

Monitoring the Cell Cycle of the Unicellular Green Algae *Raphidocelis subcapitata*

Analysis of cell growth and proliferation using CASY



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• Introduction

The freshwater algae *Raphidocelis subcapitata* are sickle-shaped single cells of $8-14 \times 2-3 \mu\text{m}$ (length x width) and reproduce asexually via autospores (1). The cell cycle consists of three phases: growth of the mother cell, cell division (which includes two divisions of the nucleus), and the release of four autospores (2). Over a period of 24h the cell number and size distribution of a synchronized culture of *R. subcapitata* was tracked, revealing phases of cell growth and phases of proliferation.

• Methods

Cells

Inoculum cultures of *R. subcapitata* strain SAG 61.81 from the Culture Collection of Algae at Göttingen University, Germany were grown in sterilized OECD growth medium ($\text{pH } 8.1$) at a temperature of $22 \pm 1.5^\circ\text{C}$ and diluted periodically.

Synchronous growth of algae cultures was achieved by a 16:8 light dark cycle with a light intensity of $7600 \pm 503 \text{ lux}$. Cultures were maintained in sterile cell culture flasks on a horizontal shaker at 55 rpm.

Sample Collection

As light directs cell division, during a period of 2.5h before and 2h after onset of light, samples were taken in intervals of 15 min. Two additional samples were taken after 9.5 and 24h.

CASY Analysis

All samples were analyzed with CASY (60 μm Capillary; 3x200 μl ; 0-20 μm ; sample volume 50 μl -2ml) for cell number, cell size and total cell volume.

Graphs of the CASY measurements were created with CASYworX 1.2 software (OLS).

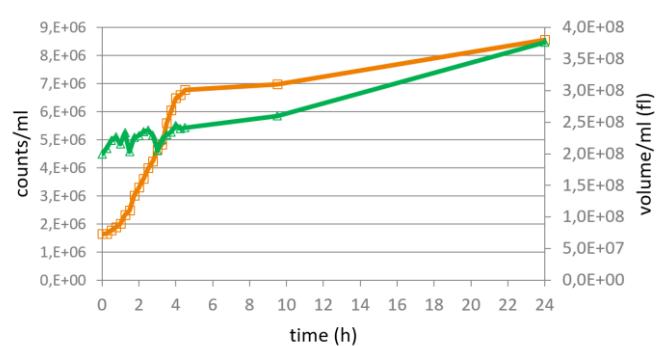
Fig. 1: Biovolume remains stable during proliferation.
About 2.5h (t=0) before onset of light, each mother cell begins to releases four autospores. About 2h after onset of light (t=4.5), proliferation has ended and the cell number has quadrupled. While the cell number has changed significantly, biovolume has remained relatively stable.
Orange line: counts/ml; green line: volume/ml

• Results

Biovolume remains stable during cell division

During cell division, the initial cell concentration of $1.7 \times 10^6 \text{ cells/ml}$ quadrupled itself to $6.8 \times 10^6 \text{ cells/ml}$ after 4.5 h, in accordance with the release of four autospores from the mother cell.

Concurrently, the biovolume (volume/ml) remained relatively stable during cell division at around $2.3 \times 10^8 \text{ fl/ml}$. However, biovolume increased again during the subsequent growth phase, to $2.6 \times 10^8 \text{ fl/ml}$ at t9.5 and $3.8 \times 10^8 \text{ fl/ml}$ after 24 h (Fig.1).



Monitoring mother cells and autospores using CASY

The cell size distributions documented the process of cell division and were characteristic for each phase (Fig. 2). CASY measurements resulted in direct visualization of the cell/autospore relation in the sample due to accurate cell size and cell count analysis.

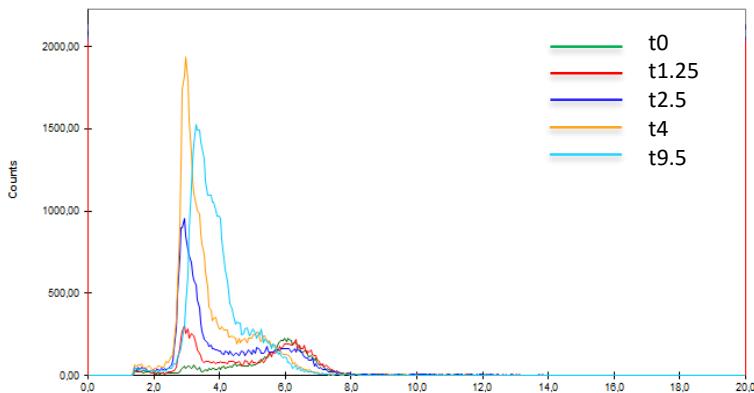
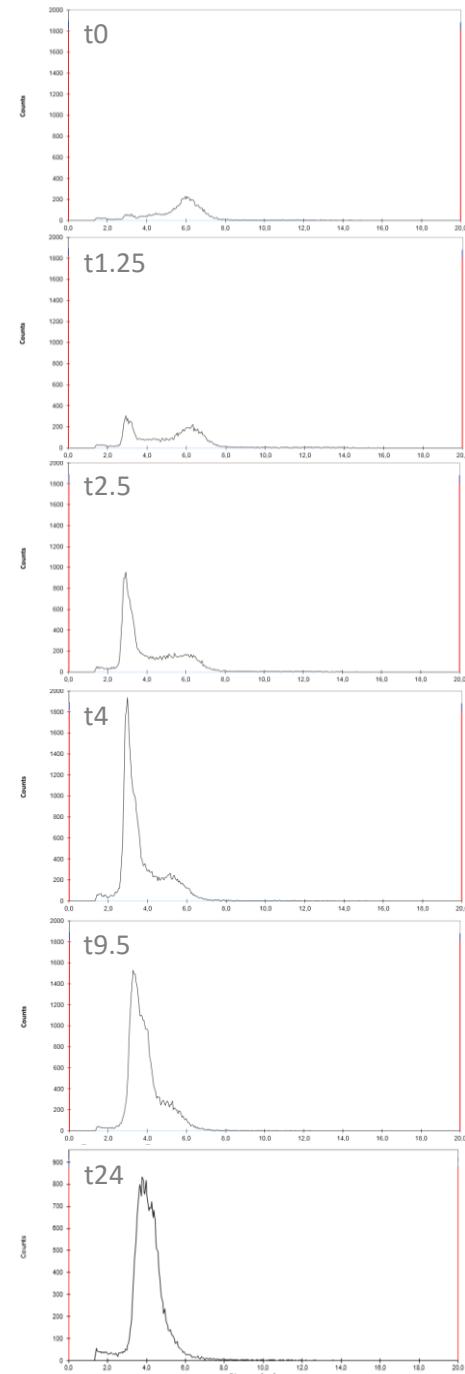


Fig. 2: Cell size distributions of algae cultures during cell division, as visualized with CASY.

At t0, the mother cells had reached their maximum size of 6 μm in diameter. During the subsequent release of four autospores, the daughter cells can be detected by the formation of a peak at approx. 3 μm . After about 4 hours (t4), the formation of the daughter cells has progressed significantly and mother cells have disappeared.

The „transitional“ peak between 4 and 5 μm represents mother cells that have released one or two autospores, but had not finished releasing all four of them. After 9½ h (t9.5), the cells had entered the next growth phase. The peak is slowly shifting to the right, as cell size and volume increases again. At t24 the culture had continued its growth which will progress until the size for the next division is reached.



• Summary

Measuring algae cultures growth pattern

Here we demonstrated CASY's capabilities to resolve a green algal cell cycle: A quick, label-free measurement allows for a comprehensive analysis of growth behavior of algae including cell number, biovolume, cell size and volume distributions.

Thus, alterations like inhibiting effects of chemical pollutants are easily assayed using CASY in ecotoxicity testing. A further application note describes analysis of growth-inhibiting effects of antibiotics on green algae.

• References

- Organization for Economic Co-operation and Development, (2011). Freshwater alga and cyanobacteria growth inhibition test. Guideline 201, Paris, France
- Machado, M. D., Soares, V. D., (2014). Modification of cell volume and proliferative capacity of *Pseudokirchneriella subcapitata* cells exposed to metal stress, Aquatic Toxicology 147, 1-6

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