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GENERAL REVIEW

New tools in cytometry

Nouveaux outils en cytométrie

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Summary Cytometry aims to analyze cells, of any type, using dedicated instruments. The quantitative aspect makes flow cytometry (FCM) a good complementary tool for morphology. Most of the identification tools are based on immunostaining of cell structure details and more and more tools are available in terms of specificities and labels. FCM is under exponential development thanks to technical, immunological and data analysis progresses. Actual generations are now routinely using 6 to 10 simultaneous immuno-labeling on 20 to 100,000 cells, at high speed and short sample preparation and can easily detect rare events at frequency below 10^{-4} cells. Data interpretation is complex and requires expertise. Mathematical tools are available to support analysis and classification of cells based. Cells from tissues can also be analyzed by FCM after mechanical and or enzymatic separation, but in situ cells can also be analyzed with the help of cytometry. Very new instruments bring spectral analysis, image in flow and mass spectrometry. Medical applications are very broad, notably in hemopathies, immunology, solid tumors, but also microbiology, toxicology, drug discovery, food and environmental industry. But, the limit of FCM is its dependence on operator from sample preparation, instrument settings up to data analysis and a strong effort is now under progress for standardization and constitution of international data bank for references and education.

MOTS CLÉS
Cytométrie en flux ;

Résumé La cytométrie vise à analyser les cellules de tout type, en utilisant des instruments dédiés. L'aspect quantitatif fait de la cytométrie de flux (FCM) un bon outil complémentaire à la morphologie. La plupart des outils d'identification sont basés sur une détection immunocytochimique des détails de la structure cellulaire et de plus en plus d'outils sont disponibles

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en termes de spécificité et de marquages. La FCM est en développement exponentiel grâce aux progrès techniques, immunologiques et en analyse des données. Les générations actuelles d'appareils utilisent maintenant couramment 6 à 10 immuno-marquages simultanés sur 20 à 100 000 cellules, à grande vitesse avec des étapes courtes de préparation des échantillons et peuvent facilement détecter des événements rares à une fréquence inférieure à 10^{-4} cellules. L'interprétation des données est complexe et nécessite de l'expérience. Des outils mathématiques sont disponibles pour permettre l'analyse et la classification des cellules. Les cellules provenant de tissus peuvent également être analysées par FCM après séparation mécanique ou enzymatique, mais les cellules in situ peuvent également être analysées à l'aide de la cytométrie. Les instruments les plus récents permettent une analyse spectrale, l'image du débit et la spectrométrie de masse. Les applications médicales sont très larges, notamment dans les hémopathies, en immunologie, pour les tumeurs solides, mais aussi la microbiologie, la toxicologie, la découverte de médicaments, l'alimentation et l'industrie de l'environnement. Mais la limite de la FCM est sa dépendance à l'opérateur de la préparation de l'échantillon, les paramètres de l'appareil jusqu'à l'analyse des données et un effort important est actuellement en cours pour la normalisation et de la constitution d'une banque de données internationale pour servir de références et pour l'enseignement.

Introduction: what does cytometry mean?

Cytometry aims to analyze cells of any type, including microbes and non-nucleated cells, using instruments and computers with most of the time mathematical assistance. The use of an instrument means signal measurement, partial automation, possible high-throughput (analysis of large amounts of cells) and high content (measuring many parameters simultaneously). It does not necessarily means fully objective measurement. Also and most of all, cytometry does not means standardized yet and we shall see that this is a major issue to be solved on the next few years, even if the question is already addressed for some time. What makes cytometry so special in the biological world is its unique feature of analyzing large amount of cells at single level and on multiple parameters.

Cytometry includes cell analysis in flow or on slides and more recently in vivo. In cytometry on flow, cells need to be in suspension injected in the instrument at high speed. Flow cytometry (FCM) is the most widely used cytometry technique, especially in medical applications. In cytometry on microscopes, cells can be analyzed on slides or in cell culture vessels and microscopic view is computer-assisted. Cells can be individual or confluent, from culture, smears or even in tissue sections. They can be analyzed in 2 or even in 3 dimensions by playing with focalization. Living cells can also be analyzed on time (4D) during culture. If cells are confluent, their frontiers are not always easy to define. They are frequently individualized by locating their nuclei that can be easily stained. More recently, cell analysis was adapted for in vivo cell studies, during surgery or directly by transcutaneous microscopy or endoscopy [1,2]. All these techniques are under very fast evolution. Here, we shall focus on recent developments of FCM.

Principles of cytometry

FCM is based on light scattering and multiple fluorescent labeling. Cells in suspension are injected in a thin, laminar flow into the system (Fig. 1). The sample stream is centralized by a second flow of inert buffer (sheath fluid) that is used for hydrodynamic focusing. Cells must pass exactly in front of the laser beams (cell alignment). Typically, the light sources are violet, ultraviolet, blue and red but yellow and green are also available. Several parallel beams can be used and signals are related to the same cell by considering the time delay between laser hits. Cell scatter is measured by photodetectors in front (forward scatter directly related to cell size) and laterally (side scatter, related to cell heterogeneity or granularity). If cells are labeled, fluorochomes are activated by the laser beams and the signals are selected by a pinhole, separated in the optical bench with dichroic mirrors and filters and measured by different photomultipliers (PMT).

Cell labeling is possible thanks to fluorescent chemicals that specifically stain some cell components or are enzyme substrates and/or fluorochromes conjugated to monoclonal antibodies. A large portfolio of fluorochromes with different spectra of emission is now available. Of course, using simultaneously different fluorochromes means that colors must be separated. This is done by a combination of filters and mirrors in the optical bench. But because spectra are overlapping, we need further mathematical corrections (compensation) that represent the tricky part of the protocol designs and settings [3,4].

The second point is that cells are analyzed one by one, in a flux, at high speed (100 to 1000 cells per seconds or more) implying the cells must be in suspension and well separated. This makes FCM particularly adapted for hematology, immunology but more applications are available for cell biology, pharmacology, toxicology and even solid tumors [5,6].

The last main point is the multiparametric level of information on each cell (classically 6 to 12 or more parameters per cell) making possible to define very precisely phenotypes and to cluster the cells in sub-communities according to their levels of resemblance. This requires specific biological expertise. Parameters can be structural or functional. Even very rare cells can be identified. Several international

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