Detect bacteria in both research and industrial samples

New applications for flow cytometric analysis.

Immunologists have long applied flow cytometry to their field of study because it is capable of delivering phenotypic statistics at the single-cell level for subsets within heterogeneous cell populations. Over the last 50 years, this technology, which began with simple 1-laser, 2-channel instruments, has advanced to include instruments with as many as 10 lasers and 50 channels. As the capabilities of flow cytometry technology have expanded, so have the applications that can now be analyzed by flow cytometry.

In part, this expansion of applications for flow cytometry is the result of the pairing of this technology with other detection platforms. The combination of fluorescence imaging and flow cytometry has led to the development of imaging cytometry, which, although much slower and less efficient than standard flow cytometry, is beneficial in applications where protein localization or cell-to-cell interaction is of interest. The combination of mass spectrometry and flow cytometry has led to the development of mass cytometry, which allows for the rapid interrogation of individual cell phenotypes using antibodies labeled with heavy metal ions instead of fluorescent dyes. While these advancements have provided additional analytical power, they also have limitations; generally they are not capable of quickly analyzing large cell populations (e.g., >1 million events) or effectively evaluating difficult sample types without extensive and potentially damaging manual processing.

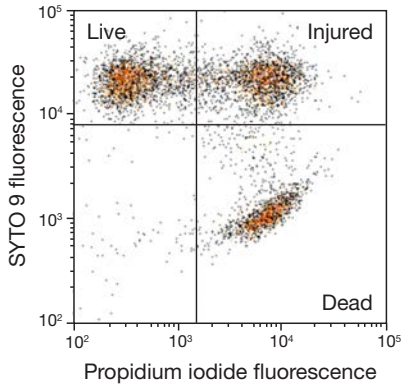


Figure 1. Staining of *Pasteurella multocida* using the LIVE/DEAD *Bac*Light Bacterial Viability Kit. *P. multocida* samples were analyzed using the Invitrogen™ LIVE/DEAD™ *Bac*Light™ Bacterial Viability and Counting Kit (for flow cytometry, Cat. No. L34856) on the Invitrogen™ Attune™ NxT Flow Cytometer at a flow rate of 25 µL/min with an event rate of approximately 5,000 events/sec. Samples were detected using the blue 488 nm laser and 530/30 nm emission filter for detection of Invitrogen™ SYTO ™ 9 Green Fluorescent Nucleic Acid Stain, and the yellow 561 nm laser and 620/15 nm emission filter for detection of propidium iodide (PI). The live, SYTO 9 dye–positive population is clearly distinguished from the dead, PI-positive population. Cells that are stained by both SYTO 9 and PI dyes are classified as injured cells, with some degree of damage to cell membranes.

Acoustics-assisted hydrodynamic focusing

While there have been many technologies that have been coupled with flow cytometry over the last 50 years, the introduction of acoustic focusing and positive displacement–based fluidics has opened the doors to new application areas in the environmental, food, and agriculture sciences, to name just a few. With its syringe-driven sampling, larger flow cell, and acoustic focusing–assisted cell alignment, the Invitrogen™ Attune™ NxT Flow Cytometer addresses many of the drawbacks of conventional hydrodynamic focusing–based flow cytometry. The Attune NxT Flow Cytometer provides faster run times (up to 10 times faster than traditional flow cytometers) and resistance to clogging, even with difficult samples that include larger or sticky cells. The anti-clogging design of the Attune NxT Flow Cytometer, attributable to its positive displacement–based fluidic system, has allowed previously incompatible samples types to be analyzed by flow cytometry. Here we describe advances in both instrumentation and reagents that facilitate flow cytometric analysis of bacteria in a wide variety of sample types.

Fluorescence-based detection of bacteria

Determining the size and viability of a bacterial population is a prerequisite for microbiology research, as well as for antibiotic, probiotic, and food and beverage industries. The traditional approach to microbial viability assessment is based on counting colony-forming units (CFUs), which requires several dilution and plating steps, appropriate growth media, and manual colony counting [1]. In addition, this method generally needs a minimum of 24 hours to complete, does not account for viable but unculturable microorganisms, and can be inaccurate due to the miscounting of cell clumps as single colonies. Thus, an alternative is needed to produce faster and more accurate counts of viable bacterial cells.

Using flow cytometry in conjunction with fluorescence-based viability assays allows thousands of bacteria to be analyzed in seconds,

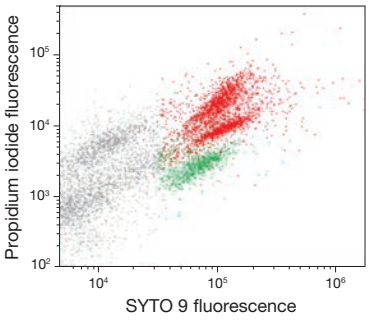
and population viability can be determined immediately. Moreover, high-throughput options allow large volumes and dilute samples to be analyzed. Early flow cytometry bacterial analyses were limited to studies of cellular aggregation because the instrumentation could not achieve enough resolution for single-bacterium identification [2]. Recent improvements in flow cytometers coupled with the availability of fluorescent bacterial viability dyes have led to increases in both resolution and the capabilities of multiparameter analysis.

The Invitrogen™ LIVE/DEAD™ *Bac*Light™ Bacterial Viability Kits provide two different nucleic acid stains—the green-fluorescent Invitrogen™ SYTO™ 9 dye and the red-fluorescent propidium iodide—to rapidly distinguish live bacteria with intact plasma membranes from dead bacteria with compromised membranes. The SYTO 9 and propidium iodide stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. When used alone, the SYTO 9 stain generally labels all bacteria in a population—those with intact membranes as well as those with damaged membranes. In contrast, propidium iodide penetrates only bacteria with damaged membranes, causing a reduction in the SYTO 9 fluorescence when both dyes are present. With an appropriate mixture of the SYTO 9 and propidium iodide stains, bacteria with intact cell membranes fluoresce bright green, whereas bacteria with damaged membranes fluoresce red (Figure 1). The LIVE/DEAD *Bac*Light Bacterial Viability Kits yield consistent results in studies that use a variety of eubacterial genera.

Wastewater analysis

Through bacterial detection and removal, wastewater treatment is intended to help protect the public from disease and infection caused by pathogenic organisms. Recycling of potable water is increasingly important, especially in areas affected by water shortages due to drought, overpumping of groundwater, or arid climates. Accurate bacterial counts can be difficult and time-consuming with traditional flow cytometers because environmental samples are often very dilute, making the time required for acquisition of large volumes of wastewater unfeasible.

With its acoustics-assisted technology, the Attune NxT Flow Cytometer allows for fast (up to 1 mL/minute) and accurate analysis of very dilute samples. In addition, total bacteria and live and dead populations can be quantified without the use of reference counting beads (Figure 2). This rapid acquisition rate supports the use of flow cytometry as a standard method for water testing in an industrial setting.



Name	Gate	Count	%Total	Concentration
All Events	All Events	29,169	100.0	29.140
cells	cells	10,257	35.2	10.247
Live	Live	1,007	3.5	1.006
Dead	Dead	2,813	9.6	2.810

Figure 2. Flow cytometric analysis of bacteria in treated municipal wastewater. A 3 mL sample of municipal wastewater was labeled using the Invitrogen™ LIVE/DEAD™ *Bac*Light Bacterial Viability and Counting Kit (for flow cytometry, Cat. No. L34856) and analyzed on the Invitrogen™ Attune™ NxT Flow Cytometer at a flow rate of 1 mL/min, allowing quick and accurate detection of a small number of bacteria. Concentrations of live and dead bacteria where determined without using reference counting beads. The two-parameter dot plot (propidium iodide vs. Invitrogen™ SYTO™ 9 fluorescence) shows well-separated live (green) and dead (red) bacterial populations; the statistics table displays the concentration measurements (cells/µL) for labeled bacteria in the acquired sample. Wastewater samples may also include small eukaryotes and types of bacteria that are potentially viable but nonculturable, each of which may be fluorescently stained with these dyes, as well as debris or other components that can contribute to background fluorescence.

Learn more about bacterial detection by flow cytometry

Find out about bacterial viability and vitality assays for flow cytometry at **thermofisher.com/flow-bacterial-viability** and about the Attune NxT Flow Cytometer at **thermofisher.com/attune**. ■

References

1. Hazan R, Que YA, Maura D et al. (2012) *BMC Microbiol* 12:259.
2. Mulroney KT, Hall JM, Huang X et al. (2017) *Sci Rep* 7:1903.

Product	Quantity	Cat. No.
LIVE/DEAD™ *Bac*Light™ Bacterial Viability and Counting Kit, for flow cytometry	1 kit	L34856
LIVE/DEAD™ *Bac*Light™ Bacterial Viability Kit	1 kit	L13152
SYTO™ 9 Green Fluorescent Nucleic Acid Stain	100 µL	S34854
Attune™ NxT Acoustic Focusing Cytometer, blue/red/violet6/yellow	1 each	A29004
Attune™ NxT Acoustic Focusing Cytometer, blue/red/violet6	1 each	A29003
Attune™ NxT Acoustic Focusing Cytometer, blue/violet6	1 each	A29002
Attune™ NxT Violet Laser Upgrade Kit	1 kit	100022777