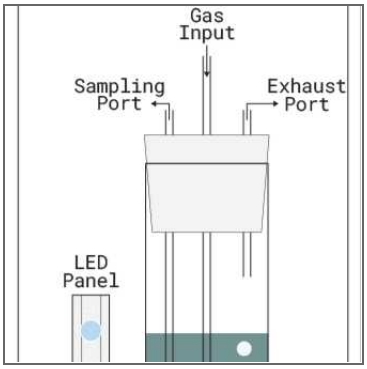

DEC 11, 2023

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We use this protocol and it's working

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Algaeorithm Classroom Guide: Constructing Mini Photobioreactors for Microalgae

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Algaeorithm

A Ashwin Mukherjee

ABSTRACT

A guide to constructing mini photobioreactors and cultivating algae in classroom settings.

MATERIALS

Product recommendations are included under each item.

Equipment

Microscope	NAME
Swift	BRAND
B082Y5G8RP	SKU
https://www.amazon.com/Trinocular-Microscope-Magnification-Siedentopf-Research-Grade/dp/B082Y5G8RP?th=1	LINK
must have 400x magnification capacity	SPECIFICATIONS

PROTOCOL integer ID:
83648

Keywords: algae cultivation, bioreactor, cell sampling, algae culture, algae, bioenergy, machine learning, computational biology

Equipment

Hemocytometer — NAME

Rs' Science — BRAND

B076ZT949V — SKU

https://www.amazon.com/Rs-Science-Improved-Hemocytometer-Methylene/dp/B076ZT949V — LINK

includes coverslips, sample vials, and transfer pipettes — SPECIFICATIONS

Equipment

Glass Tubes — NAME

DEPEPE — BRAND

B072ZQ4DS9 — SKU

https://www.amazon.com/DEPEPE-25%C3%97200mm-Glass-Tubes-Stoppers/dp/B072ZQ4DS9?ref_=ast_sto_dp&th=1&psc=1 — LINK

12 tubes — SPECIFICATIONS

Equipment

Air Pump — NAME

HITOP — BRAND

B07RJ3XLM8 — SKU

https://www.amazon.com/HITOP-Aquarium-Outlets-Aerator-Accessories/dp/B07RJ3XLM8 — LINK

2 output tubes — SPECIFICATIONS

Equipment

Concentrated Medium

NAME

Alga-Gro

BRAND

153758

SKU

https://www.carolina.com/dehydrated-media-and-media-ingredients/alga-gro-concentrated-medium-sterile-500-ml/153758.pr?question=algae+media

LINK

makes 25 liters

SPECIFICATIONS

Equipment

Freshwater Medium

NAME

Alga-Grow

BRAND

153752

SKU

https://www.carolina.com/dehydrated-media-and-media-ingredients/alga-gro-freshwater-medium-1-qt/153752.pr?question=algae+media

LINK

makes 1 liter

SPECIFICATIONS

Equipment

Chlamydomonas, Living

NAME

Carolina

BRAND

131738

SKU

https://www.carolina.com/protozoa/chlamydomonas-living/131738.pr

LINK

1 culture is enough for up to 30 students

SPECIFICATIONS

Equipment

Glass Tube Rack — NAME

Karter Scientific — BRAND

B08DWX588Q — SKU

https://www.amazon.com/dp/B08DWX588Q?psc=1&ref=ppx_yo2ov_dt_b_product_details — LINK

holds 6 glass tubes — SPECIFICATIONS

Equipment

Gang Valve — NAME

AQUANEAT — BRAND

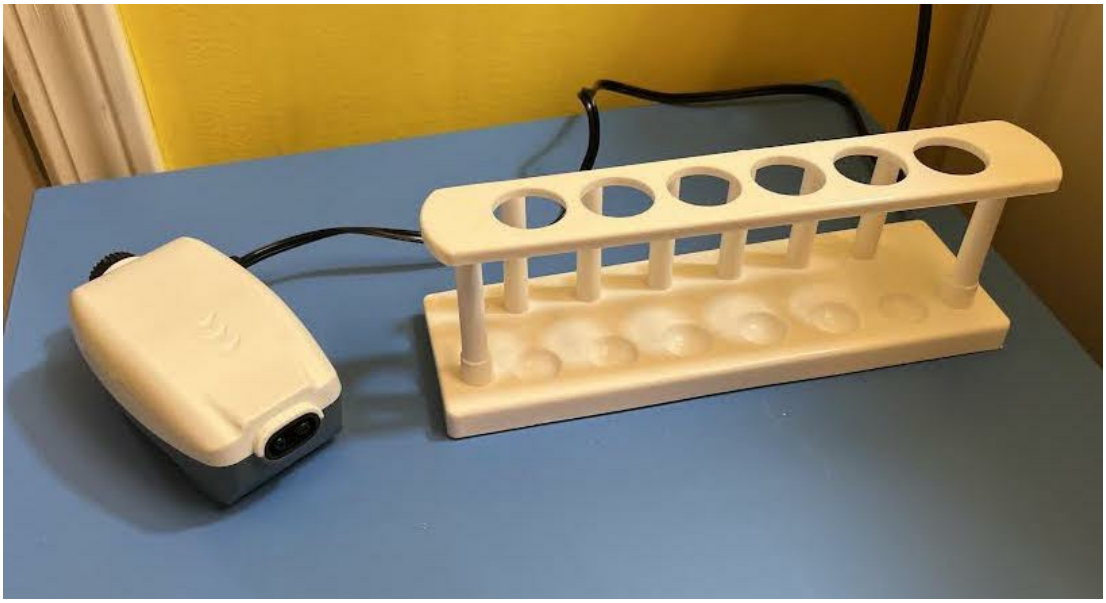
B08NPKW82P — SKU

https://www.amazon.com/dp/B08NPKW82P?ref=ppx_yo2ov_dt_b_product_details&th=1d — LINK

5 output tubes — SPECIFICATIONS

Prepare Photobioreactors

1 Place the glass tube rack and **unplugged** air pump on a table or flat surface where they will not be disturbed.

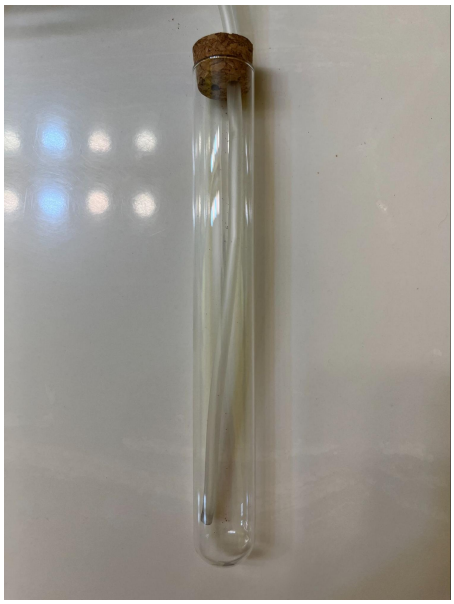


air pump and test tube rack

2 Repeat the following procedure for each reactor you plan to prepare:

2.1 Remove the cork from one glass tube and set the tube aside.

2.2 Thread a section of aquarium tubing through the larger opening in the cork. **Do not cut the tubing until you are sure it will be long enough to connect the pump to the reactor.** The amount of tubing exiting the cork **on the bottom** should be less than an inch from the bottom of the tube when the cork is placed back onto the tube.



tubing length for bioreactor

2.3 Remove the cork with tubing from the test tube and set it aside.

2.4 Using a dropping pipette (like the one provided in the hemocytometer kit), pipette approximately 35 mL of the growth media (concentrated or freshwater) and approximately 6 mL of the *Chlamydomonas* culture into the glass tube.

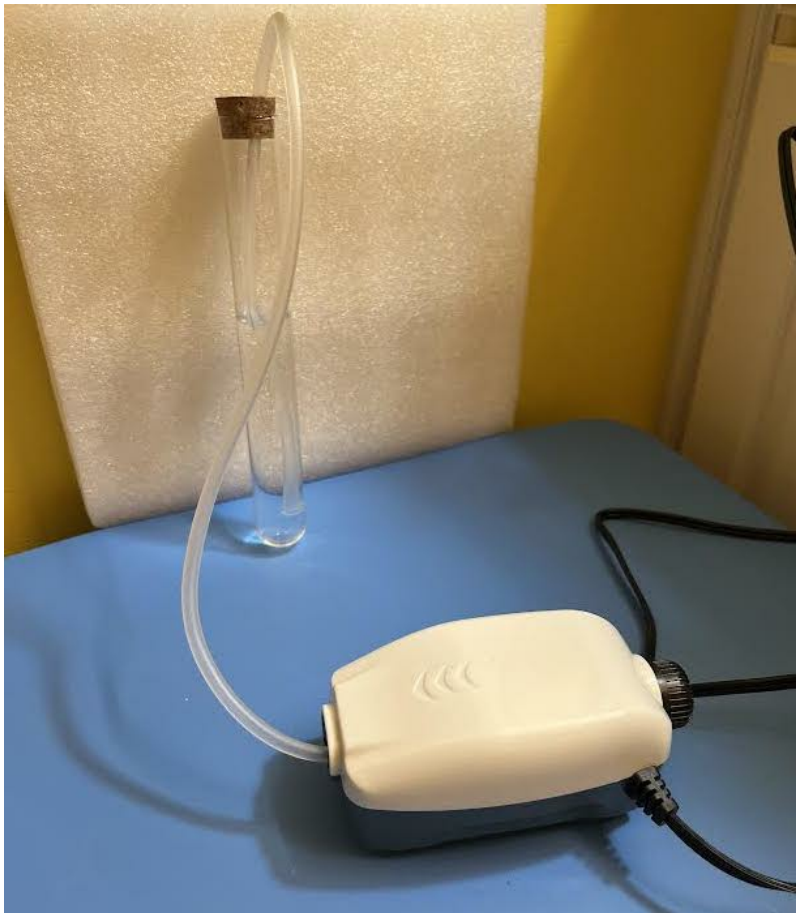
2.5 Place the cork with tubing back onto the glass tube so that the tubing is within an inch of the glass tube's bottom and place the entire apparatus onto the glass tube rack.

2.6 Connect the tubing to the pump using one of the following methods

1. If using gang valves, connect the open end of the tubing to one of the gang valves and use another section of tubing to connect the gang valves to the air pump.

bioreactor setup with gang valves

2. If not using gang valves, directly connect the open end of the tubing to the aquarium pump output.

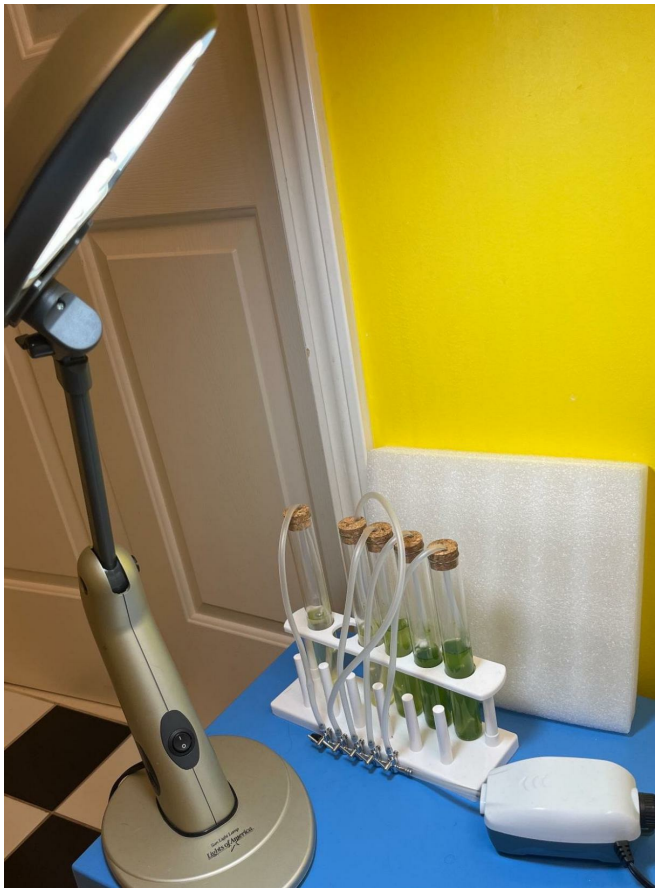


pump to bioreactor connection

2.7 Plug in the aquarium pump and adjust the aquarium pump output until the reactor is bubbling gently.

Note

If you are experimenting with varying light levels or would like to provide light 24/7, you can use a desk lamp or a similar light source. Similarly, you can adjust the pump output to investigate the effects of changing the air supply.



bioreactors with desk lamp

Sample Photobioreactors + Prepare Hemocytometer

3 Combine 3 parts isopropyl alcohol and 7 parts water in a spray bottle

4 Clean the glass section of the hemocytometer and 1 hemocytometer coverslip using the sheets provided

with the hemocytometer.

5 Place the coverslip onto the counting section of the hemocytometer.

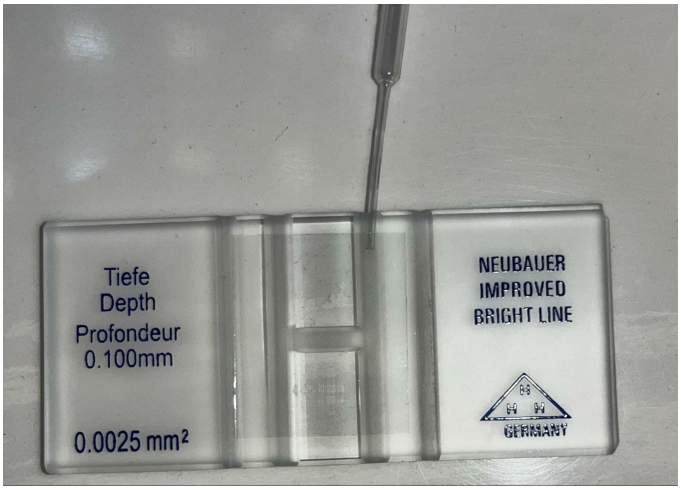
6 Unplug the aquarium pump.

7 Carefully remove the top from one photobioreactor and use a pipette to pick up approximately 10 µL or 1 drop.

8 Pipette approximately 10 µL into one side of the hemocytometer. The drop should disperse evenly under the coverslip.

Note

You can observe two samples on the same hemocytometer by using different samples on either side of the hemocytometer. To ensure that you do not “flood” the hemocytometer, ensure that there is only enough liquid to cover the observation area of the hemocytometer and that none leaks into the wells on either side.



dropping a sample into the hemocytometer

9 Set the hemocytometer aside and replace the top of the photobioreactor.

Analyze Samples

10 Place the 10x eyepieces into the binocular ports of the microscope.

microscope 10x eyepieces

11 Rotate the objective lenses until the 10x objective lens is directed at the observation area of the microscope.

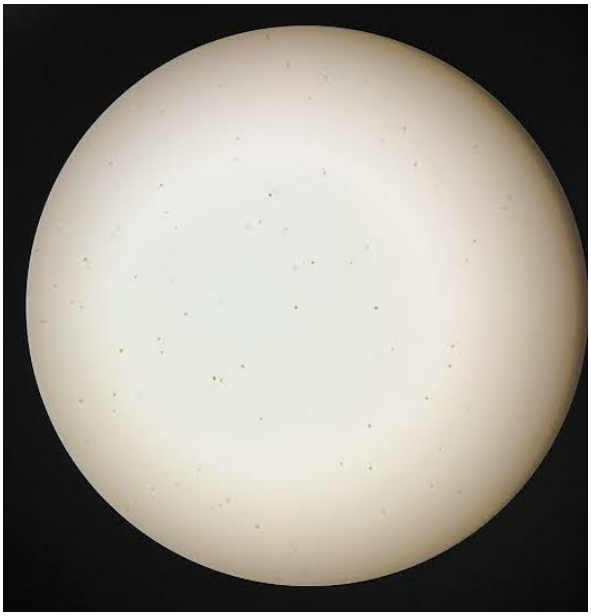
12 Use the coarse focus knob to lower the observation area as much as possible.

13 Securely place the hemocytometer in the observation area of the microscope. Turn the microscope on and adjust the light to the dimmest setting.

hemocytometer on microscope

14 Adjust the binocular eyepieces until you see one image while looking through them.

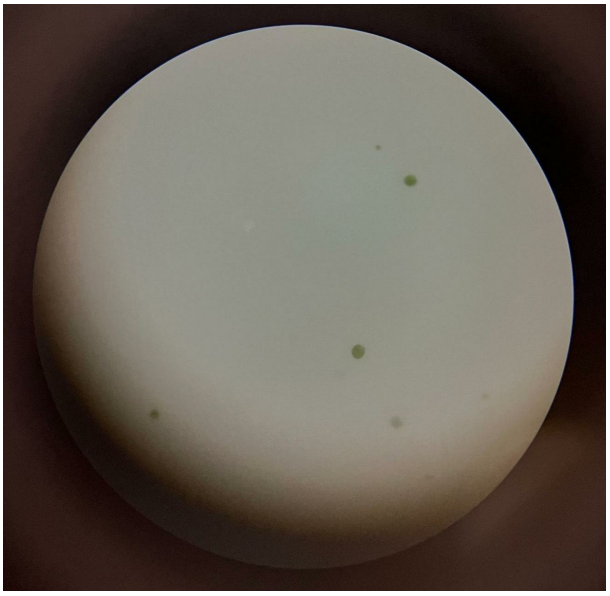
15 While looking through the binocular eyepieces, slowly raise the observation area using the coarse focus knob until an image forms. Once an image forms, use the fine focus knob to clarify the image.



microscope image (10x)

16 Rotate the objective lens to 40x, ensuring that the objective and the slide **DO NOT TOUCH**. Use the fine focus knob to refocus the image.

> **Note**
>
> If fewer than 10 cells are visible when using the 40x objective lens, you can use the 10x objective lens to collect data once a clear image has formed.



microscope image (40x)

17 Collect data

17.1 If collecting manually, follow the instructions included in the hemocytometer kit to calculate cell concentration.

17.2 If collecting automatically through Algaeorithm, follow the linked guide to capture/upload images and extract cell data.

Protocol

NAME

Algaeorithm Classroom Guide: Analyzing Microscope Images

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PREVIEW