WHITE PAPER CYTOMETRY

Flow cytometry for the analysis of microorganisms (yeasts, bacteria ...)

Introduction:

Used extensively for medical research and diagnostics applications, flow cytometry offers solutions for the counting and detailed analysis of microscopic flora such as yeasts, marine microorganisms, bacteria, spores, etc.

Principle of the method:

The detection parameters of flow cytometers are capable of providing information on the size, granularity and fluorescence emissions, either intrinsic (autofluorescence) or extrinsic (fluorescence due to the addition of probes or antibodies conjugated to fluorochromes).

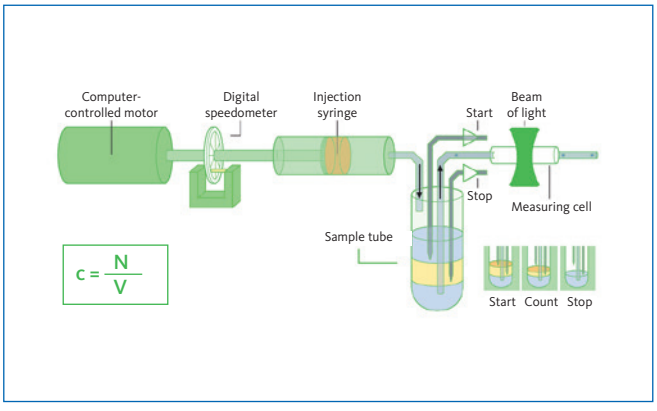
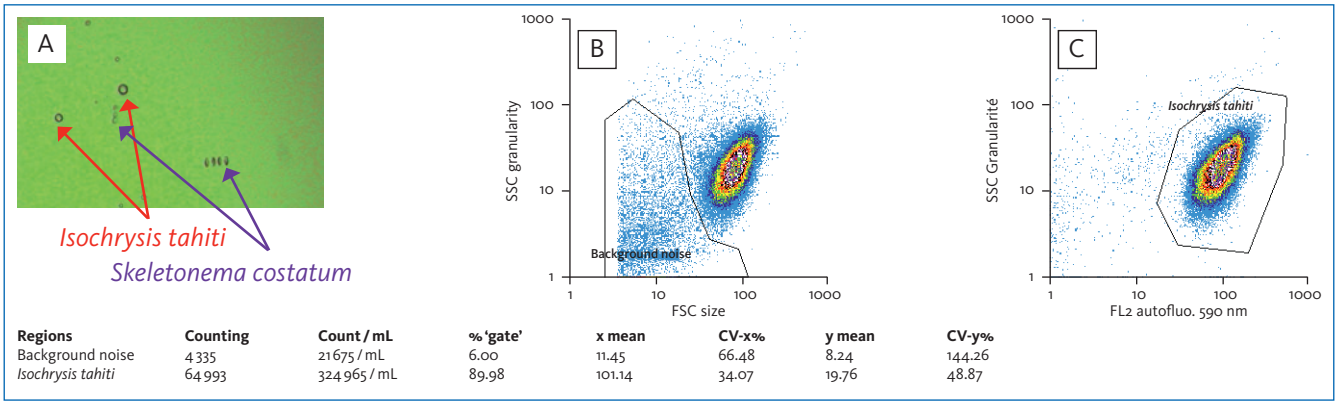


Figure 1: *Principle of Sysmex Partec volumetric mechanical counting using electrodes*

Microorganism counting:

The Sysmex Partec volumetric mechanical counting system using electrodes remains the simplest and most accurate counting method (Figure 1).

In the following example, the '*Isochrysis tahiti*' region of the last cytogram makes it possible to visualise the count of the microalgae (Figures 2A to 2C).



Regions	Counting	Count / mL	% 'gate'	x mean	CV-x%	y mean	CV-y%
Background noise	4 335	21 675 / mL	6.00	11.45	66.48	8.24	144.26
Isochrysis tahiti	64 993	324 965 / mL	89.98	101.14	34.07	19.76	48.87

Figure 2A: *Image of Isochrysis tahiti and Skeletonema costatum microalgae on the CyScope® microscope, 400 x magnification.*
Figure 2B: *Cytogram showing the size vs the granularity; the flow cytometry shows a population outside of the background noise.*
Figure 2C: *Cytogram showing the granularity vs the fluorescence emitted between 565 and 615 nm.The Isochrysis tahiti region allows counting of the microalgae of the same name: 324 965 microalgae/mL.*

Quantification of lactic acid bacteria:

Counting of lactic acid bacteria is essential for the quality assessment of yeasts and fermented dairy products. The bacteria samples are prepared and, if necessary, diluted with a peptonised saline solution or staining buffer.

These bacteria samples may be harvested from fermented dairy products or from freeze-dried cultures or cultures frozen at -20°C, which will consequently require re-suspension or thawing phases.

Performance of a double fluorescent staining protocol makes it possible to show dead and live bacteria (Figures 3A and 3B). There are various protocols available for achieving similar results in active fluorescent units, inactive fluorescent units and their ratio.

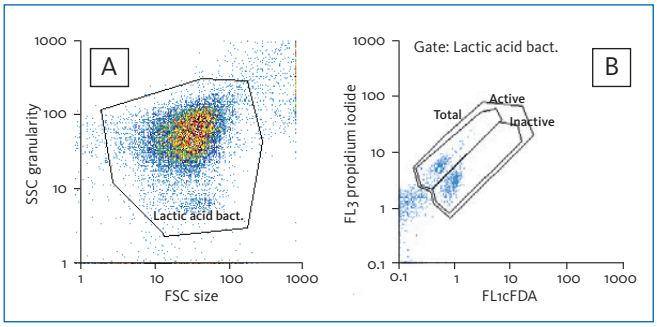


Figure 3A: *Cytograms showing size vs granularity of lactic acid bacteria.*
Figure 3B: *Cytogram showing viability staining (cFDA: FL1) vs mortality staining (propidium iodide: FL3) of lactic acid bacteria following a double staining protocol.*

Water quality analysis:

The technology employed in Sysmex Partec cytometers was specially developed to eliminate the limitations to which standard cytometers are usually subject and to create a compact, portable and easy-to-use format.

The sample is diluted to obtain a maximum concentration of 1 x 105 to 5 x 105 cells/mL, then stained with a fluorescent probe, which intercalates between the double strands of nucleic acids (Figures 4 & 5).

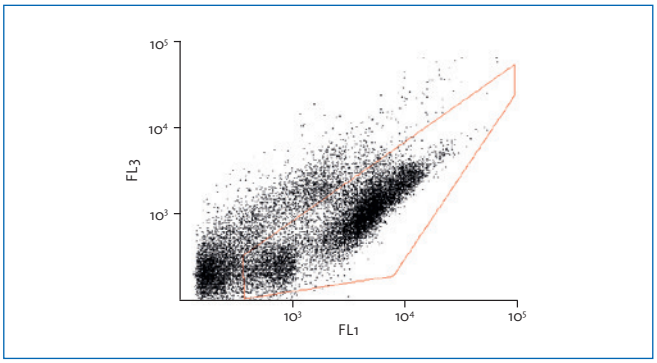


Figure 4: *Sample of drinking water stained with SYBR® Green: cytogram showing green fluorescence (SYBR® Green, FL1) vs red fluorescence (propidium iodide, FL3). The aquatic bacteria are gated in the 'red polygon' region. The signals above and to the left of this region are the unstained particles and the particles stained non-specifically with the SYBR® Green respectively.*

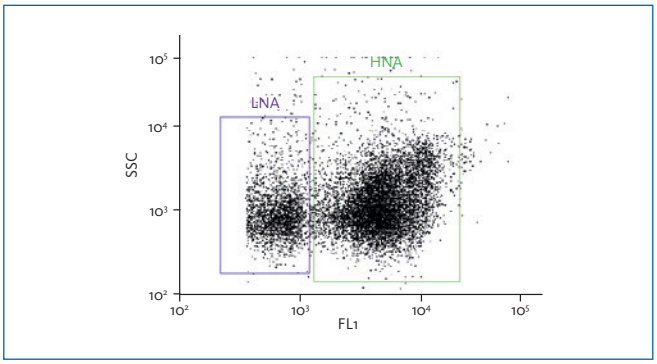


Figure 5: *The same sample of drinking water stained with SYBR® Green: cytogram showing green fluorescence (FL1) vs granularity (SSC). The aquatic bacteria, whose chromosome and plasmids are stained with SYBR® Green fluorescent, typically display different quantities of DNA. The 'LNA' bacteria in the blue region contain little DNA, and the 'HNA' bacteria in the green region contain more DNA.*

Yeast analysis: YeastControl™ solutions:

Sysmex Partec offers a range of YeastControl™ solutions for monitoring the biotechnological fermentation processes. These reagents are ready and easy to use, allowing rapid, direct results: proliferation, growth kinetics and other physiological parameters applied in brewing and winemaking, culture monitoring in biomedical research and optimisation of production methods (Figure 6).

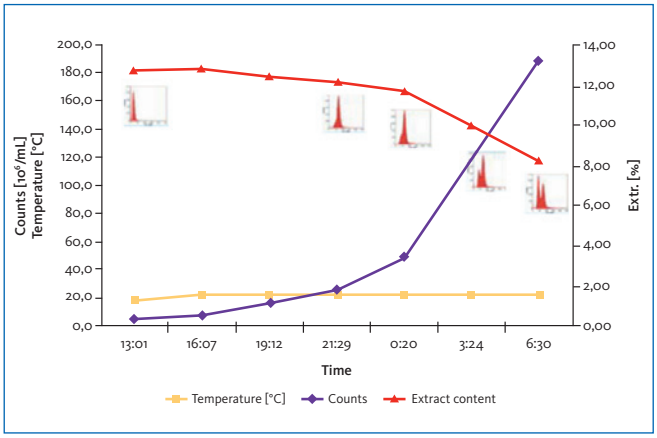


Figure 6: *Kinetics of cell counting (blue curve) compared to the percentage of their extract content (red curve) for optimisation of the fermentation method in brewing at a constant temperature (yellow curve). The superimposed cytograms show the distribution profiles of the cell cycles by means of flow cytometry.*

The YeastControl™ solutions make it possible to analyse: the cell cycle, viability (Figure 7), glycogen content, trehalose content, neutral lipid content, proteinase content and ageing (scarring of the buds on the surface of the yeasts).

Fermentation monitoring in winemaking: OenoYeast™:

Sysmex Partec offers the OenoYeast™ solution for monitoring the indigenous yeasts of white and rosé musts, tirage yeasts used for sparkling wines, the viability of the yeasts in case of languid fermentation and undesirable *Brettanomyces bruxellensis* yeasts during maturation (Figure 8).

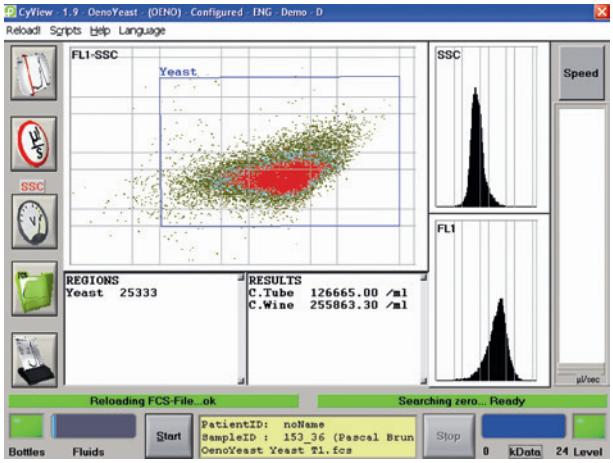


Figure 8: *Counting of live yeast with the Oenolyser® cytometer using the OenoYeast™ kit. The 'Yeast' region allows one to quantify the population of live yeasts shown in concentration in the results.*

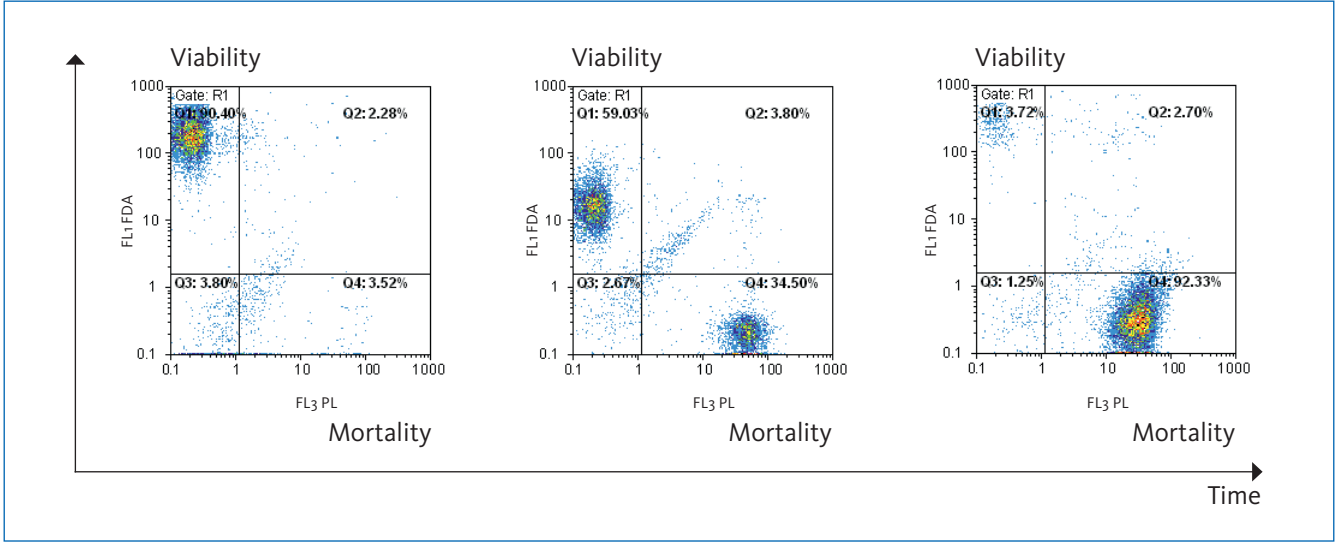


Figure 7: *Analysis of the viability of the Saccharomyces cerevisiae yeasts with the YeastControl™ kit. The Q1 quadrant quantifies the population of the live yeasts; the Q4 quadrant quantifies the dead yeasts. From left to right, the viability of the yeasts is affected over the course of time: mortality increases.*

Specific staining of microorganisms:

The investigation of blood cell populations by flow cytometry makes great use of specific stains, employing antibodies directed against epitopes, specifically characterising a cell population. This method is also starting to be used for identifying microorganisms in cultures.

Other cytometry inputs: intracellular pH, membrane potential, membrane vitality and fluidity:

Intracellular pH and membrane potential

The intracellular pH (pHi) is easy to measure with probes whose fluorescence depends on pH. An active microbial cell has to maintain a constant pHi throughout the course of its growth. This measurement therefore makes it possible to characterise the physiological state of cells over the course of the fermentation processes.

The transmembrane potential determines the exchanges between the cell and the external environment. Analysis of this potential allows the cellular state of the bacterial population to be characterised. It can be performed easily by flow cytometry with fluorescent anionic and cationic probes.

Vitality

Vitality is a parameter which characterises the metabolic performance of a microbial population. It is traditionally assessed using specific rates of growth or metabolite production. An indirect measurement based on the dependent energy excretion of a fluorochrome can be performed by flow cytometry, which makes it possible to obtain this parameter in 30 minutes.

Membrane fluidity

The membrane fluidity of microorganisms can be measured by fluorescence polarisation following staining with DPH (diphenylhexatriene). It is thus possible to follow the evolution of the membrane fluidity over the course of the culture, correlated to the modifications of the composition in membrane fatty acids. Fluidity can be measured much more simply and quickly with flow cytometry than for membrane fatty acids. The advantage of cytometry compared with the reference method in spectrofluorimetry is the ability to differentiate the measuring of fluidity in viable and dead cells using a viability/mortality/fluidity triple staining method.

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