



A40-Microbiology & A50-MiniFCM High Performance Flow Cytometers:

## An Introduction to Microbiology Applications

The 'A40-MiniFCM' and 'A50-MiniFCM' offer more power than traditional large flow cytometers in a small, portable package.

With high numerical aperture optics and a unique optical design, the A50 model is the most sensitive flow cytometer on the market, but not the most expensive.

Bacteria & archaea have a volume roughly 1000 times less than mammalian cells and therefore typically produce much smaller optical signals. For high quality data from these cells the flow cytometer's optics should be designed with this in mind. The MiniFCM is in a class of its own.

Apogee offers a range of flow cytometer models to suit different applications. For routine work with mammalian cells, our A40LC with standard sensitivity (comparable to flow cytometers from the best known manufacturers) may suffice. Microbiology users will find applications where only the high sensitivity A40 or A50 models gives the high quality results they need.

This document introduces common microbiology applications. To discuss your specific needs, write to [info@ApogeeFlow.com](mailto:info@ApogeeFlow.com)



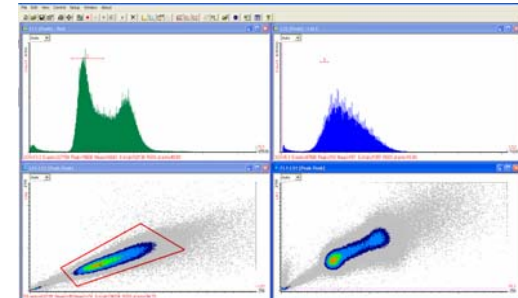


## Features:

The MiniFCM is the leading flow cytometer for microbiology applications. Bacteria, archaea, yeast, protozoa and some viruses can be analyzed in a wide range of samples (e.g. marine, bio-defense, food, water & environmental).

In addition to measuring the size/shape of each cell at up to three light scatter angle ranges, fluorescence markers offer a wide range of measurements:

- DNA content using a DNA binding dye (e.g. propidium iodide, SYTO, DAPI) or combination (e.g. mithramycin and ethidium bromide)
- Viability and vitality can be measured using
  - a membrane potential indicator such as DiOC<sub>2</sub>(3)
  - a DNA binding dye excluded by healthy, intact cell membranes (e.g. propidium iodide)
  - an indicator of esterase activity (e.g. CFDA)
- Gram staining to discriminate gram-positive bacteria from gram-negative bacteria
- Antibody techniques offer cell identification
- Consult the latest literature for more.



Up to 4 fluorescence detectors can be installed, so a combination of reagents can be used to measure several characteristics at the same time, for example DNA content using FL1 (green fluorescence from SYTO), and cell permeability using FL2 (red fluorescence from PI)

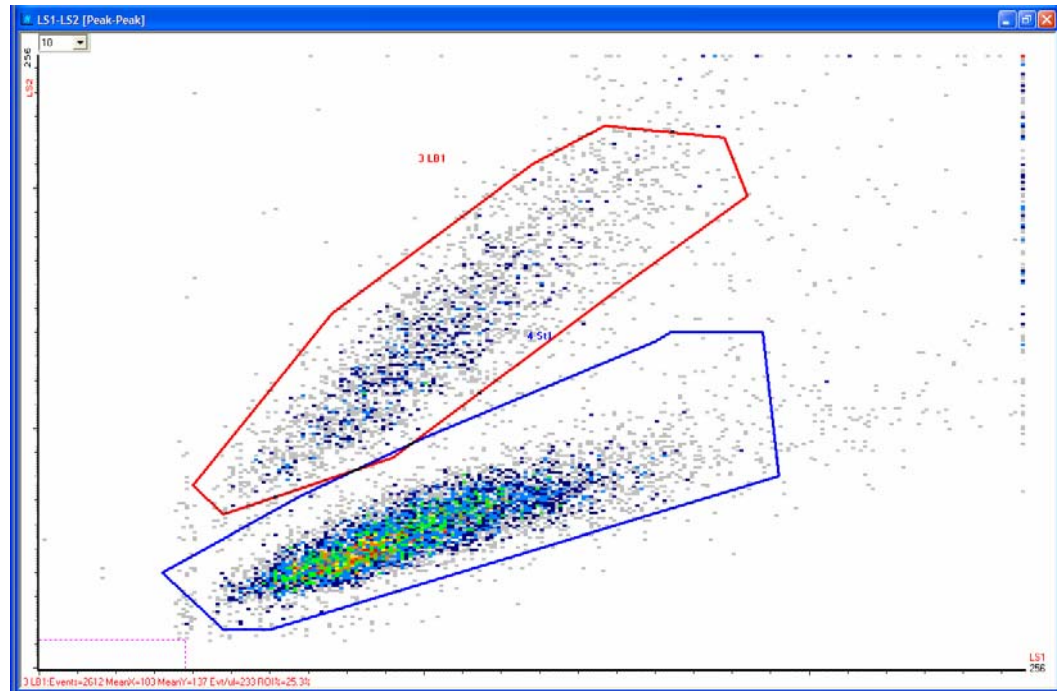


## Light Scatter Performance

Using light scatter alone, the MiniFCM is capable of counting and distinguishing different types of microbe. For example, Lactobacillus and Staphylococcus populations can be completely resolved.

On some conventional flow cytometers this mixture appears as just one population (overlapping), but the MiniFCM completely resolves the two types of cell using the two light scatter signals.

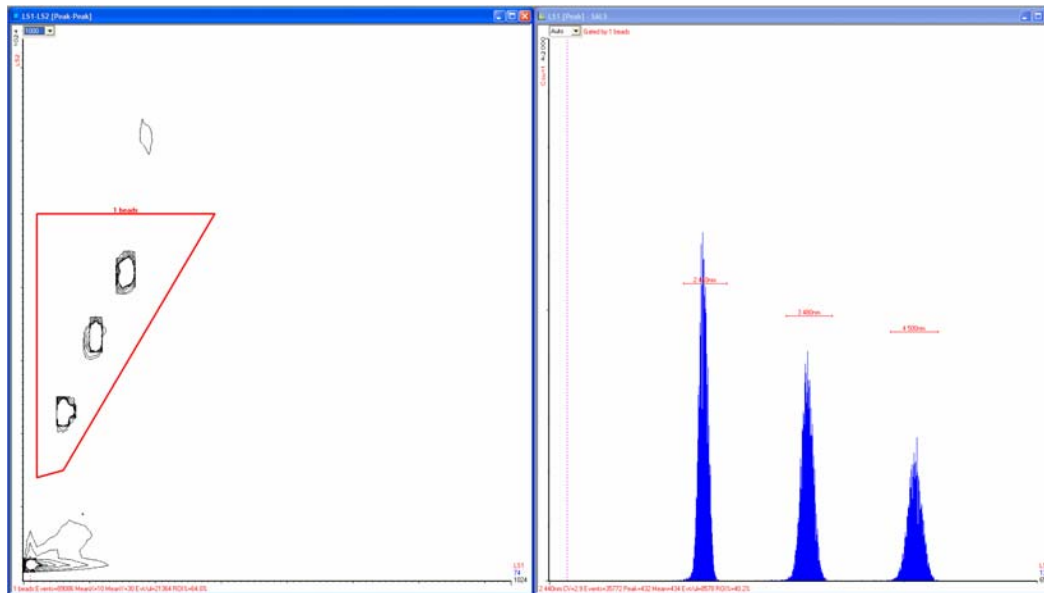
Of course fluorescence probes are often used to complement the light scatter data, but the machine's excellent light scatter resolution gives you useful cell size and shape information.





### Light Scatter Performance (continued)

The MiniFCM's unique optics allows incredibly small size differences to be measured. The data below shows resolution of 440nm, 480nm and 500nm latex test beads. The A50 model can measure, by light scatter, smaller particles than any other flow cytometer on the market, and has the best light scatter resolution of populations of small particles.



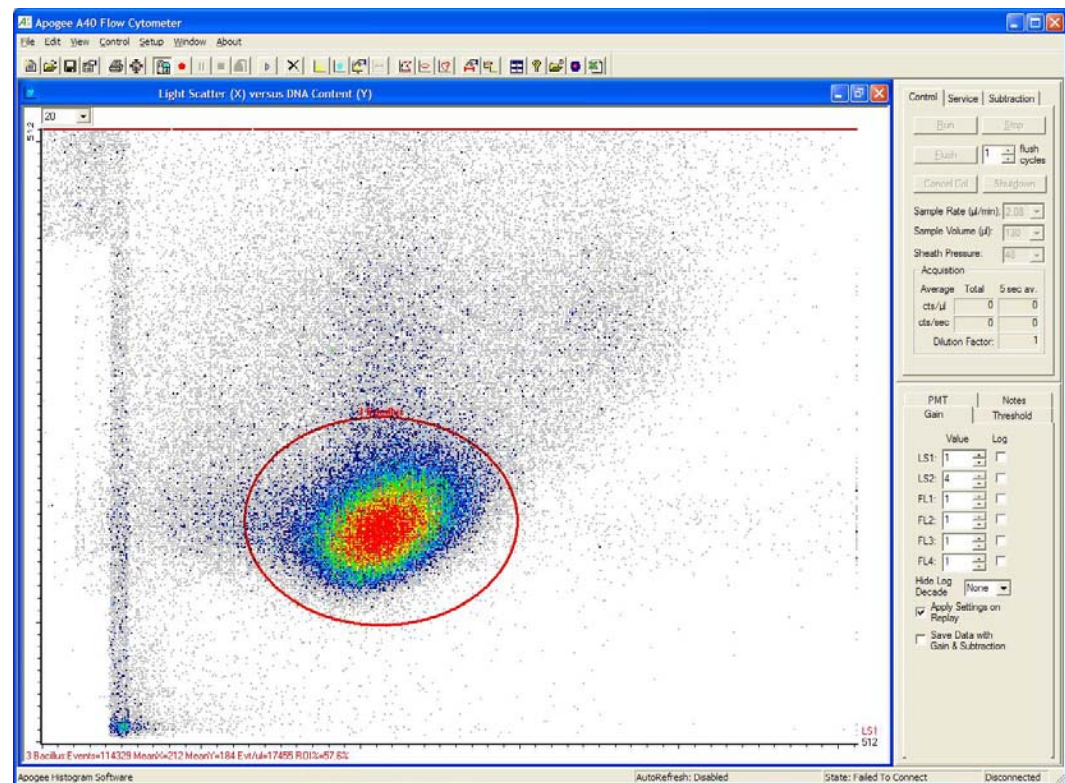


## DNA Content

Fast, convenient nucleic acid stains are available to fluorescently label DNA so that the fluorescence is proportional to DNA content. This allows discrimination of cells from background debris, cell cycle and ploidy analysis.

The cytogram shows a population of *Bacillus globigii* (a species of *Bacillus* found in soil and decomposing organic matter) prepared with a SYTO nucleic acid stain. The data is presented on linear scales with side scatter on X-axis and red fluorescence on Y-axis.

The fluorescent population of cells can be easily distinguished from background particles which do not contain DNA, and from particles with a different amount of DNA.



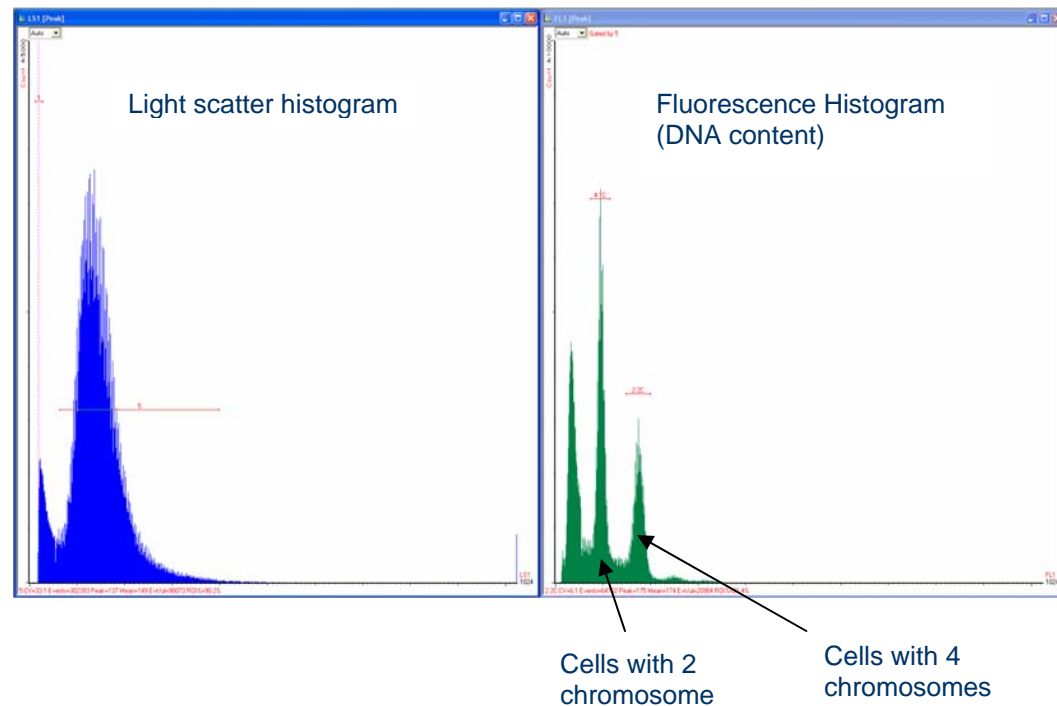


## DNA Content – Cell Cycle & Ploidy Analysis

Flow cytometry using DNA staining is an essential tool for the diagnosis of certain cancers (cancer cells often have an aberrant DNA content) and for performing cell cycle analysis. The principles apply to bacteria and archaea as well as mammalian cells. Analysis of the DNA content provides information on the proliferation of the cells, in particular on the duration of the different cell cycle phases by determining the relative number of cells.

This data is from a stationary phase sample of *Methanothermobacter thermoautotrophicus*. This archaeal extremophile (1.75Mbp genome) grows as flexible rods or curved filaments.

The DNA content histogram shows populations of cells with 2 and 4 genome copies.

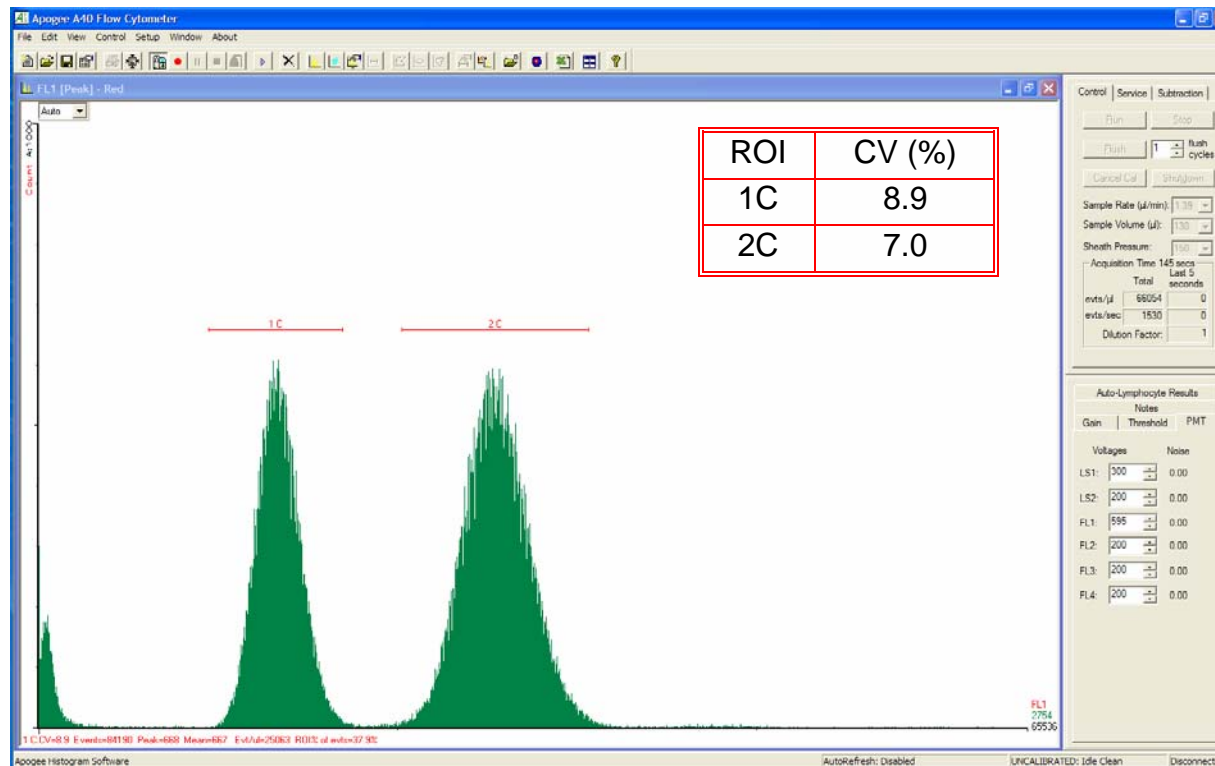


Above data acquired on Apogee A40 flow cytometer courtesy of Prof R. Bernander & Dr M. Lundgren (see Journal of Bacteriology, 2005, 187:1856-1858)



## DNA Content – Ploidy Analysis (continued)

E. coli treated to give populations with 1 and 2 chromosomes. The MiniFCM fully resolves the two populations.



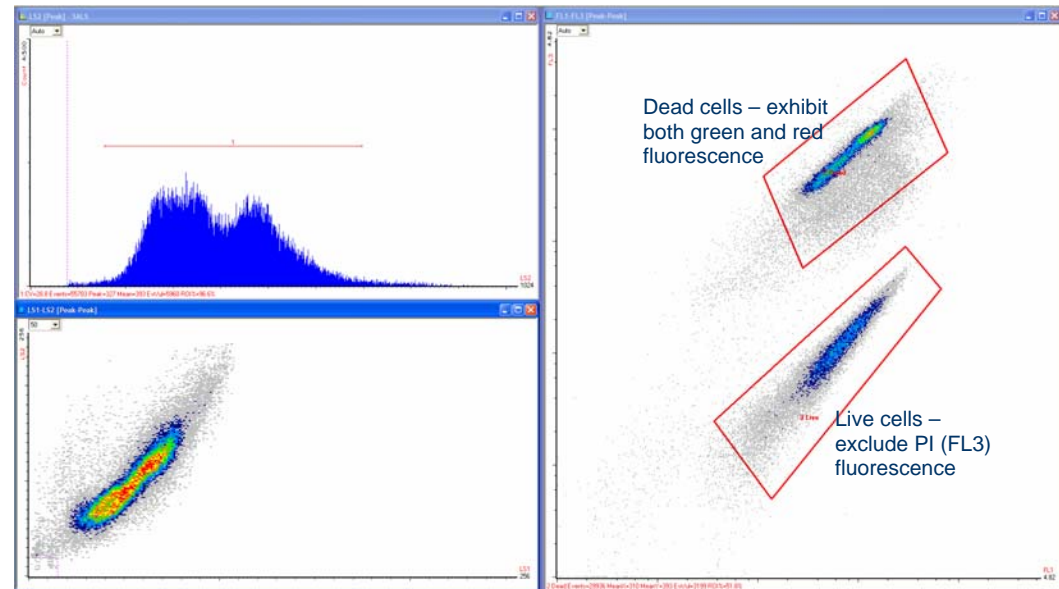


## Viability

Propidium iodide (red fluorescence – FL3 detector) and a cell permeable nucleic acid stain (e.g. SYTO 13 – green fluorescence – FL1 detector) can be used in combination to distinguish live and dead cell populations.

Here is a mixture of live and dead (heat treated) bakers yeast. Cells with intact membrane exclude the propidium iodide and therefore exhibit very little red fluorescence, but do exhibit green fluorescence from the SYTO stain.

Dead cells with damaged cell membrane exhibit both green and red (PI) fluorescence. The same method can be applied to bacteria.



### Live/Dead baker's yeast analysis

Fluorescence (right hand graph) is plotted on a logarithmic scale. So there is more than one logarithmic decade (x10) between the propidium iodide fluorescence from live and dead cells.

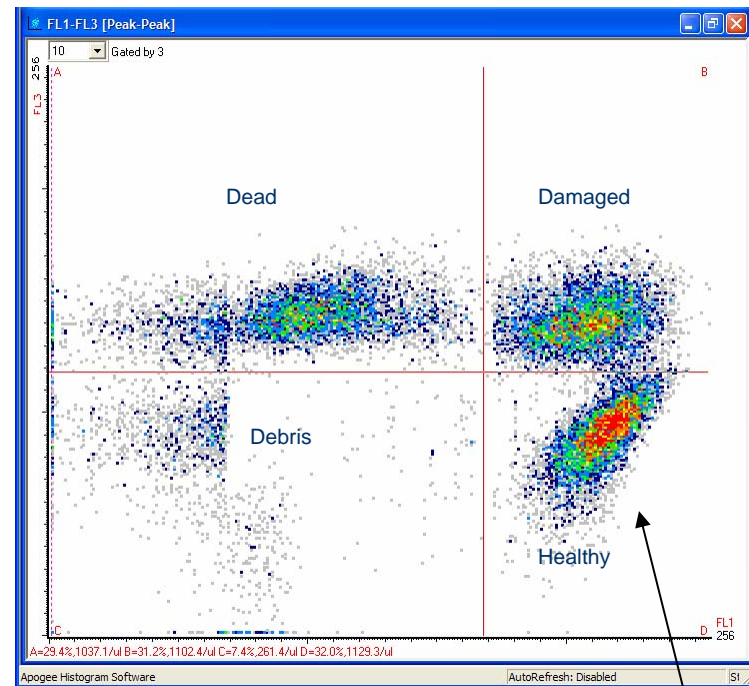


## Metabolic Activity

CFDA (carboxyfluorescein diacetate) can be used to give a viability evaluation by monitoring metabolic activity. Upon hydrolysis by intracellular nonspecific esterases, CFDA forms carboxyfluoresceinesterase and this emits green fluorescence when excited by a blue laser. Green fluorescence is collected by the flow cytometer's FL1 detector.

The cytogram shows Lactococcus prepared using

- propidium iodide to label cells with a damaged membrane, and
- CFDA to label metabolizing cells



Note: Data plotted on logarithmic scale.

Healthy cells exclude PI (FL3) and are metabolizing so emit green fluorescence (FL1)