Visualizing the Invisible: Using Microscopy to Observe Microbial Communities Over Time

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Introduction

Microbes support the larger ecological community by determining the fate of carbon and creating key compounds necessary for life (Fig. 1). By studying marine microbial communities, we can understand how these tiny organisms influence the life-sustaining cycles of our planet and build better predictions of how these systems will change with our climate.

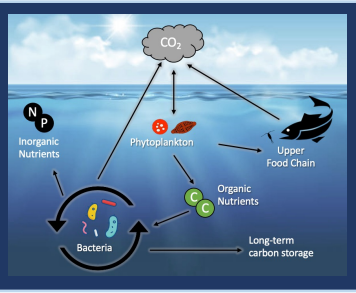
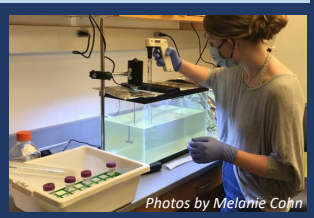


Figure 1. *Bacteria and phytoplankton cycle organic (carbon) and inorganic (nitrogen, phosphorous) nutrients and transform them for use by higher trophic levels or export them to long-term storage.*

However, microbes cannot be seen by the naked eye, so microbiologists use a variety of methods to better understand it. Molecular techniques show who is present in the community and what they are doing, but they are often quantitatively difficult and can be costly. On the other hand, imaging techniques such as FlowCam and IFCB are powerful and high-throughput, but are not universally accessible and cannot capture information about every microbial trophic level. As a result, there is a need for a single technique which is high-throughput yet accessible and holistic.

Methods



Photos by Melanie Cohn

Tank Setup

- Water collected at BT-11 Ferry Site in Neuse River Estuary, NC
- 60-Liter glass tanks fitted with acrylic mixer to create water motion
- Water divided evenly between 6 tanks – 3 different treatments in duplicate representing different organic matter sources (Fig. 2)

Figure 2. *Light treatments and organic matter sources for tanks*

Methods (con't)

Sample Preparation

- Fluorescent Approach (Fig. 3a): Used SYBR-stained and autofluorescent cells to estimate cell counts (GFP and Cy5 channels, 60x and 100x). Cells stained and vacuum filtered through a 0.22-µm pore filter and mounted on a glass slide.
- DIC Approach (Fig. 3b): Used DIC and autofluorescence to provide high-resolution images of cells (DIC and Cy5 channels, 20x and 40x). Samples suspended in 10% ethanol, concentrated, and 1 mL put in glass chamber slides.

Figure 3. *Imaging/Sample Preparation Approaches*

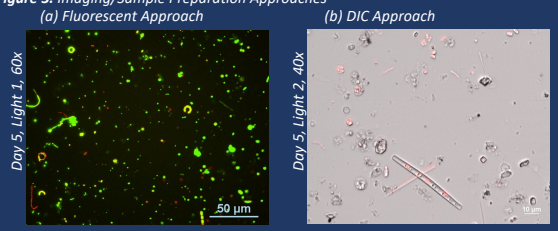


Image Acquisition and Analysis

General Analysis Workflows in NIS Elements were used to automatically process raw images, enabling their use for accurate cell counts and measurements (Fig. 4).

- Image processing steps increase cell definition and contrast so that trained binary thresholding AI can accurately outline cells.
- Minimum of 3 supervised training sessions with 1000 automated iterations per thresholding (Fig. 7). Each thresholded cell object counted and measured.
- Aggregates and debris are difficult to threshold accurately (7a), often resulting in severe overestimation (7b) or underestimation (7c) of cell counts

Figure 6. *General Analysis Workflow for image processing*

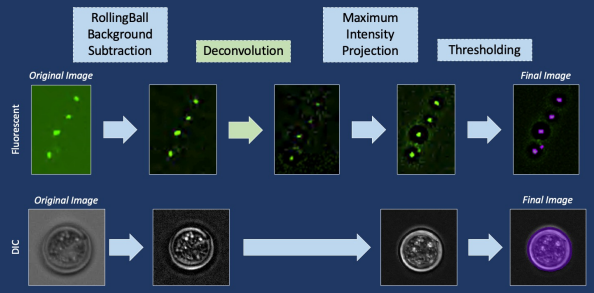
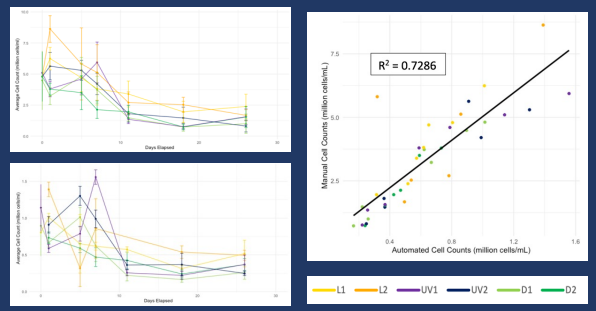


Figure 7. *Aggregates present problems for thresholding recognition and cell counts*

Results

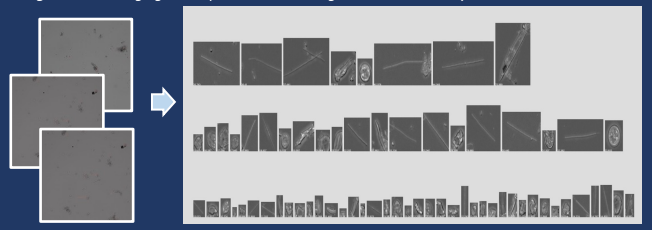
Automated methods generally reflected manual trends well (Fig. 8). Light tanks declined more slowly in cell counts and maintained a higher population for longer than dark and UV tanks. Differences in magnitude between counts are most likely due to thresholding inaccuracies which can be remedied with further thresholding trainings.

Figure 8. *Automated and manual cell counts show similar trends but different magnitudes*



Subimage grids were used to visualize changes in the phytoplankton (> 10 µm) population over time (Fig. 9). This grid is comparable to outputs of programs like FlowCam and IFCB, but uses open-source software and publicly available scripts.

Figure 9. *Subimage grids helped visualize changes in the community over time.*



Conclusions

- Automated high-throughput microscopy methods provided mostly accurate reflections of low-throughput manual methods.
- More manual thresholding trainings are needed to improve accuracy of counts.
- These methods are holistic, accessible, and provide an alternate and effective way to examine samples across a variety of treatments and timepoints to better understand how microbial communities change over time.

Acknowledgements

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