

GREEN PLANTS, RED GLOW

Looking at chlorophyll's red fluorescence as an exercise in exploring photosynthesis, agriculture, and global ecology

Abstract

Photosynthesis can be challenging for instructors to teach and uninteresting for students to learn, but this shouldn't be the case. An activity developed by middle-school educators and university scientists lets students see how red light emitted from sunlit plants is captured by satellites to measure global photosynthesis. In plants, most of the absorbed light energy is channeled into photosynthesis, and the tiny amount that is emitted as red fluorescence is not visible by naked eye but is detectable by satellites. When chlorophyll is removed from plants into a solution – uncoupled from the photosynthetic apparatus – chlorophyll still is green and absorbs light, but the absorbed light energy has nowhere to go, and a large red glow is visible. In a readily accessible 1-hour middle-school classroom activity, students extract chlorophyll from spinach using rubbing alcohol (91% isopropyl alcohol) and then observe the abundant red fluorescence upon illumination with a flashlight. This simple observation of the red glow (fluorescence) from chlorophyll provides a terrific anchor for teaching photosynthesis in a biological, agricultural and global ecology context, thereby inspiring students to better appreciate the fascinating world of plants.

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Introduction

Photosynthesis is one of the most important biological processes but can be challenging to teach and obscure for students. This article presents a one-hour, hands-on classroom activity that lets students extract chlorophyll from spinach leaves and directly observe the red light (fluorescence) emitted by the chlorophyll upon illumination. Students then learn that the same red light – emitted from plants in fields and forests around the sunlit planet – can be observed by satellites and used as a proxy for the extent of global photosynthesis. Using the 5E Model, we introduce students to the concepts of *chlorophyll* and *fluorescence* as related to photosynthesis. Although experiments involving chlorophyll often require expensive equipment, this low-cost activity uses materials that can be found at a neighborhood grocery store or pharmacy. This classroom activity was developed in collaboration with middle-school educators and XX University scientists, and tested at a Montessori middle school and a Title 1 Middle School in XX. The activity provides an anchor for teaching photosynthesis in a biological, agricultural, and ecological context and for inspiring students to see plants as fascinating and vital to life on earth.

Background Information

Photosynthesis is often summarized by the equation $[6 \text{ CO}_2 + 6 \text{ H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2]$, which of course is correct, but this focus on input (carbon dioxide and water) and output (carbohydrate and oxygen) diverts attention from the essential role of chlorophyll. Indeed, chlorophyll is not present in the equation at all, but should at least be written above the arrow to indicate its essential enabling role. Chlorophyll, the abundant green molecule in plants, absorbs light and channels the energy to drive the photosynthesis equation. Chlorophyll is located in chloroplasts – subcellular factories for solar energy utilization – which in turn are located in plant cells. In plants, most of the absorbed light energy is channeled into photosynthesis, and the tiny amount that is emitted as red fluorescence is not visible by naked eye but is detectable by satellites.

When plant leaves are treated with a solvent such as rubbing alcohol, chlorophyll is removed from plant cells to give a green solution. Chlorophyll still absorbs light, but uncoupled from the photosynthetic apparatus, the absorbed light energy has nowhere to go, and a large red glow is visible.

Engage

Figure 1 shows a satellite image. To begin the activity, ask students to describe what they see. “What do you see in Figure 1?” Make a chart of student observations and questions so that you can track them throughout the lesson. Students should look for scale, patterns, shapes, textures, colors (including shadows), and consider their prior knowledge. Observations such as “I wonder

why some regions are bright pink, others red, yet others completely dark” flow nicely into the question of how plants work that they will explore during the lesson. However, you may want to add to student questions, or guide student questions towards the disciplinary and cross-cutting topics of energy and matter. It is important that teachers NOT answer the questions at this time, students should build answers to these questions as they move through the lesson.

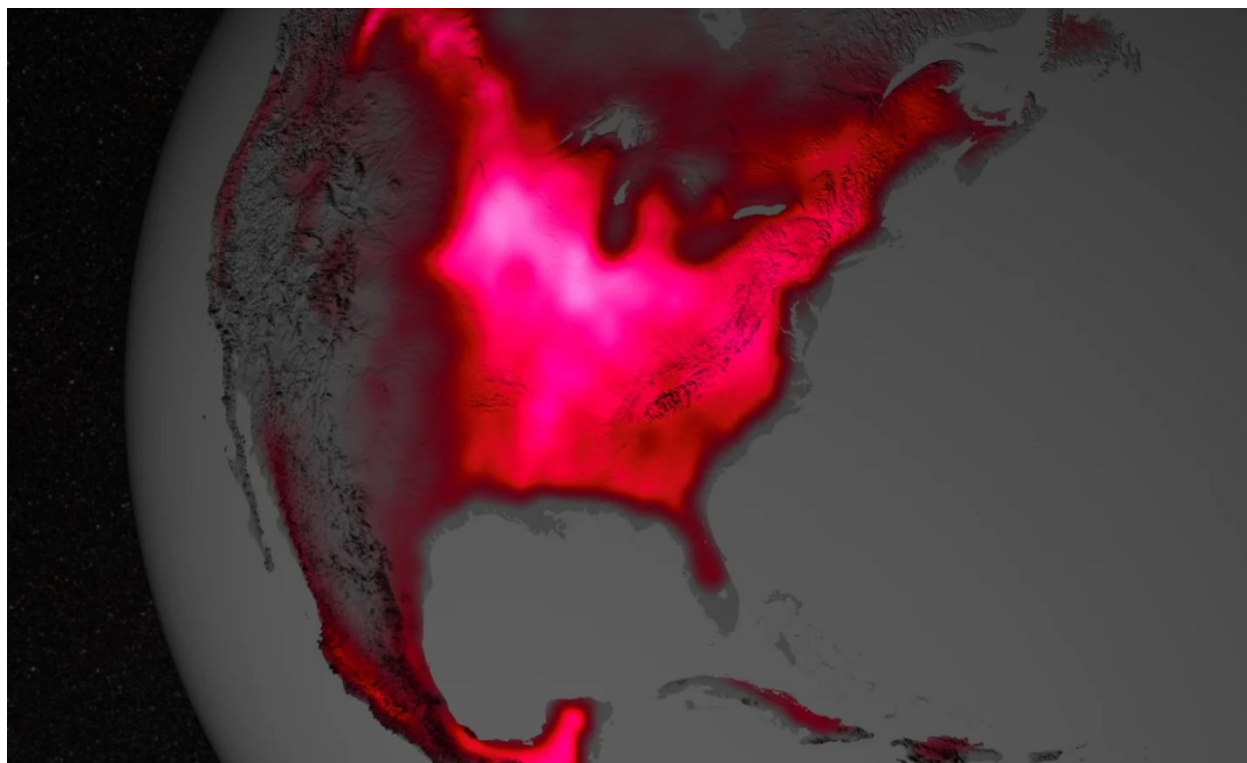


Figure 1. An image of sunlight-induced fluorescence (SIF) across much of North America from 2007 to 2011. SIF is often used as a measure of carbon fixation and crop health. Image: NASA Goddard Space Flight Center.

Explore

Say to students: If we'd like to learn more about this red glow we need to do an experiment. Before starting the process in *Figure 2*, please review safe laboratory procedures. Although handling small quantities of rubbing alcohol is quite safe, there are still several precautionary steps:

- Provide students with splash-proof eyewear and safety gloves to limit exposure.
- Pour the rubbing alcohol solutions from one container to another with care to avoid spills, which in small quantity can be cleaned up by wiping with paper towels.
- No materials used in this activity should be ingested. Note that rubbing alcohol contains ~91% isopropyl alcohol, ~9% water, and bitter additives to thwart ingestion.
- Rubbing alcohol is flammable; store and use away from any open flames. For further information about rubbing alcohol, see the Online Supplemental Materials.

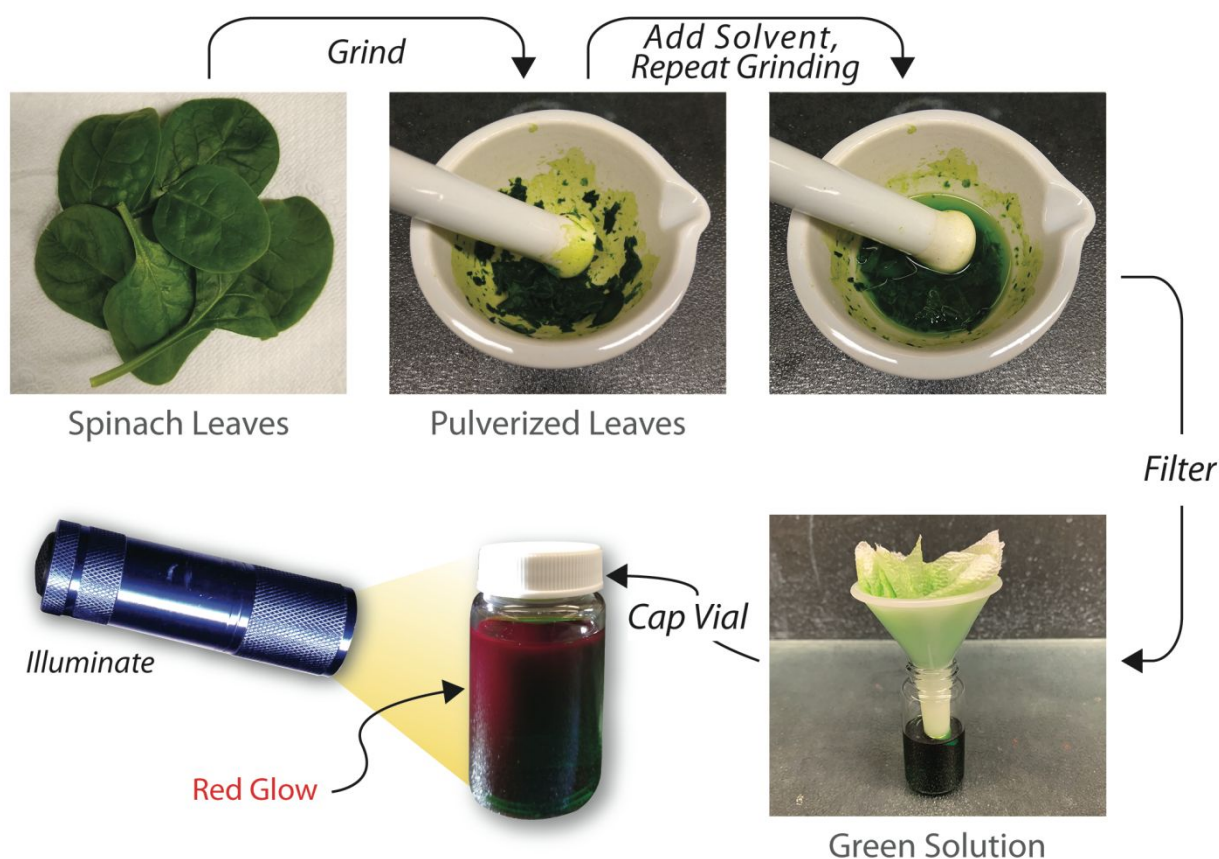


Figure 2. The individual steps for naked-eye observation of a red glow following the extraction.

1. Pulverize spinach. Students work in groups of 3-4 to break up five to seven spinach leaves into small pieces by hands or scissors. They place the broken leaves into a mortar and grind the leaves thoroughly using a pestle, in circular motions, for a minute or two. Next, they add thirty milliliters of rubbing alcohol to the leaves and repeat the grinding process. Students carefully set aside the mortar and move to the next step.

2. Assemble the filter. Students cover the mouth of a small funnel with two layers of paper napkin, paper towel, or coffee filter. Then, they place the funnel over a glass container, completing the filter assembly.

3. Strain the suspension through the filter. Students carefully pour the green suspension from the mortar into the funnel, aiming for the center of the paper filter. Unwanted leaf tissue is retained by the filter, and the green solution filters into the container. If a clear, dark green solution is not obtained, the students should pour the liquid back into the mortar, create a new filter, and repeat the straining process.

4. Observe the red glow. After the clear, dark green liquid is completely filtered into the container, students put the container in a dark space, turn on a flashlight, shine the light through the container, and observe a red glow around the edges of the container. If students have difficulty seeing the

red glow, move the flashlight back and forth, closer and further away from the container. See online resources for brief video demonstrations of each step.

5. Record. Students write down their observations, questions, and ideas. A laboratory procedure sheet for the above procedure, and an observation/assessment sheet, are provided in the Online Supplemental Materials.

Teacher Notes for Experiment

Students are very eager to begin the extraction step of this activity, so be careful to limit spills.

Students may have to strain the green suspension more than once.

Depending on how brightly lit the classroom is even with the lights off, students may need a darker space such as a cabinet or an assembled lightbox. See the Online Supplemental Materials for directions to create a simple cardboard lightbox.

Students should use a bright flashlight so the chlorophyll receives ample light energy, thus emitting a strong, red glow. Fluorescence intensity decreases as the flashlight moves away from the container. Because chlorophyll absorbs more strongly in the visible region than in the ultraviolet, a flashlight is superior than a (ultraviolet) blacklight to induce fluorescence.

Note that the amount of emitted light from chlorophyll in solution is up to 32% (i.e., for 100 molecules of chlorophyll that each absorb a photon of light, up to 32% emit a photon (red light). For chlorophyll in a healthy leaf, only about 1% or less emit (Schlau-Cohen and Berry, 2015). The latter can't be seen by naked eye, first because the intensity is very low, second because of the large abundance of natural light of the same general color.

Explain

Following the experiment and after students have written down their own observations, questions, and ideas. Students, working in their experimental groups, should spend 10-15 minutes brainstorming and discussing what they think is going on. Spinach is a plant and they should consider what plants need to live. One purpose of this activity is to find out what your students already know about plants and how they grow. Many students may be familiar with photosynthesis, but others may not. Three question prompts may be, 1. *What makes plants green?*, 2. *What do plants need/use/consume?*, and 3. *What do plants give off?* This activity should shift the focus of just learning about photosynthesis, for example, to figuring out why or how something happens. Students should be engaged in building evidence-based explanatory ideas that help them figure out how plants or trees grow as opposed to simply learning about the topic of photosynthesis. By using the phenomenon of the red glow, students are able to use the three dimensions of the NGSS to explain. Students explain phenomena by developing and applying Disciplinary Core Ideas (DCIs) and Cross-cutting Concepts (CCCs) through use of the Science and Engineering Practices.

After 10-15 minutes of small group discussions, teachers should bring the class together and ask a member from each group about the group's observations, questions, and ideas. These should be written on the board for each group. In this stage, teachers can ask a few questions to guide students' knowledge, if needed. These questions should be framed in such a way to make students

think more deeply. Possible questions are: *Why was spinach used in this experiment? Why was rubbing alcohol used? Why were the spinach leaves pulverized? What would cause the green liquid to glow red? How does the red glow relate to the satellite image (Figure 1) we viewed at the beginning of this lesson? How might the red glow (how bright or dark it is) relate to agriculture, farming, and the health of plants?*

Through the group and whole-class discussions, knowledge is individually and socially constructed as students work out their own understanding of the red-glow phenomenon while being facilitated by a more knowledgeable other (the teacher). Students bring different amounts of prior knowledge about the process of photosynthesis to the activity, which they will readily share with their groups. Through the whole class discussion, key concepts around photosynthesis will arise including light energy, chemical energy, leaves, chlorophyll, chloroplasts, carbon dioxide, oxygen, water, and others. Teachers guide a whole-class discussion about the observations, questions, and ideas from each group and see if the class can agree on some hypotheses, models, and explanations for the red glow experiment. Teachers are encouraged to use *Figure 3* in discussing the structure and function of chloroplasts. Through this lesson, students use the practices of science: asking questions, carrying out investigations, analyzing and interpreting data, construction explanations, engaging in argument from evidence, and obtaining, evaluating, and communicating information. To help the teacher facilitate learning, notes for class discussion are given below.

Teacher Notes for Class Discussion

Chlorophyll is a molecule. Yet advanced concepts about chemistry (atoms, molecules), biology (cells and organelles) and physics (energy) are *not* required to accomplish the objectives of this activity.

There actually are two dominant forms of chlorophyll in plants, chlorophyll *a* and chlorophyll *b*; here we use the term chlorophyll collectively.

When chlorophyll absorbs light, all of the energy can be used for photosynthesis, at least in principle. But in practice, some energy is released as heat, and a tiny amount is emitted as red light (fluorescence). We can't see this fluorescence from a leaf by our naked eye, but satellites with sensitive detectors can detect this red glow (SIF). *Figure 1* shows the red glow from the North American Midwestern farm belt. The more chlorophyll, the more red glow; so the intensity of the glow is a marker of the health and vigor of plants.

What happens if chlorophyll is taken out of the plant? Now part of the energy that would have gone into photosynthesis is released in abundance as red fluorescence, which the student can see in the spinach extraction activity.

The organization of components in leaves is essential for efficient capture and utilization of light energy. As seen in *Figure 3*, the characteristic green color of leaves is due to the presence of chlorophyll within the chloroplasts of plant cells. Rubbing alcohol disrupts the cell membranes (where chlorophyll is located) and dissolves (i.e., extracts) the chlorophyll.

Satellite monitoring of SIF can be used on a global scale. Examples include monitoring the health of the Amazon Rain forest, tracking rising temperatures globally, and measuring extended wet seasons across the Earth (de Sousa *et al.*, 2017).

When discussing SIF, a map of SIF should be displayed to aid visual learners.

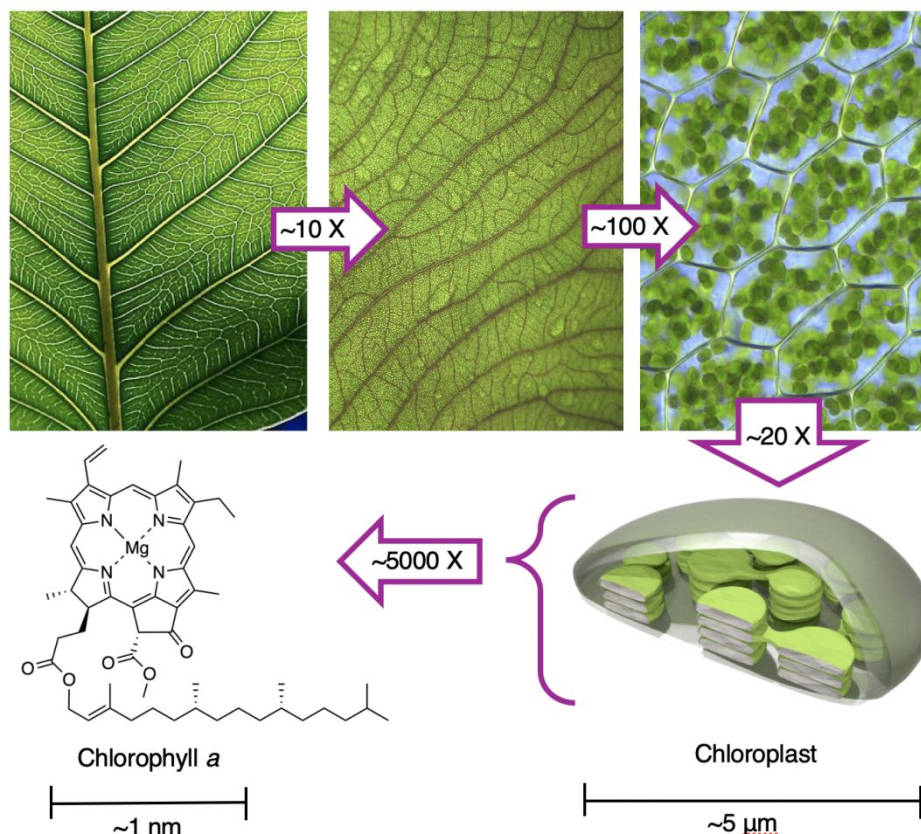


Figure 3. The “green” in leaves comes from chlorophyll molecules located within plant cells. Approximate magnification scales are given in the arrows (total $\sim 10^8$ X from 1 cm of the leaf to 1 nm, the size of the chlorophyll molecule). Images are from Wikimedia commons.

For proper explanation, students worked through extensions on their lab activity sheet. Students checked the capability of food coloring and a mystery substance (such as a popular colored beverage) to fluoresce red (see Extension activities below). Working together in a group, students discussed the observable differences between both leaves and the food coloring. Students were able to conclude that food coloring lacks the presence of chlorophyll and therefore does not glow red. This led to the discussion of their mystery substance and the conclusion that it could not contain chlorophyll for the same reasonings.

Elaborate

Related activities: Fluorescers and non-fluorescers

The following three questions pertain to elaborative activities for which procedures and materials supplies are described in the Online Supplemental Materials.

Does any green solution glow red? To test this, prepare a solution of green food coloring in water. Illumination with a flashlight should show no red fluorescence. These kinds of dyes absorb light (hence their color) but almost instantly release all of the energy as heat. If we had a sensitive thermal detector, this release of heat could be detected. This experiment provides a

counterexample to the features of chlorophyll. A counterexample with a mystery substance also can be employed, for example a popular colored carbonated beverage.

Is red the only fluorescent color? Chlorophyll is hardly unique in exhibiting fluorescence – many substances give off light after absorbing light. Common examples are provided by the dyes used in text highlighters. Immerse the tip of a text highlighter in rubbing alcohol until a colored solution results. Illuminate the colored solution with the flashlight and observed the fluorescence. State the color of the solution and the color of the fluorescence.

Is spinach special? All plants contain chlorophyll. Spinach is very attractive because it has a high concentration of chlorophyll, and the tissue is soft enough for ease of extraction. But other leaves certainly can be used – students may wish to bring in their own plant material. Some leaves (e.g., holly) have a tough outer layer for which rubbing alcohol is insufficient to break through the leaves. But the chlorophyll is still there, and if released, would also glow bright red.

Evaluate

Students are evaluated on their participation in the lab, discussion, and conclusive lab questions. Students should complete a lab write up that will be turned in to assess understanding. As part of the lab write up, students should address the following questions: (1) What molecule makes leaves green, and what is its function? (2) Where is chlorophyll located within a plant? (3) Where does the red glow come from? (4) Why is the red glow used as a measure of plant health? (5) What would farmers be able to learn from images taken by satellites? Students should be able to: (1) Explain what molecule makes leaves green and its function. (2) Describe the observable differences between the leaves and food coloring. (3) Determine if a mystery substance has the presence of chlorophyll and explain their reasoning. (4) Explain how SIF images help scientists study the effects of global climate change. (5) Explain what farmers would be able to learn from SIF images taken by satellite.

Materials

Item per each working group (1-4 students)	Cost for class of 30
30 mL of rubbing alcohol	\$5
1 coffee filter (optional napkin, paper towel)	\$2
5–7 spinach leaves (remove stems)	\$4
~25 mL glass container (or baby food jar)	–
1 mortar and pestle (or cup and wooden spoon)	–
1 graduated cylinder (or beaker or measuring cup)	–
1 small funnel (or strainer with a cotton layer)	–
1 bright flashlight	\$5
dark space for visualization	–
food coloring	\$4

mystery substance (e.g., a colored beverage)	\$2
Total	\$22

Reflection

Photosynthesis is traditionally taught with neglect of the essential role of chlorophyll in enabling the photosynthesis equation: $6 \text{ CO}_2 + 6 \text{ H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{ O}_2$. The present activity focuses attention on chlorophyll and the flow of energy: in the plant the absorbed light energy is almost exclusively channeled into photosynthesis (only a small amount of the light is emitted as a faint red glow) whereas upon removal from the plant much of the absorbed light is emitted as a strong red glow. Yet, the weak red glow emanating from plants provides a measure of photosynthetic activity, and can be monitored globally by satellite. The classroom activity coheres with A Framework for K-12 Science Education (Next Generation Science Standards) where “the flow of energy through a natural system” provides a lens on crosscutting concepts concerning Energy and Matter. The activity supports student sensemaking for (1) LS1.A: Structure and Function Within Cells, where special structures are responsible for particular functions as part of a larger element – and extraction here with rubbing alcohol completely disrupts such architectures; and builds toward (2) LS1.C: Organization for Matter and Energy Flow in Organisms, Plants, Algae (including Phytoplankton) and Microorganisms, where the energy from light enables the formation of sugars (food) from carbon dioxide from the atmosphere and water through the process of photosynthesis (mediated by chlorophyll), which also releases oxygen as a photosynthetic waste product (but an essential substance for respiration by animals). These sugars can be used immediately or stored for growth or later use.

Conclusions

Photosynthesis and the life cycle of plants lie at the foundation of life on earth. Students are introduced to a new technology of growing importance to agricultural scientists and ecologists, solar-induced fluorescence (SIF), which detects red light emitted from sunlit plants, and thereby enables visualization of photosynthetic productivity on a global scale. The “green plants – red glow” activity connects the abstract idea of photosynthetic efficiency to the hands-on experience of seeing (by naked eye) the red glow from chlorophyll upon removal from the organized molecular architecture of the plant cell. The activity described here is simple, meaningful, and captivating for students at middle schools rich and poor alike.

References

- De Sousa, C.H.R., Hilker, T., Waring, R., De Moura, Y.M., and Lyapustin, A. 2017. Progress in remote sensing of photosynthetic activity over the Amazon basin. *Remote Sensing* 9(1):48.
- Schlau-Cohen, G. S., and Berry, J. 2015. Photosynthetic fluorescence, from molecule to planet. *Physics Today* 68: 66.

Resources

Velev, K. 2018. Watching the planet breathe: the story of SIF. <https://oco.jpl.nasa.gov>

Supplemental Files – Files concerning laboratory instruction and assessment sheets, additional information for the instructor, procedures for extension activities, demonstration videos, and guide to constructing a lightbox.

For Peer Review

Connecting to the NextGen Science Standards

Connecting to the *Next Generation Science Standards* (NGSS Lead States 2013)

- The chart below makes one set of connections between the instruction outlined in this article and the *NGSS*. Other valid connections beyond the space constraints here are likely.
- The materials, lessons, and activities outlined in the article are one step toward reaching the performance expectations listed below.

Standard: MS Growth, Development, and Reproduction of Organisms	
Performance Expectation: MS-LS1-6. Construct a scientific explanation based on evidence for the role of photosynthesis in the cycling of matter and flow of energy into and out of organisms.	
Dimension	Classroom Connection
Science and Engineering Practice Constructing Explanations and Designing Solutions	Students utilize summative review to devise explanations and possible applications of learned concepts.
Disciplinary Core Idea: The chemical reaction by which plants produce complex food molecules (sugars) requires an energy input (i.e., from sunlight) to occur.	Variation in observable fluorescence is due to chlorophyll in the plant (not detectable by eye, but detectable by satellite) versus in a solution (energy that would go into photosynthesis is now released and can be detected by eye).
Crosscutting Concept Cause and Effect	Students will discover red fluorescence as a direct result of removing chlorophyll from the spinach leaves.

Connections to the *Common Core State Standards* (NGAC and CCSSO 2010)

ELA: SL.8.5 Integrate multimedia and visual displays in presentation to clarify information, strengthen claims and evidence, and add interest.
XX State standards: 6.L.1 Understand the structures, processes and behaviors of plants that enable them to survive and reproduce. 7.L.1.2 Compare the structures and functions of plant and animal cells, including major organelles (cell membrane, cell wall, nucleus, chloroplasts, mitochondria, and vacuoles). 8.P.1.4 Explain how the idea of atoms and a balanced chemical equation support the law of conservation of mass. (If the teacher uses the equation for photosynthesis) 8.L.4.2 Explain the relationship between genetic variation and an organism's ability to adapt to its environment. (For the extension activity only.)

Acknowledgements

This project was funded by the National Science Foundation Division of Chemistry (additional information to be provided following double-blind review).

Callout Box

Content Area	Biological Sciences
Grade Level	6-8
Big Idea/Unit	Photosynthesis can be assessed by the faint red glow from sunlit plants
Essential Pre-existing Knowledge	Photosynthesis provides the food for almost all life on earth
Time Required	One hour
Costs (for a class of 30)	\$ 22
Safety	Chemical splash-proof safety glasses and gloves must be worn during handling of rubbing alcohol and throughout the chlorophyll extraction process.

Supplemental Materials for:

**Green Plants, Red Glow – Looking at chlorophyll’s red fluorescence as an exercise in
exploring photosynthesis, agriculture, and global ecology**

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Laboratory Instruction Sheet: Green Plants, Red Glow

Part 1: Extract Preparation

- (1) Pick 5-7 green leaves of spinach.
- (2) Break the leaves into small pieces by hand or scissors.
- (3) Place the pieces into the mortar (or bowl).
- (4) Using a pestle (or spoon), thoroughly grind the leaves.
- (5) Fill the mortar with rubbing alcohol to less than half full.
- (6) Repeat the grinding without spilling the mixture.
- (7) Place two unfolded napkins over the mouth of the funnel.
- (8) Place a small vial directly underneath the opening of the funnel.
 - ❖ Make sure the vial and funnel do not tip over.
- (9) Pour the mixture over the napkins inside the funnel and strain.
 - ❖ Make sure leaf pieces are not included in the vial.

Stop here and wait for instructions

Part 2: Visualization

- (1) Bring your sample to the nearest light box and place it into the slot in the front.
- (2) With the flashlight on, place your sample near the light.
- (3) Use the slot at the top of the light box in order to look inside.
 - ❖ You may need to move your sample back and forth from the light.
- (4) Return to your seat, record what you saw, and write why this may have happened.

Observations and Assessment: Green Plants, Red Glow

Question 1. What molecule makes leaves green, and what is its function?

Question 2. Where is chlorophyll located within a plant?

Question 3. Where does the red glow come from?

Question 4. Why is the red glow used as a measure of plant health?

Question 5. What would farmers be able to learn from images taken by satellites?

Observation Space. Please use this area to make observations during the activity.

3. Additional Information for Instructors

A. Why Plants? – the following is provided to spark classroom discussion

1. What do plants give us directly?

- Food – vegetables, fruits
- Wood – for furniture, buildings, basketball courts, etc.
- Flowers
- Fragrances – rose, lavender, anise, etc.
- Cotton and clothes made of cotton
- Coffee and tea
- Sugar
- Spices – cinnamon, chili, clove, curry, eucalyptus, ginger, vanilla, etc.
- Oxygen, from photosynthesis
- Shade
- Emerald green planet – beauty
- Habitat for birds and other living things
- Food for insects

2. What do plants give us indirectly?

- Gasoline (derived from oil, which is produced by decay of plants over millions of years)
- Food (such as bread, from wheat; meat, from animals eating plants; fish, from fish eating phytoplankton)
- Medicines (the majority of all medicines are derived from plants)
- Removal of carbon dioxide from the atmosphere
- Capture of energy (from the sun) to feed almost the entire biosphere

B. Rubbing alcohol

Rubbing alcohol is a term that refers to various types of solutions, including those that contain 95% ethanol, 70% isopropyl alcohol, or 91% isopropyl alcohol, with the remainder in each case comprised of water as well as trace quantities of bitter-tasting additives to discourage ingestion. For this activity, rubbing alcohol refers to 91% isopropyl alcohol purchased from a local drugstore. While 95% ethanol (sometimes termed “surgical spirit B. P.”) appears equally effective, use of ethanol may not be appropriate for a classroom setting. The results with 70% isopropyl alcohol were adequate but less effective than with 91% rubbing alcohol.

Among all possible solvents, we chose “isopropyl rubbing alcohol” (~91% isopropyl alcohol, ~9% water, and bitter additives) for the following reasons:

- It is sold in most grocery stores, pharmacies, and discount stores.
- It is relatively non-hazardous, particularly compared with many other solvents; diethyl ether (flammable), for example, is used in research labs to extract chlorophyll from plants, but is inappropriate for educational activities in a classroom setting.
- It may be disposed of in a ventilated sink by flushing with excess amounts of water.
- It contains bitter additives to prevent intentional ingestion.

C. Solar Induced Fluorescence

What is SIF? Solar-Induced Fluorescence is the emission of photons by the chlorophyll molecules in plants upon illumination by the sun. “Solar-induced” contrasts the emission with other types of illumination, say by use of lasers in the field to observe fluorescence. Regardless of light source (sun or laser or flashlight), the photons emitted from chlorophyll can be easily observed by fluorimeters (fluorescence detection instruments) that are tuned to the same wavelength. We do not see the stars (other than the sun) during daytime because the light from the sun is so overwhelming. So how is solar-induced fluorescence detected out in the bright light during daytime?

It turns out that the sun emits light at all wavelengths – all colors of the rainbow. But the gases that surround the sun absorb very narrow slices of the rainbow. So, the sunlight reaching

Earth has all colors of the rainbow minus a set of very narrow slices of light that has been filtered out by the gases surrounding the sun. Those slices are referred to as Fraunhofer lines – particular wavelengths where there is no light from the sun. If we were to look for light in one of these narrow slices, we should find none, because none is coming from the sun. But in fact, there is some light in the Fraunhofer lines in the red region, due to the glow of chlorophyll. A satellite with appropriate detectors can peer into the Fraunhofer lines and measure any light that is there. In this manner, the red glow from plants on Earth can be detected in bright sunlight. The more red glow, the more extensive the photosynthesis, and the more abundant the plants.

It warrants mention that maps of SIF can be displayed in a variety of formats. Displays to emphasize the relative intensity of light often use so-called false-color, where different colors convey different intensities. Here, Figure 1 simply uses the observed red at different intensities.

Monitoring the Amazon Rainforest: Rainforests are believed to play a key role in global ecology through the abundant photosynthesis in these warm wet regions. As the world's largest rainforest and home to the greatest variety of living organisms on earth, it is reasonable to monitor the photosynthetic activity of the Amazon ecosystem.

Measuring the wet season: The length of the annual wet season is directly associated with rates of photosynthesis. Extended dry seasons decrease rates of photosynthesis, which can be gauged by analyzing SIF. During the wet season, atmospheric carbon dioxide levels are measurably lessened due to increased rates of carbon fixation and photosynthesis. There is an association between measured SIF and carbon dioxide concentrations, but it is weak and inversely associated; meaning that as carbon dioxide concentrations decrease due to increased photosynthetic activity there will be a corresponding increase in fluorescence (De Sousa *et al.*, 2017, p. 4).

D. Observations from Prior Implementation of This Activity

- Teachers may wish to preface the extraction portion of the demonstration with some background information so that students can understand the activity before they begin the extraction procedure.

- The demonstration portion of this activity is best completed in groups of anywhere from 1–4 students. The opportunity to work in small groups is a necessary condition to promote critical thinking and collaboration reflective of a research environment. When filling out the summative review document, students should be allowed to work together and share the observations that they made in their respective groups. Group size can be increased if obtaining supplies is an obstacle, but individual involvement in each step decreases with every group member added. A collaborative environment is key to allow students to construct explanations and solutions to any obstacles that they might come across (MS-LS1-5).
- This demonstration was carried out in a classroom setting that varied from twenty-three to twenty-eight students. Two instructors, one teacher and one scientist, facilitated the extraction and visualization stages much to the enjoyment of the participants. This can be completed with one teacher.
- Background information on the organization of plant tissue structure and factors contributing to chlorophyll fluorescence was given before students assembled into groups.
- After the observation stage, the significance and possible applications of chlorophyll fluorescence were relayed to the entire class. Students then spent time contemplating the role of chlorophyll in photosynthesis, and reflecting on the significance of what they accomplished.
- Possible extensions of this activity may include students bringing leaves from around their home or school and comparing the visual fluorescence between the leaves they have selected and the spinach provided. Note that all plants contain chlorophyll, and following extraction, the chlorophyll should glow red upon illumination. However, some plants have leaves with tough exteriors, making extraction difficult.
- The chlorophyll solution and/or extract resulting from the demonstration will stain objects a very dark green, so students should use caution to avoid spilling the solution on their clothes and hands.
- Spills most often occur during the grinding and pouring stages of the activity.
- If not enough spinach or other type of leaf tissue is used, red fluorescence may not be so readily observable, as the concentration of chlorophyll may vary.

- If the red fluorescence is not immediately visible following completion of all listed steps, allow the solution to sit in the dark for ten minutes (so any poorly filtered material can settle out) before exciting with a bright flashlight.
- If an ample number of mortars and pestles is not available, teachers may either allow for chlorophyll to be extracted from the leaves on standing over time. Leaves may also be placed in a blender to shred.
- Students sometimes misunderstand that chlorophyll is “turning red”; in fact chlorophyll remains green, but only releases some of the absorbed light energy as red light.

4. Extension 1 – Green Food Coloring as a Negative Control

Experimental controls are necessary to establish baseline values for comparative results of experimentation. A negative control is developed with the intent to ensure that the dependent variable is truly the cause of the experimental results. One classic example is pharmaceutical trials that use sugar pills as a placebo group. The sugar pills do not contain the drug, but the subjects go through the same experimental process. The results from the two (or more) groups are then compared. A single experimental variable, the independent variable, is changed between the two groups. The inclusion of a negative control provides a wonderful opportunity to explain how and why scientific experimentation is seen as significant and trusted. An understanding of science as a process generally follows a clear grasp of the concepts of independent/dependent variables and experimental controls.

In the context of the chlorophyll extraction activity, one suitable negative control uses green food coloring (see Figure S-1). Students may have simply attributed the fluorescence to the fact that the chlorophyll is green. If this is the case, a dropwise addition of green food coloring to a similar volume of rubbing alcohol should create an observationally similar green solution. The apparent “greenness” of the two solutions should be as similar as possible. Green food coloring is not fluorescent, so when the two are compared with a flashlight, it should be apparent that the significance of chlorophyll fluorescence is beyond the presence of a green color. This extension activity may be useful to complete before, after, or alongside the other activities described. As a preceding activity, a negative control may deepen the impact that the initial observation of chlorophyll fluorescence has for each student. Providing the negative control after the chlorophyll

extraction could be effective at dispelling inaccurate conclusions and cementing the significance of chlorophyll and its fluorescence.



Figure S-1. Chlorophyll extract (left-hand side) and green food coloring (right-hand side) in natural light (top across) and with flashlight illumination (bottom across). The green food coloring does not fluoresce; chlorophyll in solution fluoresces strongly.

Materials Supply List for Green Food Coloring as a Negative Control:

- (i) Green food coloring (Fast Green FCF) – avoid contact with skin
- (ii) ~30 mL of rubbing alcohol or water
- (iii) ~25 mL clear container
- (iv) A dark space for visualization
- (v) Sample produced from the Chlorophyll Extraction Activity
- (vi) Bright flashlight

Green Food Coloring as a Negative Control

Observations: Use this space to record your findings as you complete the activity.

Question 1. What is a negative control?

Question 2. What are the observable differences between chlorophyll and the negative control group?

Question 3. How does this information from the negative control pertain to SIF?

5. Extension 2 – Fluorescent Materials Activity

What constitutes fluorescence? Fluorescence is not the reflection of light, but the absorption and subsequent emission of light. In general, the light absorbed is at a shorter wavelength and the light emitted is at a longer wavelength. Students can find examples of this fluorescent behavior in other classroom and household objects as well. Objects such as highlighters display fluorescent behavior. Other products that exploit the principle of fluorescence include high visibility vests and tapes.

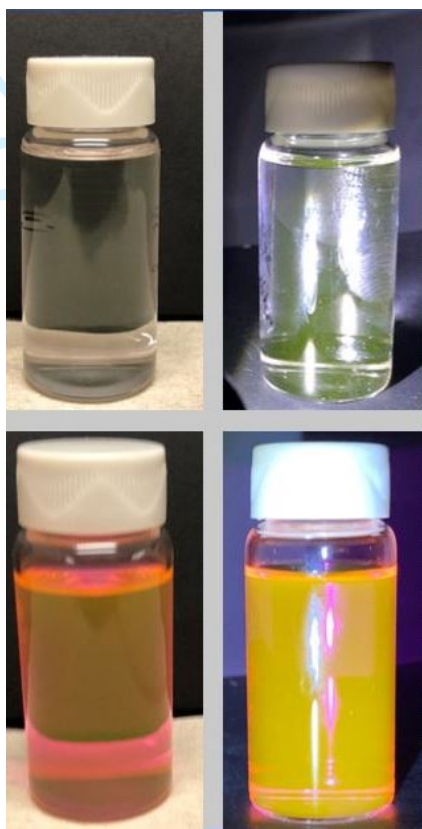


Figure S-2. Orange highlighter extract solution (top across); pink highlighter extract (bottom across). Pictures on the right-hand side are illuminated in a light box. The pink highlighter solution (bottom right) is highly fluorescent; the orange highlighter solution (top right) is not.

What materials fluoresce under a flashlight? This extension activity examines alternative materials that display fluorescent properties. Highlighters are quite accessible, and most students should be familiar with them while perhaps unaware of their fluorescent properties. Some of these

materials display different properties in their typically used state than when treated with rubbing alcohol in a similar manner to the chlorophyll extraction experiment. For this extension the highlighters selected should fluoresce with light from a handheld flashlight (see Figure S-2). The fluorescence displayed in a few of the highlighter colors is not the same as that of chlorophyll. Pink and orange highlighters contain fluorescent rhodamine dyes. The tips of most highlighters can be easily removed and placed in rubbing alcohol to create a solution. The pink highlighter shown here produced a pink solution that emitted an intense yellow fluorescence when excited. The orange highlighter solution was clear in natural light and produced a green fluorescent glow upon excitation.

Materials Supply List for the Fluorescent Materials Activity:

- (i) Pink and/or orange highlighters
- (ii) Rubbing alcohol
- (iii) Multiple clear containers
- (iv) A dark space for visualization
- (v) A bright flashlight

Fluorescent Materials Activity

Observations. Use this space to record your findings throughout the activity.

Question 1. What are the advantages of using fluorescent materials in everyday objects?

Question 2. What are the observable differences between the fluorescence in the highlighters and the fluorescence seen in chlorophyll?

Question 3. If you could make one item fluorescent for practical reasons, what would it be and why?

6. Extension 3 – An Additional Leaf

Students were asked to bring leaves, or were provided with leaves other than spinach. When working with the additional leaves, students took additional data on leaf texture, the ease of tearing, and the color in comparison to spinach. In some cases, the additional leaf (e.g., holly leaves from outside the school) were hard, more difficult to tear, and not as green. This information was taken into consideration when extracting chlorophyll, as students had to use more force when grinding. When the additional leaf suspension was illuminated, students noticed that it did not glow the same deep red as the spinach. This observation led students to dive deeper into the reasoning. They discussed how all plants have chlorophyll, but the structure of the holly leaf must be slightly different. Students were able to conclude that since a holly leaf is tougher to tear and has adapted to the harsh outdoor environment it takes more force to break down the leaf structure to extract the chlorophyll. As a class, students then discussed the importance of adaptations in the natural world. It warrants emphasis that the difficulty in extraction of chlorophyll reflects the texture of the sheath of the leaf, not the nature of the photosynthetic process or the red glow from the chlorophyll in the leaf.

7. Everything Light Box

Simply turning off the lights in your classroom may not be enough to effectively visualize the chlorophyll fluorescence in the activity. To deliver the best results for the visualization stages for all of the previously described activities, an inexpensive homemade lightbox can be constructed from materials that are readily available (see Figure S-3). A medium-sized cardboard box should suffice, but ensure that your box is an appropriate size for whatever containers hold your resulting solutions. Using a ruler, measure the sides of your box. It is recommended that the interior of the box is measured. If desired, the interior of your box can be coated with a matte black paper. This can be cut from a larger piece of black poster paper or from individual 8.5" x 11" pieces of construction paper. Painting the interior is also an option, but paper requires no drying time and requires less space to complete. At least two flaps should be cut into the box. The first flap will be on the horizontal facing side that is closest to the viewer. Cut a square, but make sure that the bottom portion of the square is not cut in order to make a flap that folds down. Corrugated cardboard boxes allow the flap to stay shut and maintain shape. Another flap can be cut open on

the vertical side of the box. Keep this flap to a size that will allow students to be able to see through while minimizing the amount of ambient light let into the box. The final step is to mount your high intensity flashlight in a way that does not point the light source in the direct path of the eyes. This can be quickly done with tape before sealing the top of the box, or through the front-facing flap.



Figure S-3. Two light box models of varying size and modifications are shown. Any box with an adequately spacious and dark interior should suffice.

Materials Supply List for the Light Box

- (i) Medium sized cardboard box
- (ii) Matte black poster board or construction paper (optional)
- (iii) Ruler
- (iv) Scissors
- (v) Tape and/or glue
- (vi) Bright flashlight