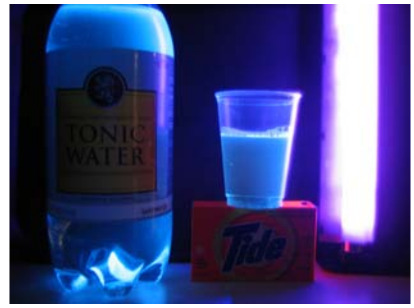
Glow up



You light up my life.

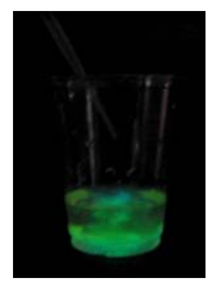
Different kinds of light can be used to study life.

Materials and Preparation
luminol
fluorescein
bleach
NaOH
clear beakers or cups
black light or UV LED
dropper or disposable pipet
(optional) tonic water, laundry detergent, extra virgin olive oil, green laser

You can get the reagents at a chemical supply house like Flinn Scientific. Black lights are available at party stores.

To make the solutions:
Solution A: 0.1 g luminol, 20 ml 2.5M NaOH (to help the luminol dissolve) in 1 L H_2O
Solution B: 0.1 g fluorescein in 1 L H_2O
20% bleach: 200 ml bleach, 800 ml H_2O

To do and notice



1. Prepare 3 solutions to be tested:
   1) 30 ml solution A; 2) 30 ml solution B; 3) 30 ml A + 30 ml B
   Notice how each solution looks underneath the room lights.

2. Turn off the lights, and use the dropper to add 5 drops of the diluted bleach solution to each container. Observe what happens in each case.

3. Take a look at all of the solutions underneath the black light. If you have tonic water or liquid laundry detergent, look at them under the UV light as well.

4. (optional) Shine a green laser through a bottle of olive oil. What color is the beam?

What's going on?

You may have noticed that solution A had a blue glow with the addition of bleach and solution B should not glow at all. The mixture of solutions A and B should have had a greenish glow when bleach was added. The types of light you see in this activity are examples of chemiluminescence and fluorescence. Detection of these two light sources serve as the basis for the majority of studies in molecular and cell biology.

Chemiluminescence is nonthermal, visible light that is produced as the result of a chemical reaction. A molecule in the reaction is excited to a higher energy state and then releases a photon as it returns to its ground state. This is distinct from thermal light that is generated by excess heat energy in a combustion reaction. When bleach is added to a solution containing luminol, an oxidation reaction occurs, and electrons in the luminol are excited to a higher energy state. As they return to their ground state, they release the energy in the form of a photon. The wavelength of the photon corresponds to the blue light that you see. When all the electrons have returned to their ground state, the mixture should stop glowing. When chemiluminescence happens in a living organism, it is called **bioluminescence**. A classic example of this is the light produced by fireflies when a luciferase enzyme catalyzes the oxidation of luciferin, another molecule produced by fireflies. The gene for luciferase can be genetically engineered into other organisms so that they too can produce light in the presence of luciferin. Since the production of light is readily quantified by modern cameras, the luciferase system is a commonly used by biologists as a reporter assay.

Fluorescence is luminescence that occurs when a molecule is excited by absorption of light energy and then emits a photon as it returns to its ground state. During this process, some energy is lost to heat or molecular motion, so the wavelength of the emission photon is longer than the wavelength of the excitation photon. This happens almost instantaneously; so fluorescence is usually only seen in the presence of the excitation light. Fluorescein is a fluorescent molecule that absorbs UV light and emits green light. It is not chemiluminescent, so the addition of bleach to solution B does not result in any light generation. However, when solution A is present, the blue light emitted by the chemiluminescence of luminol is close enough to the excitation wavelength of fluorescein that the entire solution glows green, since the fluorescein absorbs the light from the luminol reaction and re-emits it in the green range. Other examples of fluorescent molecules are quinine in tonic water and the optical whiteners present in many laundry detergents. Both of these absorb UV light and emit in the blue range. Fluorescent molecules can be excited by other wavelengths as well. Organic molecules present in olive oil fluoresce red when excited by green light. Green fluorescent protein (GFP) is a naturally occurring molecule in jellyfish that fluoresces green under UV light. Fluorescent molecules such as GFP and fluorescein are routinely attached (genetically or chemically) to biological molecules to assist in detection and monitoring *in vitro* and *in vivo*. Different lasers can supply light at specific wavelengths, so a large number of molecules can be simultaneously assayed.

References

"Oxidations of Luminol" from *Chemical Demonstrations: A Handbook for Teachers of Chemistry Vol. 1* by Bassam Z. Shakhashiri (1992)

"Methods" from *The Molecular Biology of the Cell* by Bruce Alberts, et. al. (2007)
available FREE online at: http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.part.1502

These pictures are better in color. Check them out at: www.exo.net/~jyu/activities/glowup.pdf