INSTRUMENTS

Flow cytometry in aquatic science

Julie Hall
Andrea Cumming

An increasing number of aquatic investigations are benefiting from fast and accurate cell counts on NIWA's flow cytometer.

How many algal cells will mussels eat? What proportion of plankton in a sample will die if exposed to a toxic substance? To answer these kinds of questions, researchers have traditionally had to view tiny subsamples under a microscope and count individual cells. This is still the only practical option for some kinds of research. However, a high-tech method in use at NIWA – flow cytometry – is making the task much easier and more accurate in many projects.

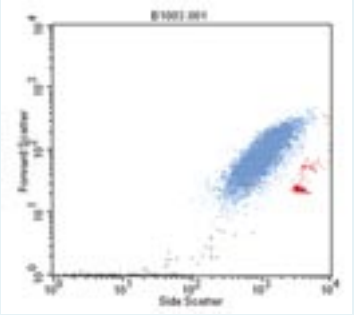
What is flow cytometry?

Flow cytometry is the measurement of characteristics of single cells suspended in solution. A beam of laser light is focused on the moving cells and light is scattered by and emitted from the cells. The information is picked up on detectors and converted into a form suitable for electronic storage and analysis. Various features of cells can be used to identify different populations or types of cells. NIWA's flow cytometer counts cells at a rate of 100 to 1800 per second and measures five different features.

The amount of laser light scattered forward gives us an estimate of the *size* of the cell.

The amount of laser light scattered sideways at 90 degrees gives us a measure of the *internal complexity* of the cell.

The other three features of cells measured by the flow cytometer depend on the fluorescence emitted when the laser light excites fluorescent material in the cells and causes emission of light of a certain wavelength. The fluorescence of a cell may come from natural pigments or from added stains. The instrument can detect fluorescence at three different wavelengths – 530, 585 and 670 nm – on its three fluorescence channels: FL1, FL2 and FL3. The graph below is an example of the identification of two distinct populations of cells using sideways and forward scatter.



Each dot is the data from one cell. The largest cells have the greatest forward-scatter signal (blue dots) and the more complex cells have the greatest side-scatter signal (red dots).

In the example shown on the opposite page (top left), cells in the blue and pink populations are distinguished on the basis of their different fluorescence signals.

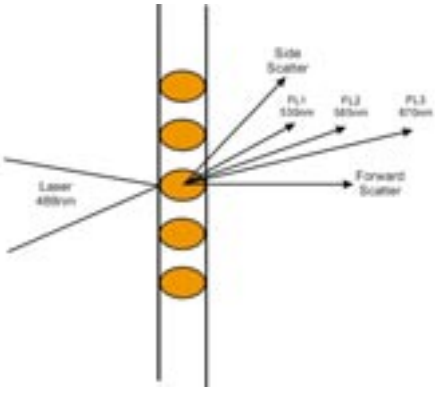
Identifying, counting and sorting

We can make similar measurements on a wide range of cell types which have naturally fluorescing pigments, like chlorophyll *a*, or we can add fluorescent stains to identify cellular features and use them to identify different populations of cells. In the example above right, the presence of algae in the sample was first confirmed by the presence of chlorophyll *a* fluorescence. Then different algal groups were identified on the basis of other cell characteristics measured with the flow cytometer.

A common device used with the cytometer is also illustrated in the figure. To enable an

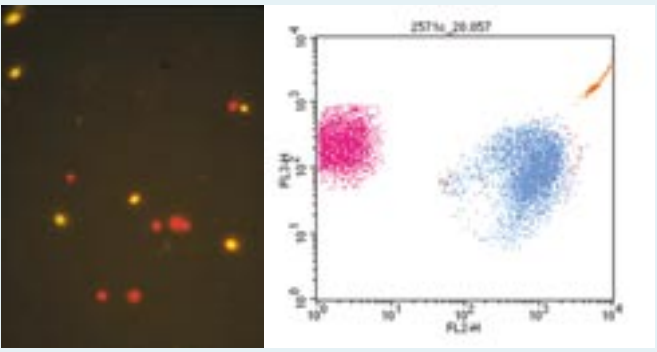
Teachers: this article can be used for Biology NCEA AS 2.2, 3.4, 3.6. See other curriculum connections at www.niwa.co.nz/pubs/wa/resources

Julie Hall and Andrea Cumming are based at NIWA in Hamilton.



far left:
NIWA's flow cytometer – the FACSCalibur – can be used in the laboratory or at sea on NIWA's research vessel *Tangaroa*.

left:
The outline of the flow cytometer with the laser which is focused on the stream of cells and the detectors which are used to measure the forward and side scatter of the laser light and the fluorescence of the cells.



left: Photo of algal cells exposed to light at 488 nm. The red fluorescence is from chlorophyll *a* and the yellow fluorescence is from the natural pigment phycoerythrin. The two cell types can be counted separately by the cytometer, on fluorescence channels 2 and 3.
right: Each dot on the graph is the data from one cell. The horizontal axis shows the fluorescence from phycoerythrin (population of blue dots, detected in the FL2 channel) and the vertical axis gives the fluorescence from chlorophyll *a* (population of pink dots, detected in the FL3 channel). The third small population of red dots is fluorescent beads added to enable cell counts to be made (see text).

accurate cell count we add a known number of fluorescent beads (the red population) to the sample. This allows us to calculate the volume of sample analysed and hence calculate the number of cells per ml of sample. The difference in the spread of the pink and blue populations compared to bead population is due to natural variation in the cells compared to the beads which are much more uniform in their characteristics.

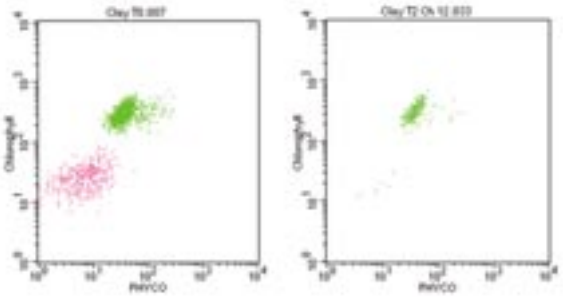
The advantages of flow cytometry over traditional microscope counts of algae are that sample preparation is quicker, counting time is reduced from 10–20 minutes per sample to about 2 minutes per sample, and we are able to count many more cells (5000–10,000 by flow cytometry compared to 400 for microsope counts), so counts are much more accurate.

NIWA's flow cytometer can also sort cells from identified populations. This allows us to identify a population of cells and then collect only this type of cell. Isolated cells can be checked by microscopy to make sure they are identified correctly by the flow cytometer.

Apart from using flow cytometry to count bacteria and algal cells (in both marine and freshwater samples), the instrument can be used to investigate the metabolic activity (or health) of cells to measure the impact of toxic substances. We have also used the flow cytometer to quantify the amount of DNA in cells. Examples of some recent applications of the cytometer are shown in the panels. ■

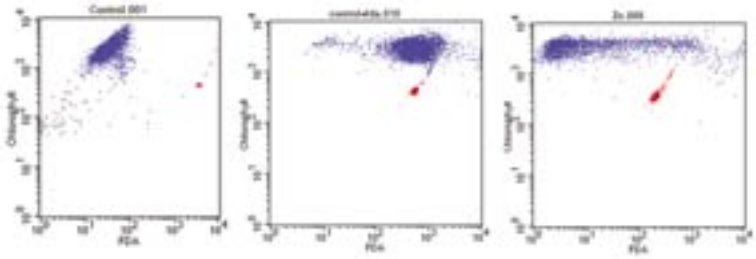
Shellfish grazing on algae

To assess the grazing rates and food preferences of shellfish we grow algae in culture and feed them to shellfish. In these experiments we started with two different species of algae and completed an initial count of the two species (left). We then allowed the shellfish to feed on the algae and then counted the number of algae left remaining in the sample (right). Each dot is the data from one cell.
This method allows us to look at both the quantity of the algae consumed by the shellfish and also the preferences of the shellfish for different foods.



Toxicity testing with algae

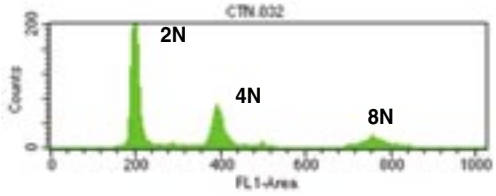
We grew cultures of algae and then used the stain fluorescein diacetate (FDA) to assess the metabolic state of the cells after exposure to a toxic substance. To identify the algal cells we use the fluorescence from chlorophyll *a* (left). We then add FDA to the cells. If the cells are metabolically active then the FDA is taken into the cell and is cleaved to form fluorescein dye that will fluoresce and be detected on the FL1 channel of the flow cytometer (middle). We then add the FDA to cells that have been exposed to the toxicant. If the cells have been affected by the toxicant and are not metabolically active, then they take up the FDA but it is not cleaved to form the fluorescein stain. So in this case the cells show chlorophyll *a* fluorescence but little fluorescein fluorescence (right). The variation in the amount of fluorescein fluorescence in the toxicant-treated cells is related to variation in the response of the individual cells to the toxicant.



Each dot is the data from one cell. The red dots are beads added at a known concentration to enable calculation of the volume of sample counted.

Assessing the DNA content of cells

During the life cycle of some species of algae the DNA content of the cells changes from haploid (N) to diploid (2N), and in some cases species can become polyploid. Flow cytometry can be used to assess the quantity of DNA in cells and hence the life-cycle stage of the cells. The algal cells are grown in culture, preserved and then exposed to the stain Pico Green which stains DNA. We can then measure the stain on the flow cytometer and identify cells with differing DNA content. Populations of cells with different DNA contents can be sorted and the different life stages of the species identified.



Acknowledgements
Thanks to Hoe Chang, Lisa Golding and Karl Safi for making their data available for this article.