Lesson Plan: Fluorescence

Background

Fluorescence is produced when a material or substance absorbs light of a given color and then gives off light of another color. The light that is given off, or *emitted*, is called fluorescence. Because the process of absorbing and re-emitting light causes energy to be lost, the resulting fluorescence has a lower energy than the absorbed light. The emitted fluorescence is also a different color than the absorbed light, since different colors of light correspond to different energies. Many animals, including fish, jellyfish, corals, butterflies and birds, contain substances or molecules that cause them to fluoresce different colors. Plants and minerals also have the ability to fluoresce. In many cases, the fluorescence can be observed under ultraviolet (UV) or blue light – different organisms or materials absorb UV or blue light and emit green, yellow, orange or red light. After the exciting light is turned off, the fluorescence is no longer observed. Many plants contain chlorophylls, which not only make the plants appear green when observed in natural sunlight, but cause them to fluoresce orange or red under UV or blue light.

Fluorescence is used by scientists to detect different molecules that they are interested in studying further. For example, DNA can be detected by its binding to compounds that are brightly fluorescent under UV light. Fluorescent proteins from jellyfish and corals have been isolated by scientists and fused to other proteins, so the proteins can be identified in cells by their fluorescence. This has made observing the proteins in cells much easier than before, and has led to important findings about the location of specific proteins in cells and insights into how the proteins work.

Fluorescence is also widely used in everyday life for many different purposes – for example, it is used in banknotes as a security measure to discourage counterfeiting, in safety signs and clothing to increase visibility, and in detergents and paper to make them appear whiter.

This lesson plan demonstrates that some materials absorb light and emit light of a different color, and are therefore *fluorescent*. It also shows that fluorescence only lasts as long as the fluorescent material is exposed to the exciting light.

Objectives & Grade Level

Demonstrate fluorescence produced by everyday objects and biological materials. Show that fluorescence differs in color from the exciting light and that fluorescence is only produced when the exciting light is on. Appropriate for middle school to advanced high school science classes; see notes for advanced students.

Materials & Equipment

- Fluorescent highlighter or marker (e.g., Bic Bright Liner, Luxor or Sharpie Fluorescent Highlighter)
  Nonfluorescent marker of the same color (e.g., some Sharpie Accent Highlighters)
  Other fluorescent substances can also be used, e.g., Petroleum jelly, Tonic water, Vitamin B2, US Banknotes
- Transparent plastic sheets
- Green plant leaves
- Mortar and pestle
  A small bowl and spoon can be substituted
- Ethanol (70 to 100%, ~3-5 *ml*)

Rubbing alcohol (70% isopropanol) can be used instead of Ethanol

- Ultraviolet (UV) or Black light lamp, White light lamp (e.g., small LED lamp)
- UV Safety glasses, Disposable gloves, Pasteur pipette, Plastic tube with cap (or Plastic bag)

Procedure

1. Fluorescence is produced when light is absorbed by a substance or material and light of a different color is given off. To demonstrate fluorescence, we will compare ink from two highlighters or markers, one of which is fluorescent and the other is not. We used a fluorescent and a nonfluorescent yellow marker. First, using each of the two markers, draw a square on a transparent plastic sheet and fill in each square with the marker.

2. To test for fluorescence, shine light from a small white light lamp onto the two squares of yellow, noting the color of the region onto which the light is directed (**Fig. 1A**). The "white" light from the lamp consists of light of many different colors, which appear colorless when all the colors are combined. Notice a difference in color of the two squares where the white light is focused – the region in the square made by the nonfluorescent marker appears yellow, but the region in the square made by the fluorescent marker appears green (**Fig. 1A**).

Yellow fluorescent markers usually contain low amounts of a fluorescent dye called pyranine mixed with other dyes to make the ink appear yellow. Pyranine makes up only a small percentage of the ink, usually less than 5%. It absorbs UV or blue light and emits green fluorescence. The green fluorescence can be observed by shining white light onto the square of yellow fluorescent ink – the yellow ink absorbs the blue light contained in the white light given off by the lamp and fluoresces green. The green fluorescence from the square made by the fluorescent yellow marker can be seen when viewed using the focused white light from a small LED lamp, even in a well lighted room.

3. To further test the fluorescence of the markers, **put on a pair of UV safety glasses** and use a UV or black light to view the two squares of yellow ink. Notice the color of the light that is given off by each square.

CAUTION! Be sure to use UV safety glasses when viewing UV light! *UV light consists of a spectrum of wavelengths, some of which can damage your eyes and skin. Although most wavelengths of UV light are not visible to the human eye, UV lamp housings often contain a filter that makes the light appear purple or black.*

Is there a difference between the squares of yellow made by the two markers when viewed under UV light?

Again, you should notice a difference between the two squares when viewed under UV light – the nonfluorescent square appears dark and the fluorescent square fluoresces green (**Fig. 1B**). This is the same color that the fluorescent square fluoresced under the white light from the small lamp, shown in **Fig. 1A**. *Note that the green fluorescence is a different color than the invisible or colorless UV light that you are shining on the fluorescent yellow square. The pyranine dye in the fluorescent yellow ink is absorbing the colorless UV light and fluorescing green. This difference in color of the fluorescence compared to the absorbed or exciting light is a hallmark of fluorescence (see **Advanced topics 1 and 2**).*

*Now turn off the UV light. Can you still see the green fluorescence? The fluorescence stops when the exciting UV light is turned off – this is another hallmark of fluorescence that makes it different from other forms of emitted light, e.g., phosphorescence (see **Note 3**).*

4. Chlorophylls, the molecules that give plants their green color, are biological molecules that fluoresce. The fluorescence will be easy to observe if you extract the chlorophylls from the leaves of a green plant. This can be done using a mortar and pestle (a small bowl and spoon can be substituted) and a small volume of ethanol or isopropanol. Add one or two leaves to the mortar or bowl (we used two medium-sized spinach leaves) and about a teaspoon (4-5 *ml*) of ethanol or isopropanol (**Fig. 2A**). Make a pulp from the leaves using a pestle or spoon. Chlorophylls are soluble in ethanol and isopropanol, and can be extracted from green leaves by breaking the cells open so they release their contents. After you make a pulp from the leaves (**Fig. 2B**), transfer the mixture to a plastic tube (a small plastic bag can be used instead) using a pipette or spoon. *Notice the color of the solution under room light – it appears green, like the leaves from which it was made.*

CAUTION! Be sure to put on your UV goggles! Now view the tube with UV light (**Fig. 2 C**). Notice the color of the solution under the UV light – *what color does it appear? Why do you think the color is different than when the tube is viewed in room light? How is the color of the fluorescence related to that of the UV light that you used to produce the fluorescence ?*

The chlorophyll fluorescence appears red, as shown in **Fig. 2C**. Red is a longer wavelength of light than the colorless UV light the chlorophylls absorb. This agrees with the Stokes shift that others have observed for chlorophylls (see **Advanced topic 1**), since the red fluorescence is longer wavelength than the UV light absorbed by the chlorophylls.

The color of the fluorescence observed for different fluorescent substances is a property of the molecules they contain that absorb light and emit fluorescence. The pyranine dye in the fluorescent yellow marker and the chlorophylls in the spinach leaves are very different molecules with different light-absorbing and light-emitting properties – because of this, their fluorescence properties differ, including the color of fluorescence they emit.

Notes

1. **Advanced topic 1**: different colors of light are due to the *wavelength* of light – the colors of light that people can see form a rainbow of colors starting with dark purple, then blue, green and yellow, and finally orange and red. The colors have corresponding wavelengths that range from around 400 *nm* to 700 *nm*, where a *nm* is equal to 10^{-9} *m*. Fluorescent compounds or substances absorb light of a given wavelength and emit fluorescence that is longer wavelength. For example, the pyranine dye in the fluorescent yellow marker absorbs UV and blue light with three peaks of absorption (λ_{abs}=370 *nm*, 400 *nm*, 460 *nm*) and emits green fluorescence (λ_{em}=510 *nm*). The chlorophylls extracted from spinach leaves are a mixture of closely related forms that absorb UV or blue light and emit red or orange fluorescence. The difference in wavelength between the light absorbed by a fluorescent compound and the light it re-emits, or its fluorescence, is known as the ***Stokes shift***. The Stokes shift for a given fluorescent compound is specific to that compound – in the case of pyranine, exciting the dye with the blue light contained in white light or by UV light will produce the same color fluorescence. For pyranine, the Stokes shifts are 50-140 *nm* for the three absorption peaks, and for the spinach chlorophylls, the Stokes shifts are ~200-240 *nm* for different forms of chlorophyll. For both pyranine and chlorophylls, the wavelength of the fluorescence is longer than the wavelengths of the absorbed light.

2. **Advanced topic 2**: the *energy of light* of different colors is inversely related to its wavelength, and is given as

$$E = hc/\lambda$$

where

E is the energy of a photon or quantum of light
h is the Planck constant or ratio of the energy of a quantum of light to its frequency, 6.626×10^{-34} *J·s*
c is the speed of light in a vacuum, 299,792,458 *m/s*, and
λ is the wavelength of the photon of light

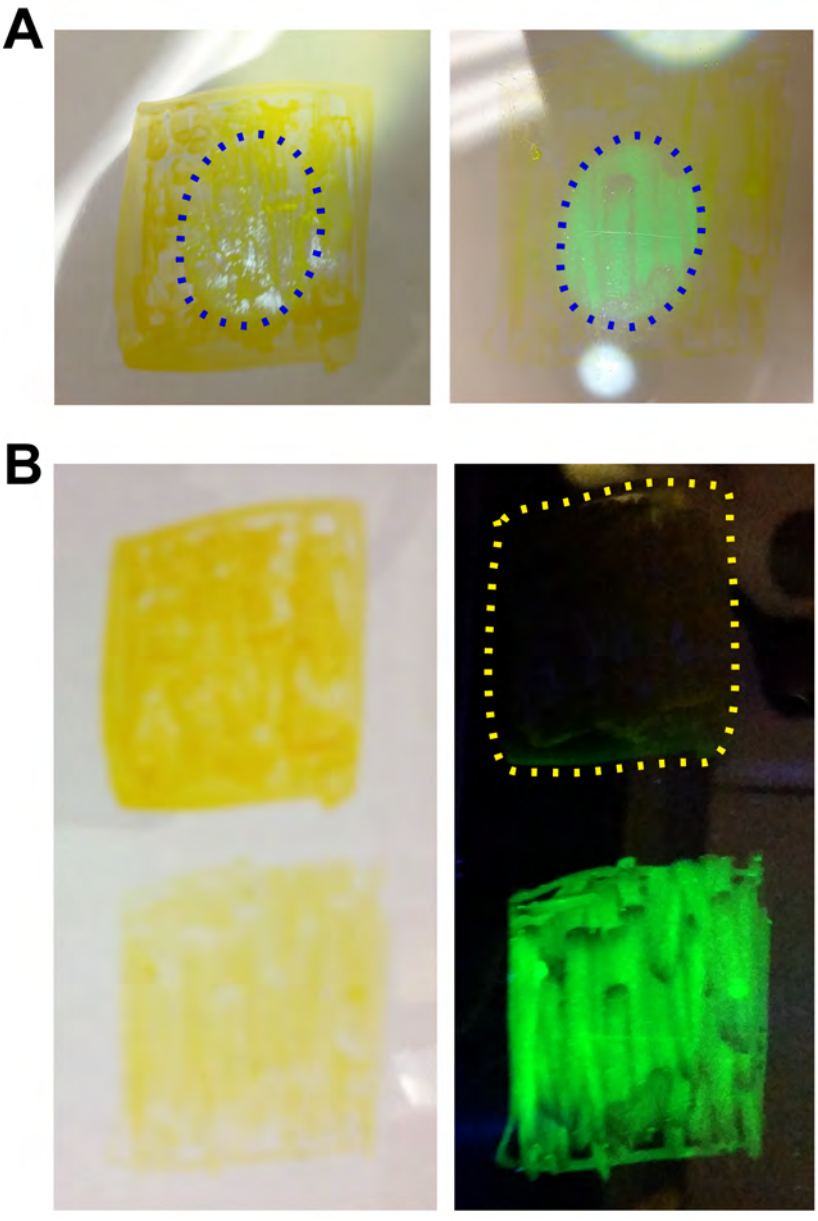
From this equation, you can see that the energy of light decreases as the wavelength increases. This means that Stokes shifts, which are the differences between absorbed light of lower wavelength and fluorescence of higher wavelength, are from absorbed light of higher energy to re-emitted light of lower energy.

3. *Phosphorescence* is similar to fluorescence and is often mistaken for fluorescence. Phosphorescent substances absorb different wavelengths of light, like fluorescent substances. However, instead of immediately re-emitting light of a different wavelength, phosphorescent substances re-emit the absorbed light over a longer period of time, which can last for minutes or even hours. Phosphorescent substances also continue to emit light, even after no longer being exposed to the exciting light. By contrast, when a fluorescent material is exposed to exciting light, it immediately begins to fluoresce, and once it is no longer exposed to the exciting light, it stops emitting fluorescence.

4. A common use of fluorescence that affects you every day is security strips in currency: the USA and other countries use fluorescent materials in their banknotes to make it more difficult for forgers to produce counterfeit bills. Security strips in newer US banknotes can be detected under UV light and differ in color (and position) with the denomination, e.g., the security strips in $5 bills fluoresce blue and those in $10 bills fluoresce red. Interestingly, higher denomination banknotes show no direct correlation between the denomination and wavelength of fluorescence – the strips in $20 bills fluoresce green, and those in $50 and $100 bills fluoresce yellow and pink, respectively.

Figures

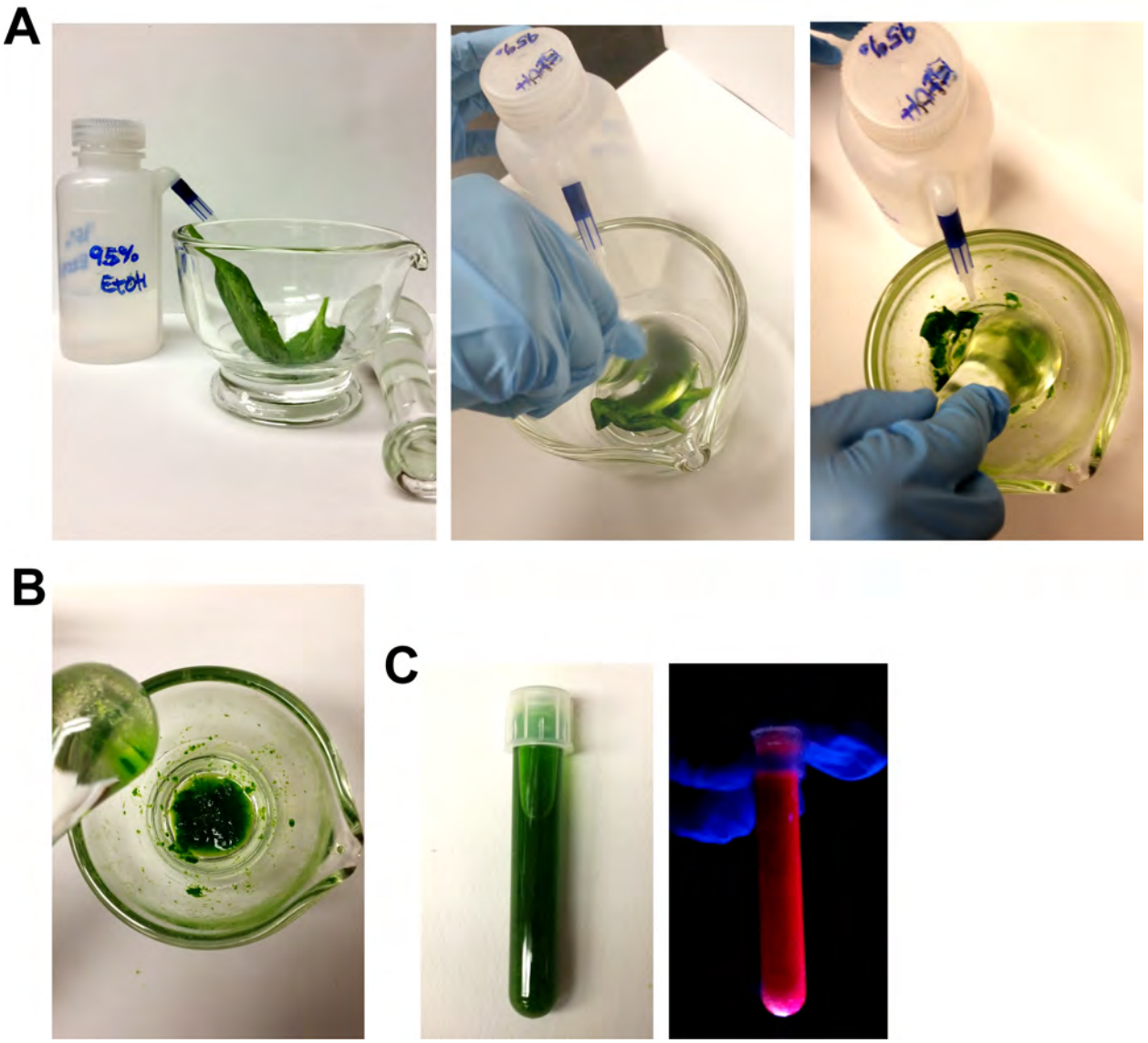
Figure 1 Observing Fluorescence Using Highlighters or Markers



A) Squares of yellow were drawn on a transparent plastic sheet using a nonfluorescent and a fluorescent highlighter or marker. The squares were then held under a white LED lamp that was focused onto a region of each yellow square (dashed blue line). The illuminated region is yellow for the square made by the nonfluorescent marker (left), but it fluoresces green for the square made by the fluorescent marker (right).

B) The same two yellow squares were observed under normal room light or UV light. The square made by the nonfluorescent yellow marker appears yellow under room light (top, left) and does not fluoresce under UV light (top, right; yellow dashed line). The square made by the fluorescent marker appears yellow under room light (bottom, left) and fluoresces green under UV light (bottom, right). When the UV light was turned off, the fluorescent square at the bottom right stopped fluorescing green. This demonstrates an important property of fluorescence – fluorescence is emitted immediately after the exciting light is absorbed, so it is only observed when the exciting light is on.

Figure 2 Observing Fluorescence Produced by Chlorophylls Extracted from Plant Leaves



A) Two medium-sized spinach leaves were placed in a mortar (left), 4-5 *ml* of 95% ethanol were added and a pestle was used to mix (middle) and crush the leaves (right). **B)** Final mixture of extracted chlorophylls. **C)** After transferring to a plastic tube, the extracted chlorophylls were observed under room light (left) where they appeared dark green, like the starting spinach leaves. When the extracted chlorophylls were observed under UV light (right), they fluoresced red.

References

Causes of Color https://en.wikipedia.org/wiki/Fluorescence

Fluorescence Wikipedia https://en.wikipedia.org/wiki/Fluorescence

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