C1. **Evolution of flow cytometry over the last ten years**

Lydia Campos[1,3], Carmen Mariana Aanei[1], Tiphanie Picot[3], Denis Guyotat[2,3]
*1- Laboratoire d'Hématologie, Hôpital Nord, CHU de Saint Etienne, France*
*2- Département d'Hématologie, Institut de Cancérologie Lucien Neuwirth, Saint Priest en Jarez, France*
*3- Laboratoire de Biologie Moléculaire de la Cellule, UMR 5239, Lyon, Université Jean Monnet, Saint Etienne, France*

In the last 10 years, flow cytometry (FCM) benefited of a tremendous progress in its different aspects. Major contributions have been achieved in the field of **instrumentation**, **reagent development, and software analysis tools, leading to the** identification of specific subsets of cells with unique biological functions in normal and pathological conditions. The evolution from 3-4 color analysis to 8-10 or more has allowed a better specificity for the desired cells, specially the detection of low frequency populations.

**Instrumental technological advances**: high powered and smaller lasers provide stable excitation sources in a wide variety of wavelengths (emitting blue, red, green, violet and yellow light). More sensitive measurement allows better resolution of dimly-staining populations from background.

Advances in **affinity reagent technology** have enabled the flow cytometric detection of numerous proteins and other molecules through the development of new monoclonal antibodies, peptides/MHC multimers, recombinant receptor and ligand binding proteins, and aptamers.

Fluorescence chemistry (tandem dyes, nanocrystals), allows fine cell analysis up to 20 parameters. Parallel advances in instrument calibration methodology, have facilitated the application of multiparametric flow cytometry analysis of the biology of quiescent, activated, growing, differentiating, proliferating, dying and dead cells, as well as cell signaling and cytokine production.

All this generates very complex data sets that demand sophisticated tools of analysis, storage and data representation. However, **data analysis strategies** are still relatively underdeveloped. The multitude of data available is rarely analyzed easily. So, a need for plug-and-analyze software has emerged. New analysis tools development remains an important step as they will permit to analyze and compare several parameters in a multi-well format simultaneously and this for several cell types.

In the last few years, new bench-top flow cytometers have appeared. They combined analytic power into ergonomic, ultra-compact and easy- to-use analyzers.



**Perspectives**

In recent years, there was not only innovation in flow cytometry but also in the field of image-based cytometry. The maturation of multispectral imaging cytometry in flow imaging and the slide based laser scanning cytometers offers the possibility to have real time analysis of cells and tissues. Technological innovations are bringing the next generation of cytometers. In this context, the development of microfluidic, lab-on-a-chip (LOC) technologies is one of the most innovative approaches toward the advancement of cytometry. These technological advances have given rise to new platforms for the characterization of single cells.

These technologies include the:

FISHMAN-R is a Japanese analyzer that enables flow cytometry on a microfluidics chip. It could detect particles sized from 0.5 to 20 µm (bacteria and cells). Sample acquisition and data analysis are performed using the same software (PC). The Celigo is the first adherent cell cytometer, which analyzes cells in their environment with minimal sample manipulation. It analyzes adherent and non-adherent cells in brightfield and 3 fluorescence colors. The high-throughput acquisition is achieved by using an F-theta lens with a high speed galvanometer mirrors. It has a CCD camera. The Celigo software performs both image acquisition and analysis. The LEAP is a microplate-based cytometry system for non-invasive in situ process, allowing the use of various adherent and non adherent cell types.

**Imaging flow cytometry: FlowCAM** was the first bench top digital imaging analyzer for particle or cell measurements in solution, originally developed for oceanographic research.

The **ImageStream** is a multispectral imaging flow cytometer that combines the strength of FC and fluorescence microscopy in a single platform. It can digitally image millions of cells directly in flow. This technology enables the identification of single cell by fluorescence and morphology (5 excitation lasers and 12 channels of detection).

The new **FlowSight** based on the same technology, combines a brightfield lamp, 4 excitation lasers and 12 channels of detection to analyze simultaneously brightfield, darkfield and ten fluorescence colors per cell at a rate of 2,000 cells /second.

Other **optical developments** appear such as **COST coding**, which is a new way to detect **multifluorescent wavelengths** using a single photodetector. This method of discriminating multiple fluorescent colors with a single photomultiplier holds promise to significantly reduce the cost and size of the total system. User can differentiate 11 fluorochromes in FC by using a single PMT.

**Mass cytometry is a completely different, non-fluorescence based approach. The Cytometric Time of Flight (CyTOF)** cell analyzer is a high throughput mass cytometer for individual cell analysis based on a novel technology. The instrument detects the stable isotopic tags attached to antibodies using labeling kits. There are > 100 stable isotopes and the mass spectrometer provides highly precise resolution between detection channels (w/o compensation). In the instrument stained cells are nebulized into single-cell droplets and introduced into the plasma. The resulting charged atomic ion clouds are immediately transferred into the high vacuum of the mass spectrometer. The cytometer is configured as a quadrupole-time-of-flight (qTOF) instrument. The quadrupole acts as a filter allowing only the heavier elemental ions, which consist primarily of the reporter "masses", to be quantitated by TOF mass analysis. A thousand cells are analyzed per second. Mass cytometer allows as many as 45 parameters to be measured for each cell. With phospho-flow cytometry is possible to measure the phosphorylation status of proteins critical to intracellular signaling cascades at the single-cell level.

This new technology allows simultaneously examined internal functional markers and cell surface markers to put together a more complete picture of cell signaling. It has increased understanding of cell expression and maturation pathways during hematopoiesis.

We expect that FCM will continue to decrease in size and energy consumption and will increase in detection and precision measurements.



**In conclusion**, the evolution of flow cytometry technology, has allowed an important understanding of cell biology. All this new technology is now used in clinical research but it would be applicable in clinical laboratories with adequate standardization inter-laboratories.

**References**

Chattopadhyay P, Roederer M. Cytometry: today's technology and tomorrow's horizons. Methods 2012, 57:251-258.

Wlodkowic D, Darzynkiewicz Z. Rise of the micromachines: microfluidics and the future of cytometry. Methods Cell Biol 2011, 102: 105-125.

Nolan GP. Flow cytometry in the post fluorescence era. Best Practice & Res Clin Haematol 2011, 505-508.