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Educational Resources: Homemade Fluorometer

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Homemade Fluorometer

Abstract from the initial JCE Classroom Activity (1): "This activity highlights fundamental aspects of fluorescence phenomena using a homemade fluorometer constructed from items readily available in the home. The activity investigates the fluorescence of highlighter marker ink using a flashlight or white LED as a light source, colored cellophane sheets as filters, and the human eye as a detector. The activity relates to the concepts of excitation and emission, Stokes shift, and the dependence of fluorescence on the intensity of light."

Related Indiana state standards

SEPS.1 Posing questions (for science) and defining problems (for engineering)
SEPS.3 Constructing and performing investigations
SEPS.6 Constructing explanations (for science) and designing solutions (for engineering)
3-5.E.3 Construct and perform fair investigations in which variables are controlled and failure points are considered to identify aspects of a model or prototype that can be improved.
6.PS.4 Investigate the properties of light, sound, and other energy waves and how they are reflected, absorbed, and transmitted through materials and space.
6-8.E.1 Identify the criteria and constraints of a design to ensure a successful solution, taking into account relevant scientific principles and potential impacts on people and the natural environment that may limit possible solutions.
7.ESS.1 Identify and investigate the properties of minerals. Identify and classify a variety of rocks based on physical characteristics from their origin, and explain how they are related using the rock cycle. (i.e. Sedimentary, igneous, and metamorphic rocks)

- Mineral connect through the discussion section because fluorescing under short wave or long wave UV light is a physical characteristic of quite a few minerals.

Main Activity

Supplies

- One cardboard box for each fluorometer
    - Boxes down to the size of a tea box work if you have small enough sample containers. Smaller sample containers/boxes do need smaller slits to reduce the extra light.
    - For using a small box such as a tea box: using a small box and a test tube can allow you to only make one slit. Create a slit for light to enter in the side of the box, around 1-1.5 inches from the bottom, and cut a hole in the top of the box for the test tube to poke through. Color the area below the test tube black using a marker. Students can look down the test tube to see if the solution fluoresces with less construction.
- Some sort of Exacto knife, box cutter, or scissors to cut slits in the sides of the boxes
- Tape (to seal the boxes)
    - The initial activity instructions state to seal the box completely and cut a hole to add and remove the sample from the top. We tested a couple homemade fluorometers where we simply left the box lids loose and held them shut if necessary while testing and it was perfectly functional. Sealing the lid with tape is not necessary.
- Rulers (for measuring slit location/size)
- Light sources in multiple colors (white, blue, and at least one red, green, and/or yellow)

    - LED lights work best, as they have the greatest spectrum. White incandescent does not work as well because it does not have a lot of light in the blue spectrum.
    - If you have access to colored LED flashlights, those work the best for colored lights.
    - Colored and white LED Christmas lights also work well
    - If you only have access to white LEDs, colored filters can be used to create colored light
        - If you happen to have actual colored lenses, they work well if they don't allow any white light through
        - The original activity suggests using multiple layers of colored cellophane to create homemade filters, which we did not test
        - We did test using colored water as a filter, which worked. Add 3 drops of food coloring to approx. 100mL water to make a heavily colored solution, then pour it into some clear-sided container approx. 1-1.5 inches wide. Place this in front of the entrance slit outside of the box.
- A yellow highlighter (this did not permanently ruin the highlighter in our tests)
    - Despite the activity instructions, especially if using darker filters/dimmer lights/a smaller sample container, dip the highlighter in the water until it is relatively concentrated.
    - In our tests, we used a Sharpie brand highlighter.
    - If you don't have access to a fluorescent highlighter or want a more concentrated water solution, Fluorescein (Na salt) can also be dissolved in water to create a bright solution.
        - Warning: Fluorescein should not go down the drain or be handled without gloves. If the solution gets on skin, rinse it immediately under water
- Coffee filter (optional)
    - We did not filter the highlighter solution and it did not appear to have any particulate matter that interfered with results.
- Sample container
    - Some sort of clear container shorter than the box
        - When testing, we used a juice glass for the larger fluorometer and a short glass cylinder for the other
        - Other sample container options include cuvettes or test tubes (with some sort of test tube holder in the fluorometer)
            - Small test tubes work well with a small box such as a tea box, because then a small hole can be cut from the top and the test tube can slide in and out.
            - The test tube does stick out from the top, so it needs covered with a hand or some sort of tube
- Distilled water
    - All of our tests in lab were conducted using DI water as we didn't have access to distilled water.
- 1 400 mL beaker (for preparing the highlighter solution) and 2 250mL beakers (for step 7 demonstrating light passing through the solution from the top)
    - Depending on the number and size of your sample containers, you may need more than 400 mL of highlighter solution. The juice glass held around 100 mL, while the short tube in the tea box only needed around 5-10 mL. Sample containers also only need filled to a little above the level of the entrance and emission slits.

Pre-activity questions

- What can you think of that's fluorescent (other than highlighters)?
  - Different plastics, possibly someone's shoelaces/shirt, some sticky notes.
- What color do you think this solution will fluoresce? Why?
  - A probably guess is yellow for the yellow highlighter

Discussion topics/topics to know

- Fluorescence
  - In grades below high school they likely have not discussed this
  - For younger grades, fluorescence can partially be described as "glowing with the lights on"
  - For older grades, the term "phosphorescence" can also be introduced and compared
    - Glow in the dark vs neon
  - In general, comparing solid objects that fluoresce can work as a good introduction to the idea.
    - See if any students have neon shoe laces, pencil bag, or shirt and demonstrate how it fluoresces if possible.
  - Fun Fact: Fluorescence was actually named by the scientist G. G. Stokes after the mineral fluorspar, now known as Fluorite, which fluoresces under UV light (aka black light) (3)
- How colors and wavelengths of light work in relation to energy
  - Can be developed with the idea of blue/red shifts for more advanced groups
  - If the students haven't discussed light yet, include a quick intro that purple moves the fastest and red moves the slowest
- Energy transfer/electromagnetic radiation
- If the class is discussing minerals (standard 7.ESS.1), talk about how some minerals fluoresce under UV light and identification qualities of that
- [Biology application] With photos of samples using fluorescent dyes/from a florescence microscope, talk about how this can determine identity or be used as a dye to target specific areas.
  - Encourage the class to talk about how easy it is to see fluorescence and how contrast makes identity easier
  - Can connect to talking about the parts of a cell

Instructions available from the *Journal of Chemical Education,* DOI: 10.1021/ed084p1312A, article "Fluorescent Fun: Using a Homemade Fluorometer" (1)
As a note, when testing in lab, steps 7 & 8 did not give clear and conclusive results.

Post-activity questions

- Why do we view the emitted light from the fluorescence at a 90 degree angle? Why not from beside the light source or at a 180 degree angle? (paraphrase from the original activity)
  - Looking at a 90 degree angle means that you don't see the light from the light source, while looking from beside the flashlight may make you see a reflects (and the light source blocks it), while viewing straight across just shows you the excitation light.
- [If the students have already learned about wavelengths and energy] How is the excitation light's wavelength related to the emitted fluorescence?
  - The excitation light wavelength is always shorter than the emitted fluorescence.

- How could this fluorometer be improved? Or if you could design your own fluorometer with no limit to supplies, what would you add?
    - Possibly less light entering and escaping (seal it better), black inside, better light sources, more or less concentrated solutions.
- How could the fluorescence be quantified? What kind of detector could be used?
    - A detector made for light. A camera could work to compare with the RGB values, or there is a piece called a photomultiplier tube that senses light.
- Other substances also fluoresce, including some whiteners in laundry detergent, certain plastics, some minerals, and the spice turmeric. Why would a scientist want to know if something fluoresces? How can fluorescent materials be used?
    - Fluorescence can identify materials or stains. Fluorescent materials can also tag substances to make them more visible, similar to the function of highlighters.

Optional additions beyond the original activity

One or more of these extensions can be added on to make the activity longer, more engaging, and/or more grade appropriate. None of this content came from the original “Fluorescent Fun: Using a Homemade Fluorometer” (1).

More fluorescent items

- Turmeric also fluoresces with white and blue light (2)
    - The powder mildly fluoresces orange at close range.
    - Turmeric is mildly insoluble in water, so the solution should be made with rubbing alcohol and filtered.
    - Despite what “Fluorescence using Turmeric” says, we did not have any success with changing fluorescence by changing the water to alcohol ratio in the solution or changing the solution’s pH. Turmeric does work as an indicator, changing to red in basic solutions (above a 7.4) (2).
        - Vinegar and diluted ammonia were used as the acidic and basic solutions, as those are simple to procure. The vinegar solution may have fluoresced more red, but it also became cloudy and therefore reflected parts of the flashlight beam. The ammonia solution also may have fluoresced more red, but it was difficult to distinguish whether red light came from fluorescence or the solution itself filtering the emitted light.
- Laundry bluing (used in most detergents) makes white shirts glow. With a UV lamp, a white shirt appears to fluoresce blue.
    - This also connects with the idea that higher-energy light is required for fluorescence. Red light doesn’t cause the fluorescence to appear.
- Other colors of highlighters can also be compared.
    - We tried green, blue, pink, and orange. The orange resisted bleeding out into water (we had more luck with a 50/50 water/alcohol mix) but was mildly fluorescent orange. It fluoresced with light from blue through yellow (so only orange and red light don’t cause fluorescence). Pink had very mild pink fluorescence on paper under blue light. Both blue and green did not fluoresce.
        - Orange was an unknown brand, and blue, green, and pink were all BIC Brite Liner. In addition, we tried a pink Sharpie standard highlighter with the same results.

        - Sharpie brand green highlighter does fluoresce the same color as Sharpie-brand yellow. Sharpie orange highlighter uses the same chemical and therefore does not fluoresce orange.
    - We also tried a pink Sharpie Gel highlighter with interesting results
        - It dissolved off of paper in 70% ethyl rubbing alcohol
            - There was mild diffusion in water, but it wouldn't fully dissolve and the solution ended up cloudy either due to residue in the beaker or a reaction.
            - Without paper, just scraping a few shavings off of the end into rubbing alcohol works.
        - The solution appeared a pale pink but fluoresced green (the same as yellow highlighter), implying that they both use the same fluorescent chemical.
- Rhodamine B is a pink solution that fluoresces orange with blue-yellow light
    - Demonstrates fluorescence well as it fluoresces orange no matter the color of the light.
    - This works as a nice contrast with yellow highlighter/fluorescein because Rhodamine B fluoresces with green and yellow light, where the other doesn't.
    - A quick demonstration of how fluorescence works can also happen here.
        - Don't show the color of the blue flashlight and shine it at the liquid. It will appear to glow orange.
        - Have students guess the color of the light. They will probably guess orange, or possibly white.
        - Show the blue flashlight and explain how fluorescence doesn't depend on the excitation frequency.
            - You can also add an orange filter to the blue light and show how the light then becomes green, showing that the Rhodamine didn't filter the light.
    - Warning: This should not be poured down the drain in large quantities or touched without gloves

Sticky Note Fun (Secret Messages and Searching)

Standard bright pink, bright orange, and yellow-green sticky notes all fluoresce under blue light. This can be used for a variety of activities. (Make sure to test the sticky notes to make sure they fluoresce before planning this activity.) Both activities fit in with a forensics theme, as they can both talk about how fluorescence is helpful for identification and heightening contrast/finding a substance.

- Secret messages
    - When dried, it is practically impossible to see yellow highlighter marks on yellow or orange stick notes. However, under blue light, the contrast is great enough that the message appears!
    - This does not work with a standard orange highlighter and orange sticky notes, as the orange has enough ink to show up.
- Searching
    - Sticky notes can be hidden around the classroom or given to students. Then, with the lights out, students can use blue flashlights to find the fluorescing notes. The goal can either be to match a student's note with another, or find the most.

Compare and Contrast - Identification and Forensics

Multiple different solutions can be made to show how fluorescence can be used for identification. This can also pair well with paper chromatography and ink analysis.

Background

This can be framed as a forensic investigation comparing which highlighter was used on an assignment left at a crime scene. One person claimed to use green highlighter, while someone else mixed blue and yellow. Here we have the "control" set of yellow and blue, and the testing set of green vs blue/yellow. The students are now investigators trying to get the ink from paper to analyze if it fluoresces.

Time: 10-20 min

Supplies

- Multiple sets of 1 yellow, 1 green, and 1 blue water-based highlighter
  - Only the yellow should fluoresce under blue light
  - Most highlighters are water-based – if you aren't sure, place a dot on a piece of paper and get it wet.
  - WARNING: Green Sharpie-brand highlighter fluoresces the same color as yellow. The experiment could be modified to compare 2 green highlighters for that, but don't use green sharpie-brand highlighter with the original activity.
- 4 approx. 1x2 inch pieces of notebook paper per group
  - Thin paper towel can be used as a substitute.
- 4 100mL beakers per group
  - These could be replaced by plastic disposable cups if needed.
- 1 blue LED light per group (can technically be shared)

Pre-activity questions:

- Do you think the blue/yellow mix will act more like the blue highlighter or the yellow highlighter?
  - It's going to end up acting like the yellow but look more like the blue, but any answer here.
- Which solutions do you think will fluoresce? Why?
  - Only the blue/yellow and yellow will fluoresce, but any answer here.

Instructions

- For each set of pieces of paper, cover both sides with highlighter – 1 yellow, 1 green, 1 blue, and 1 blue covered in yellow
  - This can be done before the activity if there is a time limit
  - Diffusion does occur faster with wet highlighter, but can occur with dry.
- Fill the 4 beakers with water
- Place the pieces of paper each in separate beakers with water and let them sit
  - After about 5 minutes, the ink should have mostly diffused into the water
  - If it doesn't appear to have done anything, try reaching in and stirring the paper around. Often, the ink will be sitting just above the surface of the paper and not be noticeable until the paper is moved/water is disturbed.
- Have students guess which ones will fluoresce and, if the students don't know, have them predict which of the two green solutions is a mixture of blue and yellow
- Turn the lights out and shine the blue light on the solutions. The blue/yellow mix and the yellow solutions should look identical, while the blue and green solutions don't fluoresce.

Post-activity questions:

- Did anything surprise you about the results?
  - Possibly that neither blue nor green fluoresced, or that the blue/yellow and yellow both looked the same under blue light.
- Other substances also fluoresce, including some whiteners in laundry detergent, certain plastics, some minerals, "neon" clothing, and the spice turmeric. How would fluorescence help forensic scientists?
  - It can detect and identify substances at a crime scene.

Possible Addition

- You can also add a "fake" yellow solution through a yellow water-based marker, food coloring, or a mixture of ink to approximate the color of the highlighter water. Comparing the two yellow solutions can be the goal instead of comparing blue/yellow vs green, or it can just add another layer.
- A permanent marker solution can also be used to attain a good green color. This marker will only dissolve in alcohol, so either that paper can be placed in alcohol, or it can serve as a "dud" for the students.

Connections to Engineering

- Depending on age levels, to encourage creativity and work toward engineering standards, students can build their own fluorometer using available supplies to either try and improve the design or completely work independently
  - If students are told to design their own, explain the important parts first and possibly show an example of a fluorometer that's not made from cardboard
- A more complex spectrofluorometer can also be built using more supplies, as seen in "Fluorescence Spectroscopy in a Shoebox" (5)
- A related activity for more computer-minded students or more quantifiable data is using 3D printed fluorometers (4)
  - Students can use the software to build their own or they can be pre-printed

Sources

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