## Module:  Invisible Forest

**Courses:** Marine Science, Oceanography, Integrated Science, STEM. This lesson may also fit within these courses: Biology, Honors Biology, Chemistry, Earth Science, Life Science, Physical Science, Physics, AP Chemistry, AP Physics, AP Biology, AP Environmental Science College Program in the High School.

**Unit:** Photosynthesis, Ecology, Biogeochemical Cycling, Energy, Photosynthesis, Phytoplankton, STEM application, skills building

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**Lesson Title:**  Lab 3.5 Phytoplankton Spectrophotometry and Fluorescence

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**ESTIMATED TIME:** 3.5-4.0 x 50 min, (+ 50-90 min for optional Spec Skills Lab)

**Objectives:** NGSS Standards within Lab 3.5

***What Students Learn:***

- A sense of size and scale, variety, and structure of organisms in the Invisible Forest
- UV light reveals chlorophyll fluorescence in phytoplankton
- Electrons in atoms and molecules can absorb light energy and re-emit light in fluorescence.  In chlorophyll, light excites an electron which either fluoresces or is chemically captured to do work in photosynthesis
- To use spectrophotometer to study phytoplanktonic chlorophyll light absorption and fluorescence
- Student phytoplankton results are comparable to published scientific data for *Prochlorococcus.* All oxygenic photosynthesizers with chlorophyll (a) will absorb and fluoresce light in the same way as seen in lab
- Chlorophyll fluorescence is used to measure water sample contents, cell counting in flow cytometers, and satellite imaging to study the Invisible Forest/global photosynthesis

***What Students Do:***

- Microscope observations of phytoplankton in visible light, and its fluorescence under UV light
- Observe examples of fluorescence and apply electron theory to model chlorophyll's ability to chemically capture light energy for life
- Prepare phytoplankton for spectrophotometer.  Can use as living cells in water or use a simple  freezing method of extracting chlorophyll with alcohol.

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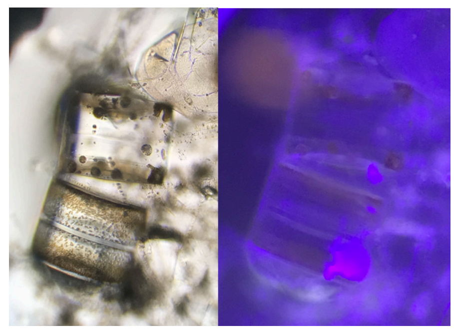
- Use spectrophotometer to measure wavelengths of planktonic chlorophyll light absorbance and fluorescence
- Compare results to published data for *Prochlorococcus* and apply to all oxygenic photosynthesizers
- Apply concepts of light and chlorophyll to understanding scientific instrumentation and measurements of oceanic ecosystem, The Invisible Forest
- Extension: Guided activity to observe visible light with diffraction gratings. Create a colored scale of visible and near visible wavelengths for their notes, linking color, wavelength, and energy
- Extension: Guided lab: Learn how to use spectrophotometer: blank calibration, absorption with dyes, and quinine fluorescence

### Introduction to Phytoplankton Spectrophotometer Lab

The Invisible Forest, of the vast open oceans, is the foundation for half of Earth's bioproductivity and atmospheric oxygen. Microscopic and living beneath the water's surface, this realm of life is just now being recognized for its importance to Earth's biosphere and atmosphere. In **Lesson 2** and **3** students were introduced to scientists studying the ocean's changing primary productivity world-wide. They do this through water sampling and satellite imagery using principles of chlorophyll fluorescence. Here, students have an opportunity to observe phytoplankton and investigate chlorophyll fluorescence with a spectrophotometer in greater depth. Guided activities and labs provide background to understand visible light, UV light, fluorescence, photosystems, and spectrophotometer function and use. Ultimately, students are led to understand how information from satellite imagery and other oceanographic instrumentation allows us to study Earth's Invisible Forest. In **Lesson 4** students will apply their understanding of fluorescence and spectrophotometry as a tool for gathering data in order to learn more about the diversity of phytoplankton in transects and layers of the ocean.



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### Overview of Instructional Activities:

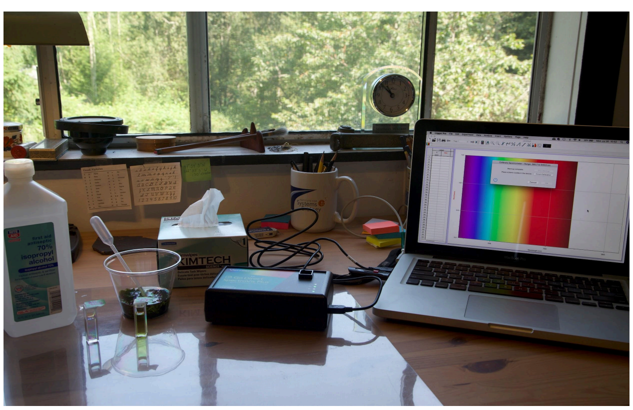
| Lesson | What students learn: | What students do: |
|---|---|---|
| [Phytoplankton Spectrophotometer Lab] Part **1A*: Observing Plankton with Microscope (35 min)** **—**Microscope observations with white and UV light. *[Making observations and Asking questions]* **Part 1B: Preparing Plankton for Chlorophyll Extraction and Spectrometer (20 min + 24 hr. freezing)—**Filtering plankton procedure. *Read **Phytoplankton Spectrophotometer Lab teacher guide** for advanced preparations. | - There's an invisible world of life in a drop of water.<br>- UV light gives unexpected additional information about plankton and chlorophyll.<br>- Laboratory filtration technique.<br><br>***Note:** Prepare or gather phytoplankton cultures to use for the Labs several weeks prior. | - Collect or culture fresh or seawater plankton, or have access to available plankton<br>- Observe plankton cultures and phytoplankton chlorophyll fluorescence in microscope with visible and near UV light.<br>- Extract planktonic chlorophyll with filters and freeze in alcohol (24 hours) to make plankton extract. |
| **Visible Light Spectrum and Fluorescence worksheet** **Guided Activity A (30 min)** (questions 1 - 5) introduces wavelengths and light spectrum. **Guided Activity B (30 min)** explores Fluorescence (questions 6 - 13) with a Demo and/or Fluorescence slide show and Energy capture complex in cell diagram —*Observing phenomena and [Developing and using models]*<br>(See **Visible Light and Fluorescence Teacher guide** for more details.)<br><br>**[Optional] Lab: Introduction to Spectrophotometer (extension) (+50–90 min)—***Using instrumentation [Analyzing and interpreting data.]* | - Learn which light wavelengths correspond to specific colors used in photosynthesis and are visible with a spectrophotometer.<br><br>- Learn spectrophotometer function, calibrating blanks, absorbance of food dyes, and fluorescence of quinine. | - Color a model spectrum matching light color to wavelength and energy content.<br>- Place visible, UV, and IR light in relation to visible spectrum and rest of electromagnetic spectrum.<br>- Use diffraction gratings to observe visible light spectrum and relate wavelengths to color<br>- Develop an operational definition of fluorescence and model of electron movement in fluorescent materials. Apply to model of chlorophyll in photosystem II.<br>- Observe fluorescence in quinine, minerals, ultrabrite papers, & chlorophyll |
| **Part 2: Phytoplankton Spectrophotometer Lab (50 min)—**Measuring phytoplankton chlorophyll absorbance and fluorescence with spectrophotometer —*[Planning and carrying out investigations. Analyzing and interpreting data.]* | - Lab procedures for using spectrophotometer.<br><br>- Interpreting absorbance and fluorescence results from spectrophotometer | - Measure phytoplankton chlorophyll absorbance and fluorescence with spectrophotometer. |
| **Part 3: Spectrophotometer Lab Analysis and Conclusions** | - Absorbance and fluorescence are used in a variety of | - Compare absorbance and fluorescence data to Sally |

| | | |
|---|---|---|
| **worksheet (30-50 min)** —*[Analyzing and interpreting data. Constructing explanations. Obtaining, evaluating, and communicating information]* | scientific instrumentation to understand ocean photosynthesis and measure phytoplankton. | ‘Penny’ Chisholm’s data on *Prochlorococcus* and apply understanding to any photosynthesis with *chlorophyll a* and remote sensing imagery. |

**Instructional Activities:**
**Phytoplankton Spectrophotometer Lab and Fluorescence [*Simplified Lesson Plan (Option)* 4 x 50 min classes]**

**Prerequisites*:** Students know and understand spectrophotometer function and use for absorption and fluorescence. Students know visible light and UV spectrum and how color, wavelength, and energy are related. Students know the relationship between chlorophyll fluorescence and electron capture in photosystems II & I. *(*if students are not familiar with these concepts use* ***Visible Light Spectrum and Fluorescence*** ***(Activity A) and*** *(***Activity B)*** *to introduce the necessary background)*

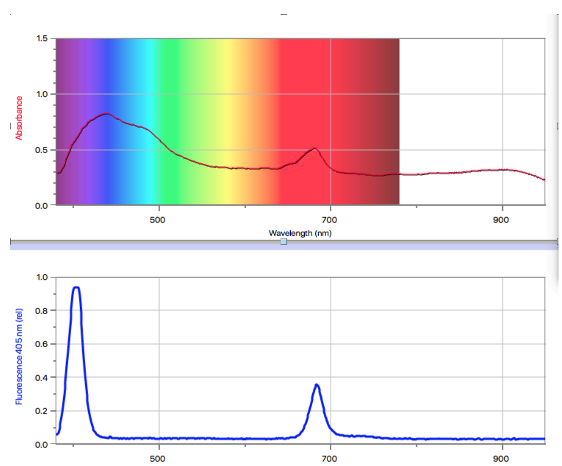
**Before Class:**
Go to Teacher Lesson Plan/Phytoplankton Spec and Fluorescence Lab* for detailed directions on how to use Lab Materials. And planning examples for short and extended lesson sequences. *Gather or prepare plankton cultures several weeks before class**. (50 min).** Print and copy 1 per group: Phytoplankton Spectrophotometer Lab_student procedure, and 1 per student: Spectrophotometer Lab Analysis and Conclusions_ worksheet Visible Light Spectrum and Fluorescence_student worksheet, Prepare to show: Fluorescence slide show**,** and Energy capture complex in cell_diagram



**Day 1:** -Hand out **Phytoplankton Spectrophotometer Lab Part 1A (35 min)** Begin lesson sequence with observing plankton with a microscope* in white and UV light *(*can be included as part of **Lesson 2**).* Obtain a fresh or seawater plankton sample. Make a wet-mount slide of the plankton. Examine plankton with a light microscope at 40x and 100x. Look for diatoms and other algae. Look for green chloroplasts within the cells. Generate wonder and questions with seeing the phenomenon of microscopic ecosystems and chlorophyll fluorescence. Next, in **Part 1B** of this **Lab** students prepare phytoplankton culture for measuring fluorescence in a spectrophotometer — which they will use in **Part 2.**

**Day 2:** For students who need further background on visible light wavelengths and how fluorescence works in a living organism use the following guided activities in **Activity A: Visible Light Spectrum (30 min)** and **Activity B: Fluorescence (30 min) -** Step 1: Hand out the Visible Light Spectrum and Fluorescence_student worksheet.Remind students the instruments they learned about in **Lesson 3,** make measurements using principles of the interaction of light and matter as well the spectrophotometers they are going to use in the following days' **Lab.** The **Lab** will provide practical experience to understand the science of measuring global primary productivity *(and thus breathable oxygen production).*
Step 2: Begin with reviewing the visible light spectrum *(**Activity A:** questions 1-5)*. First observe white light using diffraction gratings and have them color in a spectrum.
Step 3: Then use the worksheet to guide them to match wavelengths in nm with the qualitative (observations) of visible light, matching color/wavelength with energy.
Step 4: Next, for *(**Activity B:** questions 6-13)* focus in on key red and blue wavelengths of photosynthesis and the instrumentation used to study light quantitatively. Show students a selection of fluorescent materials *(quinine water, minerals, ultrabrite papers, & chlorophyll)* to view under violet and near violet/long wave UV light. Or show them *Fluorescence slide show.*
Step 5: Guide students to develop a chlorophyll model showing absorption of light, energy capture in photosystem, or re-emission of light as visible fluorescence. The *Energy capture complex in cell_diagram* can be used to trace the system as you walk them through it. These concepts are applied to understanding chlorophyll molecules in photosystems. *Adapt available material including the Fluorescence slide show**,** and Teacher slideshow script , Demo of fluorescence and Energy capture complex in cell_diagram from **this lesson** to suit one, fifty minute class. Refer to **Visible Light and Fluorescence Teacher guide** for additional background for teaching the guided activity.)*

**Day 3:** - **Phytoplankton Spectrophotometer Lab Part 2 (50 min)**.
Step 1: Remind students that chlorophyll, and thus primary production in ocean ecosystems, is measured with spectrophotometers directly with water samples and remote imaging *(as introduced in **Lesson 3)***. The ability to study light energy and its interactions with matter lets them do science with instruments similar to those used in oceanography. In this **Lab** they are measuring planktonic chlorophyll for absorption and fluorescence, to deepen understanding of photosynthesis, and the instruments used to measure this. Another instrument, the flow cytometer *(also in **Lesson 3)*** uses fluorescence and light diffraction to count, measure, and identify single celled phytoplankton, including Prochlorococcus.




Step 2: Using the phytoplankton prepared earlier in **Part 1B of this Lab**—measure phytoplankton or chlorophyll extract for light absorbance and fluorescence with a

spectrophotometer. *(If students are unfamiliar with Spectrophotometers use the **Optional extension Lab*** *for practice, before they begin to measure phytoplankton fluorescence.)*
Step 3: Students use the data they collect to develop a deeper learning model for understanding chlorophyll's ability to capture light energy converting it into life's chemical energy.
**Day 4:** - **Phytoplankton Spectrophotometer Lab** **Part 3 ( < 50 min)** This final step will give students direct experience with data and instrumentation used to measure the Invisible Forest / oceanic primary production. Use Spectrophotometer Lab Analysis and Conclusions_ worksheet to analyze their chlorophyll spectrophotometer results. Students will compare their results to Dr. Chisholm's lab results for *Prochlorococcus* and go to NASA Ocean Color website to learn how satellites use light and fluorescence to measure global chlorophyll. Review the students' analysis worksheet responses using Spectrophotometer Lab Analysis and Conclusions_Teacher Key.

**OPTIONAL ACTIVITIES (Extensions):**
***Optional extension Lab (+50 min):** Introduction to Spectrophotometer _Teacher with Introduction to Spectrophotometer Lab_Student procedure and Introduction to Spectrophotometer Lab_student worksheet
This is an intro lab using water, food dye, and quinine to teach students spectrophotometer usage and design/function of the instrument.

**Resources:**
Phytoplankton Spectrophotometer Lab_student procedure
Spectrophotometer Lab Analysis and Conclusions_ worksheet
Spectrophotometer Lab_Analysis and Conclusions_Teacher Key
NASA Ocean Color website
Visible Light and Fluorescence_Teacher guide
Visible Light Spectrum and Fluorescence_student worksheet
Visible Light Spectrum and Fluoresence_teacher key
Fluorescence slide show and *Teacher slideshow script*
Energy capture complex in cell_diagram
Fluorescence slide show_Teacher script

**Extensions:**
**Optional Extension Lab:**
Introduction to Spectrophotometer _Teacher
Introduction to Spectrophotometer Lab_Student procedure
Introduction to Spectrophotometer Lab_student worksheet

**References:**
College professor's take on light, chlorophyll, and photosystem. Nice explanation.
http://plantphys.info/plant_physiology/light.shtml
Background on NASA Ocean Color satellite bandwidth and how chlorophyll is measured.
https://oceancolor.gsfc.nasa.gov/SeaWiFS/TEACHERS/sanctuary_3.html