Development of innovate flow cytometry methodologies applied to the field of microbiology

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Résumé

Introduction and objectives:
Flow cytometry has evolved significantly over the past two decades. The miniaturization of optical systems (lasers, optical benches), fluidics (capillaries) and a sample loader (with High-Throughput Screening, HTS) have made this technology increasingly accessible to a broad range of users. What once required a dedicated room with complex pumping systems and specific currents is now condensed into simple, compact benchtop cytometers.

The increase in the number and power of lasers, now commonly found in most cytometers on the market, has significantly multiplied the number of detection parameters while greatly enhancing sensitivity thresholds.

Initially reserved for the acquisition of fluorescence associated with eukaryotic cells, the detection of smaller particles such as bacteria has opened up new possibilities for cytometry in the field of microbiology. Rapid analysis and quantitative characterization of micro-organisms are attracting growing interest in many areas of microbiology.

In this context, our eBioCyt UPS1401 platform, a "Unité Propre de Service" of the University of Strasbourg, has been developing fluorescence detection assays linked to different bacteria strains (Gram positive & negative, cocci or bacilli) since 2013 (1).

More recently, we have contributed to the development of measurements of the differential binding of fluorescent probes to several bacterial strains, as well as measurements of the effect of naturally occurring substances on bacterial toxicity.

Materials and Methods:

Bacteria were incubated with various innovative probes, combining Nile Red with different sites of the antimicrobial peptide Ubi29-41. Fluorescence intensity measurements were carried out using flow cytometry. Additionally, bacteria were incubated for 24 hours with different compounds, followed by staining with propidium iodide. Samples were analyzed using capillary flow cytometry (Guava EasyCyte Plus, Merck Millipore).

Results, discussion and conclusion:

*Intervenant

This work illustrates various applications, such as measuring the differential binding of fluorescent probes depending on the bacterial strain. Indeed, the synthesized probes showed varying levels of fluorescence labelling depending on the bacteria. The peptide probe UNR-1, incorporating the fluorophore as a minimalist non-natural amino acid (Alared), demonstrated the highest efficiency (2; 3).

This type of study could provide valuable alternatives to conventional bacterial infection diagnostic tests, being highly efficient and compatible with HTS methodologies. The research also demonstrated the possibility of generating dose-response curves for various molecules with bacteriotoxic activity. The development of advanced software has enabled rapid and highly accessible analyses for a wide community of scientific users.

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3 Weiss L, Bonnet D, Dziuba D, Karpenko J. Flow Cytometry Analysis of Perturbations in the Bacterial Cell Envelope Enabled by Monitoring Generalized Polarization of the Solvatochromic Peptide UNR-1. *Anal Chem.* 2025 Jan 14;97(1):622-628. doi: 10.1021/acs.analchem.4c04953. PMID: 39810345.

Mots-Clés: Flow cytometry, microbiology, bacteria, toxicity, fluorescent probes