



Towards Bioregenerative Life Support for Extended Human Exploration: Experiment Development for Testing the Fitness of Algae in Space

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Introduction

Food, fuel, and fresh air will need to be regenerated for astronauts during missions longer than a year. Microbes such as single-celled algae and bacteria have the potential to provide all of these necessities in a closed-loop bioreactor, or bioregenerative system.

The goal of this research project is to identify methods for growing and measuring the growth of the microalga, *Chlorella sorokiniana*, on filter membranes. *C. sorokiniana* has proven spaceflight ability and is a nutrient dense "superfood." Algae's capability for photosynthesis also makes *C. sorokiniana* an important resource for gas exchange. Finally, growing this planktonic alga on membranes rather than in tanks significantly reduces the mass and water demands of a bioregenerative system, both of which are important constraints in extended space flight.

This experiment was designed such that it can be reproduced in the European Modular Cultivation System (EMCS), a piece of equipment designed to monitor plant growth aboard the International Space Station (ISS).

Why the European Modular Cultivation (EMCS) System?

The European Modular Cultivation System (EMCS) is the only piece of equipment that allows for the simultaneous testing of plant growth in microgravity and 1g controls on the ISS. Plant or algae growth is monitored by a camera placed within the EMCS. This project developed a method of controlling light conditions and analyzing photo data such that ground control growth and monitoring experiments could be replicated in classrooms, using simple materials such as foam core and free software.



European Space Agency Astronaut, Samantha Cristoforetti, conducting maintenance on the EMCS. Photo Credit: NASA/ESA

Results: RGB Photo Analysis Can Be Used to Determine Growth

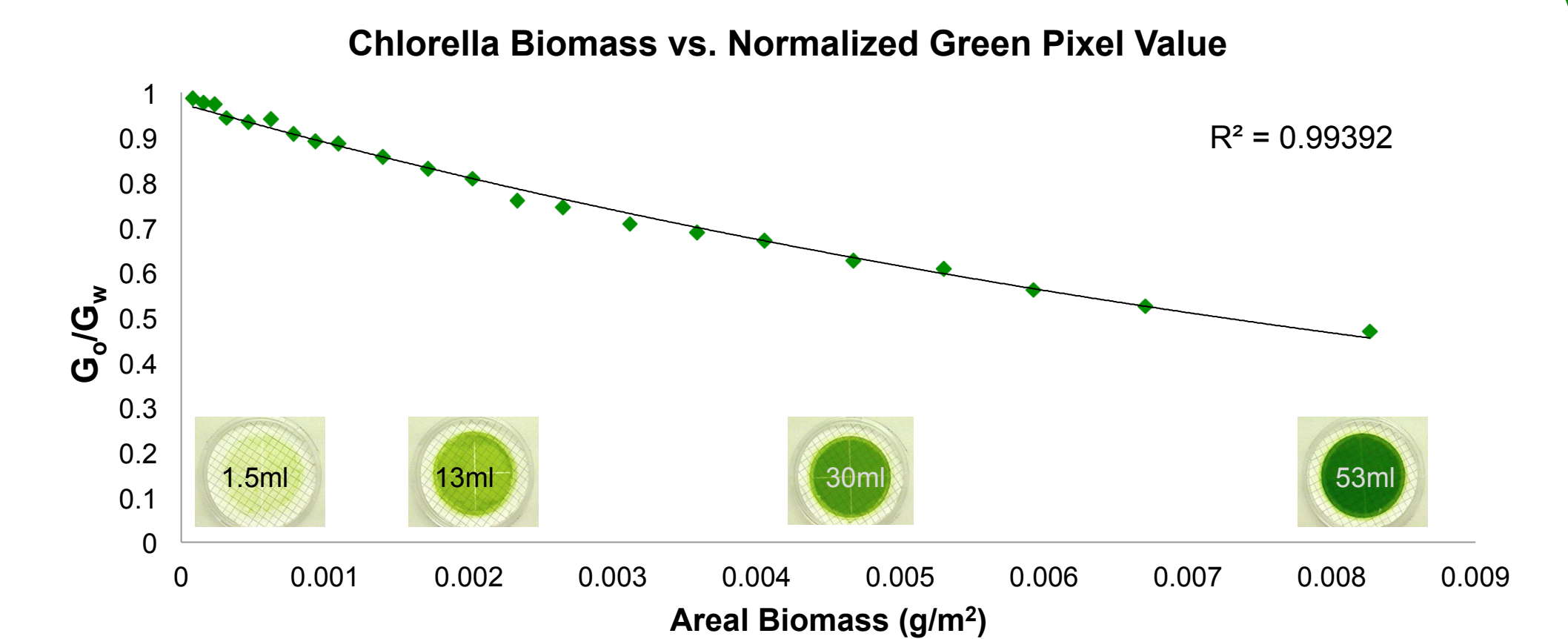


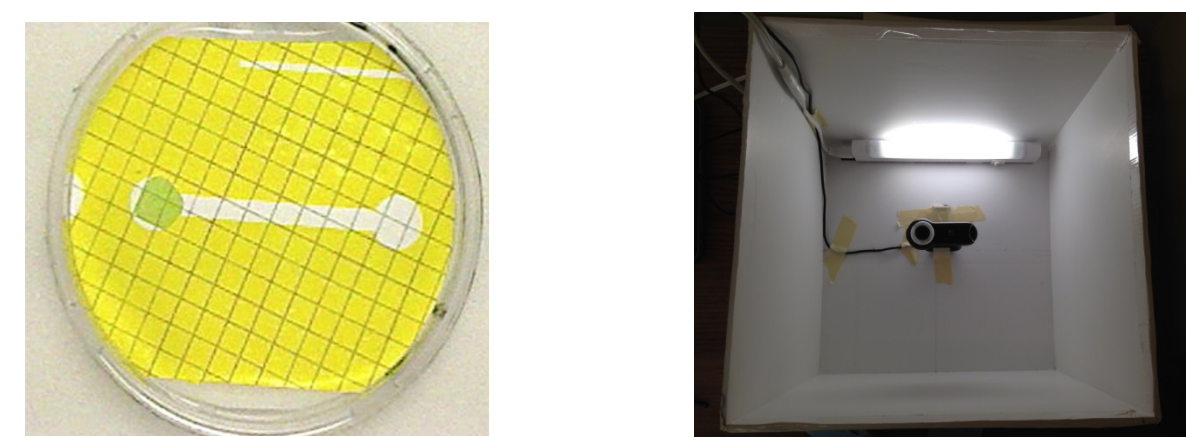
Figure 2. The y-axis, G_o/G_w , represents the intensity of green pixels within the algal region of the filter (G_o) divided by the intensity of green pixels within the white region of the filter (G_w). This controls for any green pixel intensity resulting just from the filter and not from algal growth.

Green pixel intensity and algal biomass have an inverse relationship. Just as the black background of this poster appears darker than this white box because the black ink pigments absorb more light and, hence, reflect less light to our eyes, the darker algae filters reflect less light to the camera.

Methods



Growing Algae in Systems Qualified for Spaceflight: Seed cassettes, Experiment Unique Equipment (EUE) approved for the EMCS Experimental Containers (EC) (left) contain membranes made of polyethersulfone (PES) resting on chromatography paper (middle). *Chlorella sorokiniana*, a single celled alga (right), is pipetted onto the membrane.



Measuring Growth: Ideally, growth of the algae would be monitored by the linear distance they grow along a "racetrack" printed on the filter material (left). The EMCS also contains a RGB color camera that could be used to determine the biomass of algae on the filters through measurements of the green color value of the pixels. We used a webcam in a photo box with a lamp for this study (right), but the principle is the same.

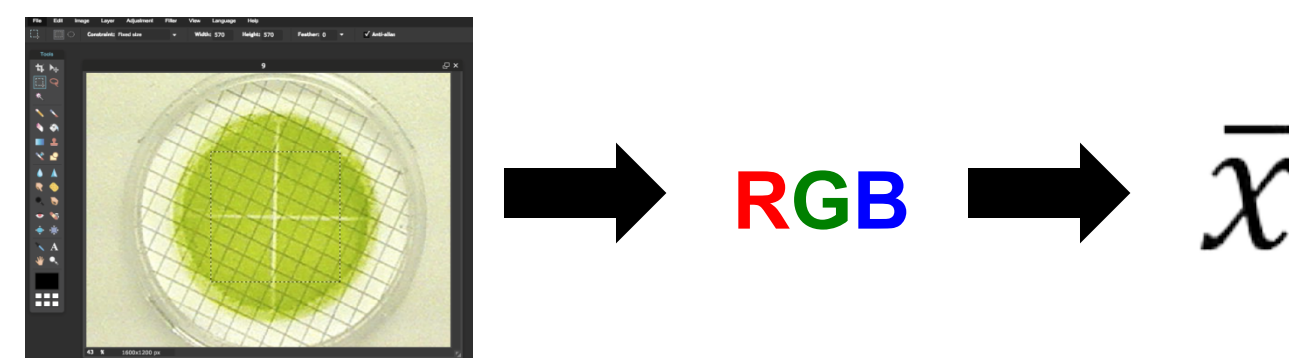
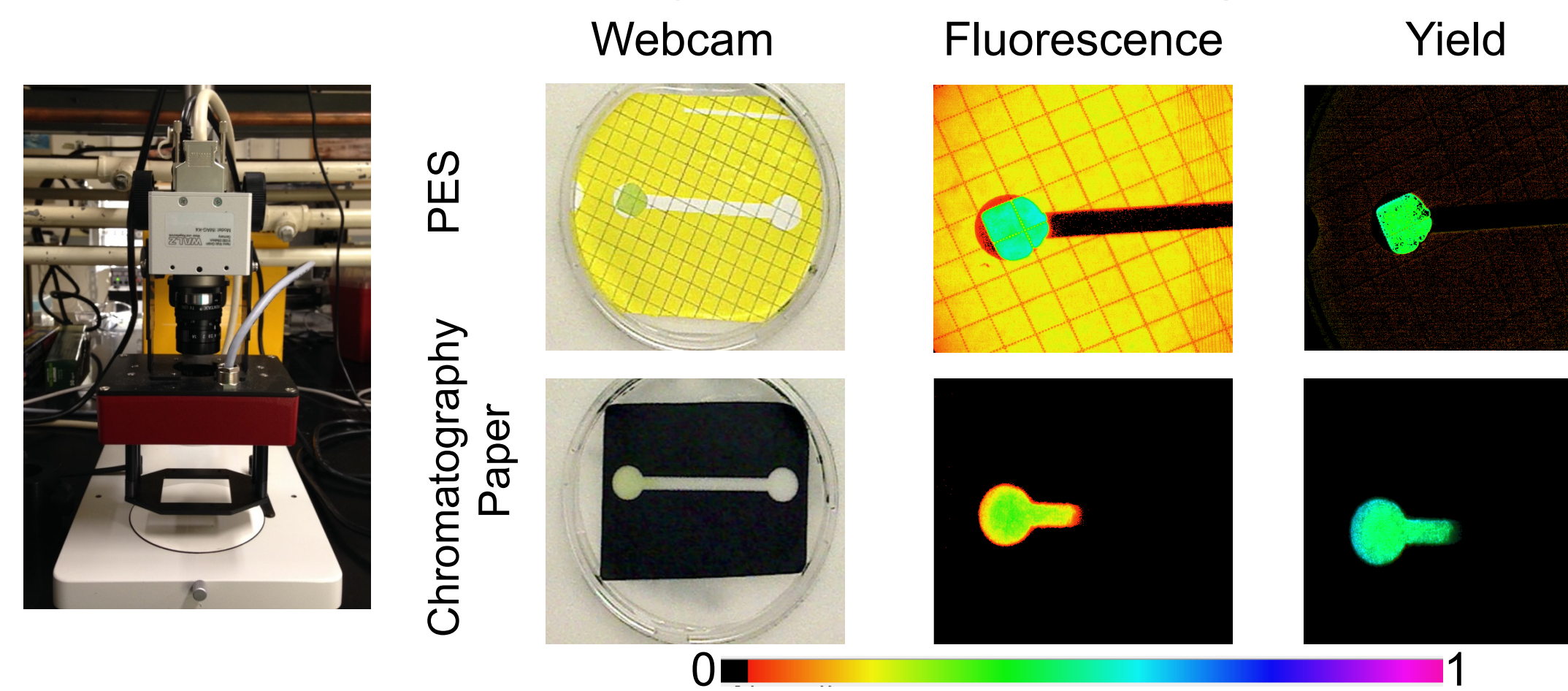


Image Analysis: Software freely available on the Internet (Get RGB, Beckman Institute) was used to analyze photo box images.

Ground-based Laboratory Verification of Spaceflight Measurements



An Imaging PAM fluorometer (left) was used to measure the photosynthetic efficiency (yield) of algae on the racetracks and to measure fluorescence, an indicator of biomass. These measurements can be used to optimize system performance.

Results: Algal Growth and Productivity

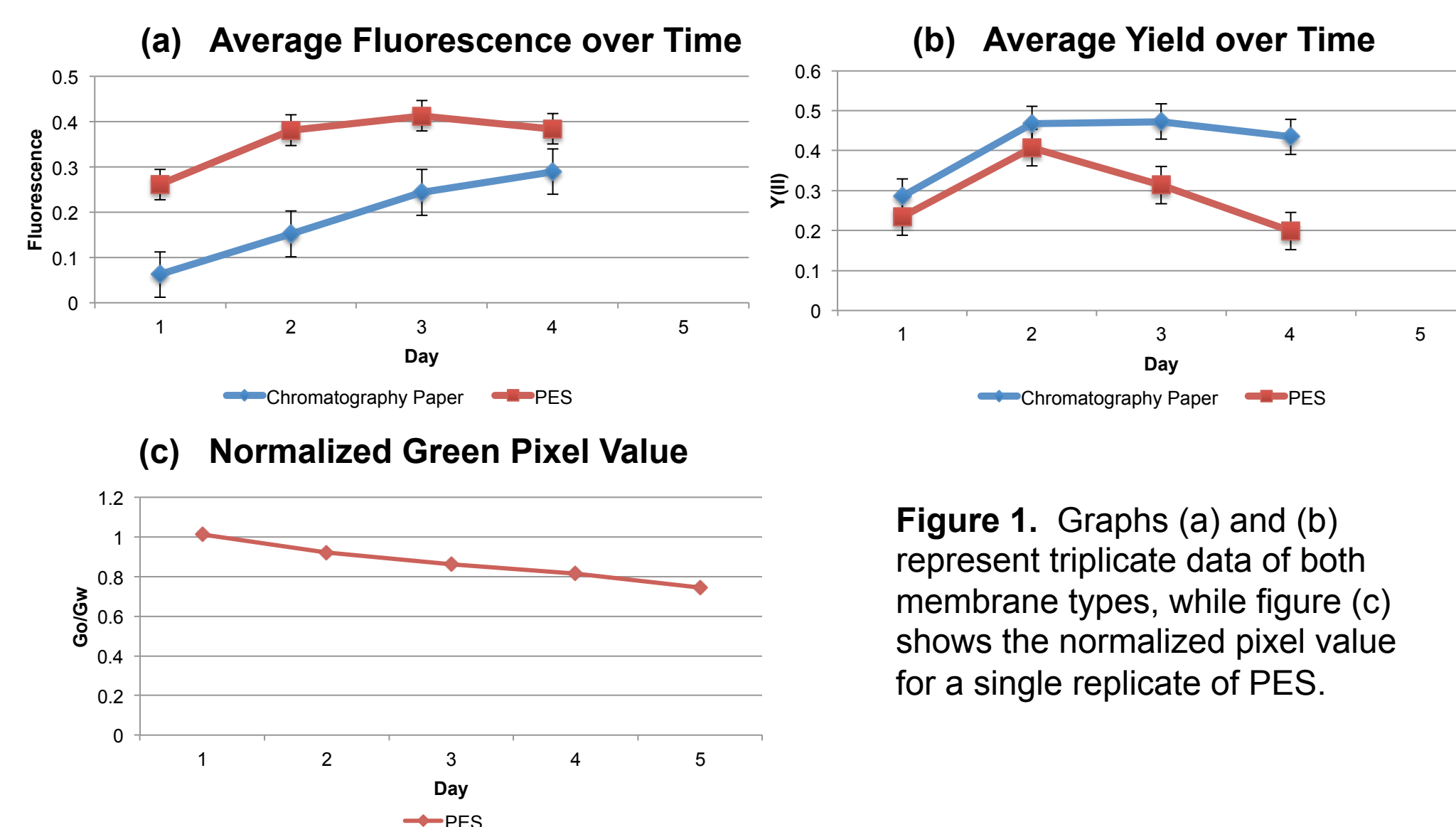


Figure 1. Graphs (a) and (b) represent triplicate data of both membrane types, while figure (c) shows the normalized pixel value for a single replicate of PES.

Discussion

This work shows that *C. sorokiniana* growth on membranes can be monitored via image analysis. In Figure 1, F increases in value over time (a) just as normalized green pixel value decreases over time (c). Increases in F have been shown to correlate with increases in biomass. Here, decreases in normalized pixel value have been shown to correlate with increases in biomass as well (Figure 2). Further replication of this work is necessary, however, as Figure 1c represents a small sample size. Normalized green pixel analysis shows promise as a good estimator of growth for *C. sorokiniana* on membranes and is a potential method for monitoring growth in microgravity conditions aboard the EMCS. Productive algal growth in microgravity may provide consistent self-generation of food, fuel, and fresh air for future astronauts on extended space missions.

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