**Centrifuging Algae Samples
Required time varies based on number of samples and model of centrifuge.**

**Objectives:**

* Students will become familiar with the popular scientific instrument, the centrifuge.
* Students will be able to relate the centrifuge and algae cells with G-forces.

**Background:** Because the algae was pulled out of a natural pond, the collection vessels likely are filled with many things that are not algae like pond water, sticks, and bacteria. In order to remove the algae from the water and other materials, centrifuging is necessary. If your school does not have a centrifuge available, you may be able to get permission to visit a local college or research institute and use theirs. It is also recommended that the instructor perform the actual centrifugation, because of the fact that the machine is very expensive and dangerous if not used correctly. A micro centrifuge may also be used, but very small amounts of algae can be obtained from using one.

**Set:** Look back on and discuss the pond sampling with the students. Allow them to hypothesize on which algae will grow the best, what it will look like, etc.

**Materials:**

* + **Ultracentrifuge**
	+ **Algae samples**
	+ **Ultracentrifuge tubes**
	+ **Spatula**
	+ **Graduated Cylinder w/ dH2O**
	+ **Balance**
	+ **Paper towels**
	+ **A couple markers.**
	+ **Paper**
	+ **Microcentrifuge tubes**

**Getting Ready:** Ask students some questions to get them thinking about the purpose of centrifuging the samples.

* So right now, you have a bottle of pond water.  Is there algae in it?
* Can we see it well enough to pull it out and start a new culture?
* What should we do about that?

Introduce students to the ultracentrifuge. Make sure they know the following:

* It is powerful.
* It is expensive.
* It can injure you or the building if not handled correctly.
* It spins samples very, very fast to separate algae biomass from water.  What’s left is a more concentrated algae sample and we can add that to a special growth medium to accelerate growth.
* The disk spins at up to 14,000 RPM’s.
* Let each student take a peek inside to get an idea of what it looks like.

**Methods:**
\*Note: This procedure may differ slightly with different centrifuges.\*

1. Have students swirl their container of pond water around to evenly suspend all algae.
2. Have each student carefully fill a large centrifuge tube with pond sample.
	* Write their name and ultracentrifuge tube number on a piece of paper so the samples can be kept straight.
	* Close old algae containers and set them aside; we will use them for plating and isolation.
	* Use dH2O to balance them all off of one students sample and explain why it is so important for them to be balanced (unbalances tubes can off set centrifuge and cause damage).
3. Make sure the lid of each ultracentrifuge tube is closed tightly and that it is completely dry.
	* Double check and explain significance (important to keep inside of the instrument dry).
4. Open top and unscrew rotor.
5. Load samples in a balanced configuration.
6. Screw rotor back on and make sure it is tight by lifting up and feeling for any “looseness.”
7. Close lid, latch door, double check.
8. Turn on the machine with the power switch.
9. Turn bottom (time) dial to “hold.”
10. Wait for vacuum gauge to turn green.
11. Set “speed” dial to desired speed (11,000 rpm)
12. Once 10 and 11 are complete, press start
13. Watch “rpm” gauge to reach desired speed, keep time short (timer does not work, time must be kept manually

*\*As samples are spinning, the instructor can discuss some interesting things about the centrifuge with the students, for example, figure out how many G-forces the samples are spinning at:*

* *Ask students what they know about G-Force and how many they think the centrifuge is spinning at?*

*G = 1.12 x radius x (RPM/1000)^2*

*radius = 137 mm RPM = 11,000 (numbers may vary based on models)*

*G = 1.12 x 137 x (11,000/1000)^2*

***G = 18,500***

1. Once desired time has spun, press stop
2. Once RPM gauge reaches 0, turn “time” dial to 0
3. Wait for “vacuum” gauge to read clear then open top door
4. Repeat if necessary
5. Remove samples
6. Re-secure lid to rotor (not too tightly)
7. Close but do not latch top door
8. Turn power switch to “off”
* There should be a green/brown mass called a pellet at the bottom of each tube. Show students pellet.
* Compare a tube that has been centrifuged to a tube that hasn’t.
1. Drain off as much excess liquid as possible, leaving only the pellet.
* One interesting thing to talk about at this point is the durability of the algae. Algae is one of the few organisms on earth that can be spun at 18,500 g-forces and live. Its durability is due to its tough cell wall. This is also a slight curse when it comes to extracting lipids, because without breaking through the cell wall, lipids cannot be removed from the cell.