

Use of FlowCam to enumerate and differentiate between marine protists

PREAMBLE. The method outlined below is based on Day et al. (2012).

The FlowCAM is a flow-cytometer that employs continuous digital imaging to measure the number, size and shape of microscopic particles in a fluid medium. It can be employed to identify and enumerate target protists in a mixed culture/ environmental sample.

1. Switch on system to start the computer. Open up software on desktop 'Visual Spreadsheet' and tick the relevant objective size. Click 'OK'

Choice of objective will be dictated by size of particles to be visualised and/or enumerated.

2. Assemble objectives and flowcells. For 4x objective – 300 Flowcell
10x objective – 100 Flowcell + collimator
20x objective – 50 Flowcell + collimator.

While handling the flowcells take extra care as they are very delicate and snap very easily.

2. Fix pump tubing to the pump and clip on the cover. Enter the sample volume and duration of each sample run.
3. Attach the Flowcell line to the bottom tube going into the pump system and attach waste line to the top tube exiting the pump system, placing the end of tubing into the waste bottle. Attach the sample funnel in to the top of the Flowcell tubing and sit in the holder provided.
4. Enter 'Setup' tab on the controller and open 'Context'. Enter settings for your samples. Enter OK.

Settings will vary from sample to sample, depending on Flowcell used, speed of pump desired, density of sample etc.

5. Add sample (~ 2ml) to the loading funnel and start the pump. Once the fluid is in the Flowcell, stop the pump. On the controller go back to 'Setup' and then click on 'Setup and Focus' – as soon as you click, switch the pump back on.
6. Adjust the Flowcell stage until the edge is visible on the Controller screen. Once located, secure the stage by screwing the pin down. Using the dials on the Flowcell stage, move the window into the middle of the Flowcell. Now use the soft focus located on the right hand side of the FlowCam body. Once your cells/materials of interest are in focus, stop

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Protists: Mixed algal – protozoan culture, or mixed environmental or lab sample with uni-cells/ small colonies/ short filaments of different diameters/ cell size

Apparatus: Bench Top VS IV FlowCAM (Fluid Imaging Technologies, Inc., Maine); 4x objective – 300 Flowcell, 10x objective – 100 Flowcell + collimator, 20x objective – 50 Flowcell + collimator; appropriate incubators/ culture systems; a class I biological safety cabinet.

Cell culture medium: Modified Provasoli, f/2, or other appropriate medium.

Solutions & standards: deionised water for system washing; plastic calibration beads - 10, 20, 50 and 100 µm diameter (Fluid Imaging Technologies, Inc.

Plasticware: membrane filters (0.5µm pore size); Universal tubes (20ml); disposable pipettes.

Chemicals used routinely are of Analar grade purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Day JG, Thomas NJ, Achilles-Day UEM & Leakey RJ (2012) Early detection of protozoan grazers in algal biofuel cultures. *Bioresource Technol.* **114**, 715–719.

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the pump and close down the run window. If working satisfactorily, close the run window and switch the pump off.

To make sure all your settings are correct, go to 'Setup' and click on 'AutoImage Mode (No Save)' and switch the pump on immediately.

7. Start the run by going to the 'Analyse' tab and clicking on 'AutoImage Mode'. Type in any notes and click 'OK'. Save the run under the 'FlowCam Masterfiles'.

As soon as you press 'Save' switch on the pump.

To calibrate for size measurements of the FlowCAM use 10, 20, 50 and 100 μm diameter plastic beads (Fluid Imaging Technologies standard calibration beads) and the interactive calibration procedure in the FlowCAM software.

8. After the run flush through the system to clean all the tubing, before adding your next sample. Then add next sample and repeat the above procedure.
9. Once all analyses have been completed, flush the Flowcell out with deionised water, ensuring tubing is cleaned properly. Empty all tubing of sample/water before taking apart the components, storing them appropriately and disposing of the waste correctly using standard microbiological procedures.
10. Data can be downloaded into Excel, or other data processing packages as is required.