N. Dean, Overview of Flow Cytometry Instrumentation *in* Current Protocols in Cytometry (1997) 1.1.1-1.1.8, John Wiley & Sons, Inc.)

III. THE SIMULATOR

The training on the flow cytometer simulator is not intended to replace the traditional training, it is meant to enhance its efficiency and efficacy.

The prototype was designed to be used not only by beginners but also by more proficient trainees as well as trainers. Its purpose is twofold:

1- to provide trainees with a cost effective interactive tool which they can use as often as they wish, increasing their knowledge and experience without the need to occupy the real equipment and the teacher;

2- to provide trainers with the possibility of preparing simulations which can be used in direct training sessions or included in hypermedia courseware.

Such a simulator should be an application that might be perceived by the user as a virtual cytometer, based on an adequate graphical environment and having associated models of the fundamental parts of this type of equipment; allowing the user to act on those parts and obtain the corresponding results. Although there are several different types of cytometers available on the market, all of them are based on the same physical principles, thus developing a general simulator which could be "tailored" to each specific case, does not seem too difficult.

The choice of platform on which to implement such an application was much facilitated by the fact that all flow cytometers have as fundamental part a personal computer. Since this is currently a Windows platform in most cases, the simulator is under development for this type of platform.

An expert in flow cytometry (experienced also in training other users) was actively involved along the complete process of design and development of this prototype, as it is usual in participatory design [5].

In this section we will refer the type of simulations which are possible using this simulator, the main functionality offered to the user, as well as the more relevant aspects of the user interface.

A. Some possible simulations

A real flow cytometry experiment can be divided in the following phases:

1 - preparation of a suspension of unstained or stained cells that is to be analysed by the cytometer. This may imply labelling of some of the substances of cells with fluorescent probes, controlling the cell concentration, etc.; this part of the experiment is not considered in the developed simulator.

2 - preparation of the cytometer to the acquisition. This phase is generally named the protocol definition. It allows the user of the cytometer to select the optical filters to be used, the characteristics of light to be measured (scattered, fluorescent), which of the measured parameters shall be graphically displayed during acquisition, the gain and voltage of the photomultipliers and several other settings. The result of changing all these settings are, or shall be in the near future, simulated.

3 - acquisition of data. During this period the user can track the development of the acquisition by observing some of the measures in graphical form (as scatter plots, usually referred in this context as "histograms"). It is also possible to observe the shape of each pulse by the use of a virtual oscilloscope connected to the output of the photomultipliers. The simulator mimics the acquisition phase by reading (and if necessary processing) data from previously prepared files.

4 - the final phase of the experiment consists in the off-line analysis of the measured data. There are a lot of commercial and noncommercial software packages available to perform this task. The simulator has some capabilities of data analysis.

B. User interface and its main aspects

In this type of application the user interface is very important; its usability can be an essential factor to the success of the simulator; thus special attention must be given to its design and implementation, following as much as possible the principles and guidelines available in the literature [5,6,7].

The flow cytometer itself seemed the obvious choice for the metaphor of the simulator's user interface; thus the graphical environment reproduces the essential characteristics of a general flow cytometer.

In order to accommodate different types of users which can have variable task and system experience, from very low to very high (i.e. from beginners to experts), several dialogue styles were used (direct manipulation, menus and function keys) integrated as smoothly as possible

To make the simulator as easy to learn and to use as possible by all those prospective users, two main modes were developed:

* a guided, less flexible, mode for beginners, which presents to the user a sequential dialogue (offering both semantic and syntactic assistance), adequate for users which are inexperienced users of this specific application as well as naive flow cytometer users (i.e. typically trainees in their first attempts to use the simulator);
* a less structured, more flexible, mode for experienced users which have semantic knowledge, as trainers and advanced trainees.

The main functionality offered to the user (the same in both modes) is:

* acquisition- corresponding to virtually acquiring data corresponding to the cell populations contained in a sample;
* protocol setting- allowing the selection of parameters to be used for data acquisition and visualization;
* visualization- allowing the visualization of the "histograms" corresponding to the cell measurements previously selected;
* tools- allowing the use of several tools (as an oscilloscope and a bank of optical filters);
* archive- management of the sample archive;
* help;

All this functionality is available through a menu bar, function keys or direct manipulation alternatively.

As referred, most virtual operations corresponding to phases 2 to 4 described in the previous section are or shall be integrated in the simulator. The preparation of virtual samples to be acquired by the virtual flow cytometer is not under consideration for the moment; nevertheless, the development of an independent module for such preparation would possibly be an interesting help to trainers.

Figures 2 to 6 show several different aspects of the user interface which are presented to the user in both guided or free modes allowing him/her to prepare the virtual cytometer, follow the acquisition and analyse the virtual results.

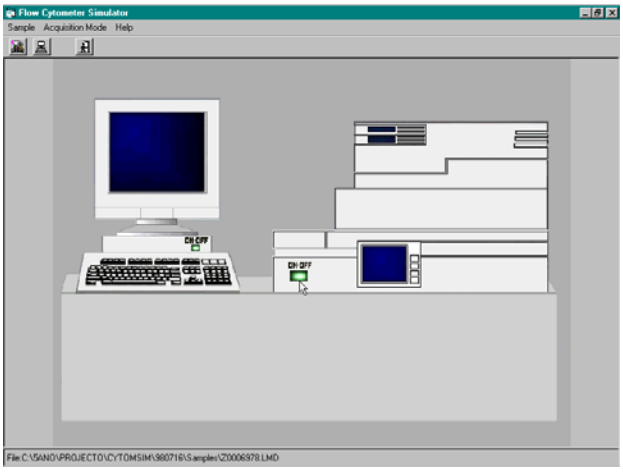

Figure 2- Main screen depicting the abstract scheme of a general flow cytometer

The main screen (shown in figure 2) depicts an abstract scheme of a flow cytometer (in order to represent the general features of all the cytometers and not the specificities of any particular type). On this screen the user has to turn on the cytometer and the computer; then he/she can proceed to the preparation of the acquisition, making all the necessary choices (signals to be visualized, gains and voltages of the photomultipliers, etc.) on the protocol screen (figure 3). After making these choices the user selects the virtual sample and starts acquisition.

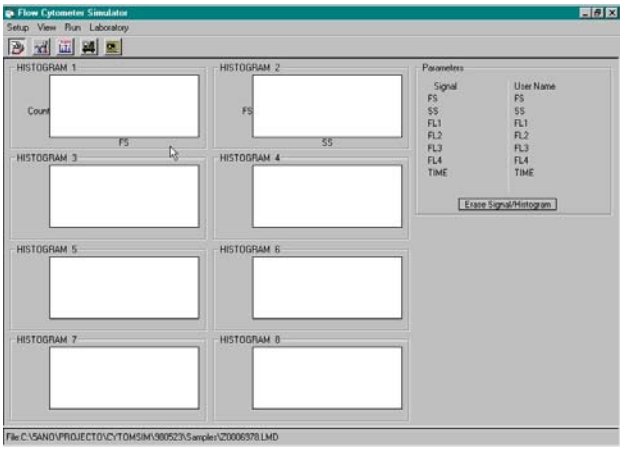

Figure 3- Protocol screen (choice of acquisition settings)

During the acquisition the pulse shape of two signals can also be visualized on a virtual oscilloscope (figure 4), which allows the user to select not only which signals should be displayed but also the time scale (all through direct manipulation of buttons similar to the ones of a real oscilloscope). Also during the acquisition the "histograms" can be visualized (figure 5).

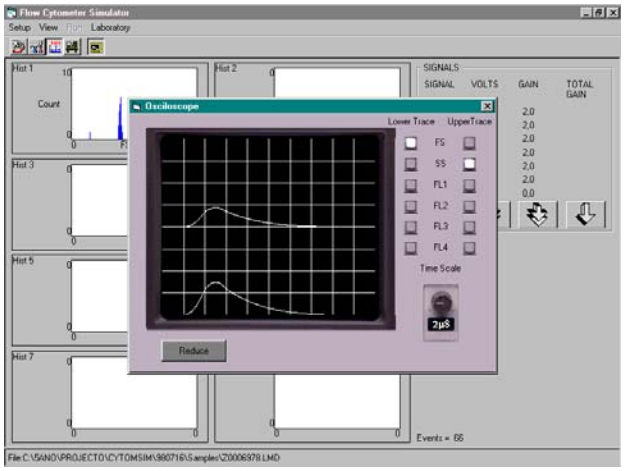

Figure 4- The osciloscope displaying two signals simultaneously

Finally, after the acquisition process is finished some simple analysis can be performed. Several types of graphical visualization of the acquired data are available and can be used with different resolutions. Gates can be applied on some graphics, so that data which complies with some characteristics defined by the gate can be examined separately (figure 6).

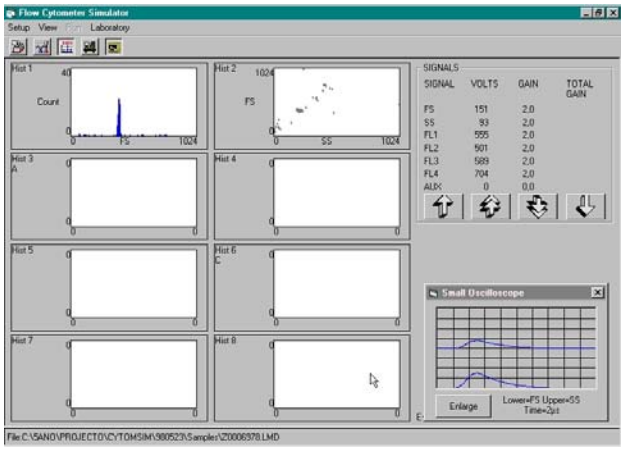

Figure 5- Visualization of the "histograms" (and minimized oscilloscope)

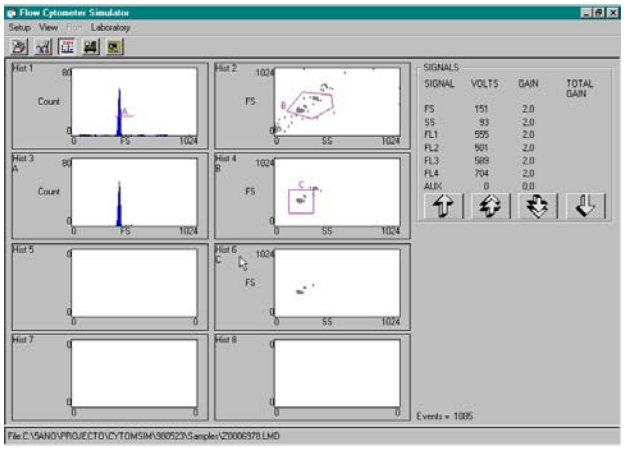

Figure 6- Analysis of results using gates on the "histograms"

IV. EVALUATION

Evaluating the interest of this simulator, as is the case of other simulators [3], involves assessing if and how much the training in flow cytometry is improved by the use of this tool. This will have to be performed in IPATIMUP, an institution which regularly trains people in this area.

V. CONCLUSIONS

In this work we have discussed the interest of flow cytometry simulators in direct or distance training and have presented a prototype of a flow cytometer simulator which is under development.

Currently, and to the best of our knowledge, there are no other simulators for this type of equipment; the existing training applications are unsophisticated, allowing only some simple simulations of specific aspects of the flow cytometer. Our goal is to develop an actual simulator which could be called a virtual flow cytometer, offering a graphical environment which imitates the cytometer and conveys to the user the feeling of using the actual cytometer. Having just the simulator can already be a great help to train people, however its usefulness can be enhanced if the resulting simulations can be used in hypermedia courseware or trough the Internet.

The performance of the simulator, in making easier the training of future flow cytometry users, it not yet assessed. This evaluation will have to be performed at IPATIMUP.

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