Flow cytometry and fluorescence for assessing the cell health of algae and cyanobacteria

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Introduction

• What is Flow cytometry?
• What can we measure?
• What results can we obtain?
What is Flow cytometry?

the measurement of particles or cells as they flow in a fluid stream through a beam of light

Uses the principles of light scattering, light excitation and the emission of fluorescent stains/molecules
Fluorescent stains

- Fluorescein Diacetate – metabolic activity (esterases) FL1
- SYTOX Green – cell membrane integrity FL1 (530 nm)

- Natural pigment autofluorescence
  Chlorophyll a (green algae/cyanobacteria) FL3 (>650 nm)
  Phycocyanin (cyanobacteria) FL4 (>670 nm)
  Allophycocyanin (cyanobacteria) FL4

- The two stains selected fluoresce at a different wavelength to natural autofluorescence, hence data is collected with different detectors.
What can we measure?

- a particle’s relative size (diameter range 0.5 - 40µm)
- relative granularity or internal complexity and
- relative fluorescence intensity
Change in fluorescence of FDA

Cells selected on the basis of size (FSC) and granularity (SSC)

Results recorded as fluorescence intensity as geomeans (FL1)
Change in fluorescence with FDA

Use of controls allow cells to be divided into groups:

FDA – live, unhealthy, dead

SG – live, dead
What results have we obtained?

*Microcystis aeruginosa* dosed with *CuSO₄*
Percentage of live cells

- FDA
- FDA apoptotic
- SG

Healthy         Unhealthy         Healthy

[Cu] mg/L

0.00 0.06 0.13 0.19 0.25 0.38 0.50 0.63 0.75 0.88 1.00

% Live

0 20 40 60 80 100
Autofluorescence values are similar to stains

**Chlorophyll a FL3**

- FDA
- SG
- auto

**Phycocyanin and Allophycocyanin FL4**

- FDA
- SG
- auto
Cell counts live/dead based on SG staining
Summary

• Large numbers of cells can be examined by flow cytometry
• Fluorescent stains are useful for assessing the health of algae and cyanobacteria
• FDA shows subtle effects with decreasing esterase activity within cells before cell membranes lyse
• Autofluorescence could be used as a quick indication of cell health but this does requires further validation
• Cell counts alone may or may not give an idea of cell health
Peter Hobson, Lyudmila Tsymbal and Jenny House

Thank you