Shining a Light on Evidence

by Jackie Smith, 2022 CTI Fellow
William Amos Hough High School

This curriculum unit is recommended for:
Honors Forensic Science, Advanced Forensic Science

Keywords: infrared light, electromagnetic spectrum, evidence, visible light, spectroscopy, blood, gunshot residue, ink alterations, Beer's Law, toxicology, document analysis, bloodstain analysis, infrared photography

Teaching Standards: See **Appendix 1** for teaching standards addressed in this unit.

Synopsis: This unit looks at the visible and infrared sections of the electromagnetic spectrum and their use in detecting latent evidence at crime scenes and testing it in the lab. Visible light is used in a type of spectroscopy which can identify an unknown substance and determine its concentration. Infrared light can be used to visualize hard to see evidence like blood and gunshot residue on dark colored clothing. Further, that evidence can be photographed with an IR sensitive camera and preserved for later investigative use. After discussing the science of light, this curriculum unit introduces the reader to several labs that can be done in the high school classroom to reinforce these concepts.

I plan to teach this unit during the coming year to 150 students in Honors and Advanced Forensic Science classes.

Shining a Light on Evidence

Jackie Smith

Introduction

Rationale

Forensic Science has captured people's imaginations in recent years and provides a great opportunity to tie together so much of what students have learned in high school. Forensics incorporates material from biology, chemistry, physics, earth science, algebra, trigonometry and even civics. It gets students excited about science and encourages critical thinking and the development of problem-solving skills.

Several units in Honors Forensics and Advanced Forensics, the first- and second-year courses offered, focus on the use of light to detect and document evidence as well as to analyze it. Drugs can be identified, and their concentrations determined using ultraviolet/visible light spectroscopy. Document forgeries and gunshot residue can be detected using infrared light. Additional latent evidence, or evidence that is not visible to the naked eye, such as body fluids can be found at crime scenes with the aid of ultraviolet light. These are the parts of the electromagnetic spectrum most important to forensic science. This paper will focus on the use of visible light in spectroscopy and infrared light in photography for the detection and analysis of evidence.

Demographics

William Amos Hough High School is a large suburban high school of over 2500 students located in the small town of Cornelius, North Carolina just north of Charlotte. We opened our doors in 2010 to serve the northern part of the Charlotte-Mecklenburg School District. Eighty-four percent of our graduates go on to either two- or four-year colleges while 16% join the military. Twenty-six percent of our students are minorities and 18% are free or reduced lunch students. Honors Forensic Science classes averaged 30 students during the 2022-2023 school year. The Advanced Forensic Science class was slightly larger with 35 students this year.

Hough offers a comprehensive college preparatory program in the arts and sciences. Classes are taught at the Standard and Honors levels, and 26 Advanced Placement courses are offered in conjunction with the College Board. Students may explore their interest in the sciences through electives. Hough offers Honors Forensic Science and Honors Advanced Forensic Science to meet that need. With the overwhelming popularity of forensics in pop culture, these courses grab students' interest while teaching them valuable lab and critical thinking skills. The first-level course covers many of the basic areas of forensics such as crime scene processing, DNA analysis, blood spatter analysis, footprint analysis and toxicology. The second-level course builds on some of the basic materials but extends them further and includes new topics such as Forensic Botany, Accident Reconstruction, Counterfeiting and Art Forgery. Honors Forensic Science is a prerequisite for Advanced Forensic Science. This curriculum unit has components geared

towards each class. It will examine the use of infrared light in locating and documenting latent evidence at crime scenes and analyzing evidence using visible light in the lab.

Unit Goals

This unit will introduce students to light spectroscopy, where discrete wavelengths of light are passed through a substance and the amount of light absorbed by the material is measured. A plot of the wavelength versus the absorbance gives a unique spectrum for every substance. Students will be introduced to Beer's Law which relates the absorbance to the concentration of the solution. They will learn how to use a calibration curve to determine concentration. The labs associated with these concepts will also reinforce the need for students to be precise in their measurements to obtain meaningful results, something students tend to get careless about.

Students will study the properties of infrared light and how it can be used to visualize otherwise invisible evidence. They will experiment with their cell phones and an IR filter to attempt to detect blood on dark clothing and ink alterations. Finally, they will work with their cell phones and a filter to attempt to photograph gunshot residue with infrared light also on dark clothing. They will come away from these activities with a greater understanding of light and its value in forensic evidence detection and analysis.

Content Research

The Electromagnetic Spectrum

Visible light is one small part of the electromagnetic spectrum (EMS). The EMS is all known forms of electromagnetic radiation arranged by wavelength.[1] For forensic purposes, the most important parts of the EMS are visible light, infrared radiation and ultraviolet radiation. These different forms of electromagnetic radiation have characteristic properties such as visibility/invisibility, wavelength, color and frequency.[2]

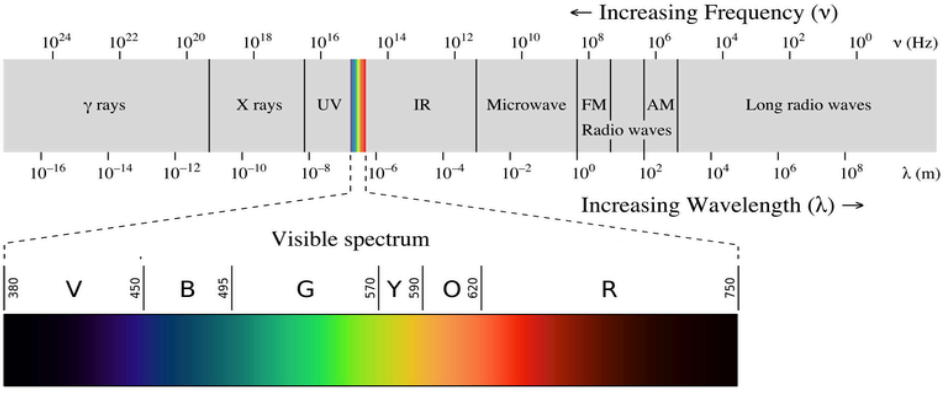

Figure 1. The Electromagnetic Spectrum.
By Philip Ronan, Gringer [CC BY-SA 3.0 (https://creativecommons.org/ licenses/by-sa/3.0)], via Wikimedia Commons

[1] "Alternate Light Source Imaging | ScienceDirect," 2.
[2] "Alternate Light Source Imaging | ScienceDirect," 2.

All light travels in waves. A wave is simply the movement of energy. Figure 2 below illustrates the different parts of a wave.

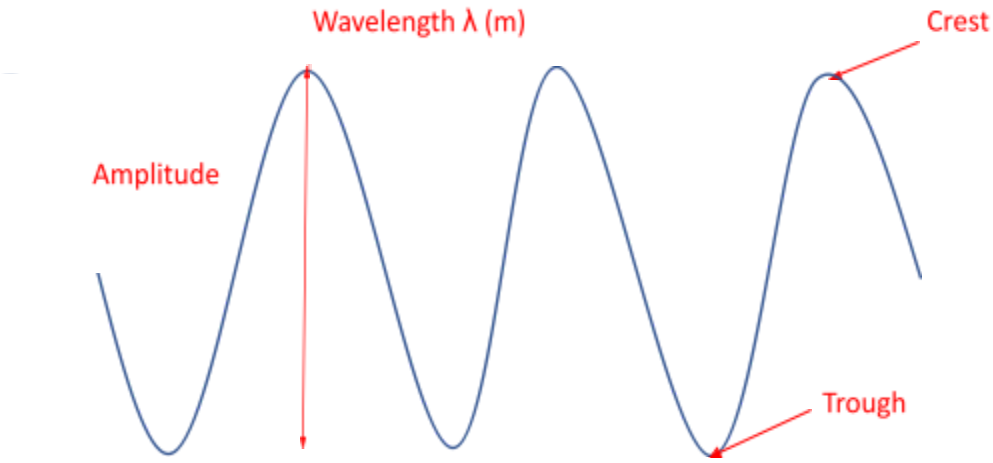

Figure 2. Parts of a Wave.

The distance between the same spot on two successive waves is called the wavelength λ and is measured in meters.[3] Nanometers are commonly used for scale. A nanometer is one billionth of a meter (that's nine zeros!) Blue light has a wavelength of approximately 450 nm. For comparison, human DNA has a diameter of 2.5 nm and an atom has a diameter of about 0.15 nm. Waves travel at different speeds in different media. The speed of a light wave, c, in a vacuum is approximately 3.0 x10^8 m/s.[4] The frequency of a wave, ν, is the number of waves that pass a given point in a unit of time.[5] For example, at the beach, the ocean waves may pass a pier at the rate of 40 waves per minute. The metric unit of frequency is cycles (number of waves) per second or the hertz (Hz). Waves with longer wavelengths will have lower frequencies because it takes them longer to pass the given point (See Figure 3.)

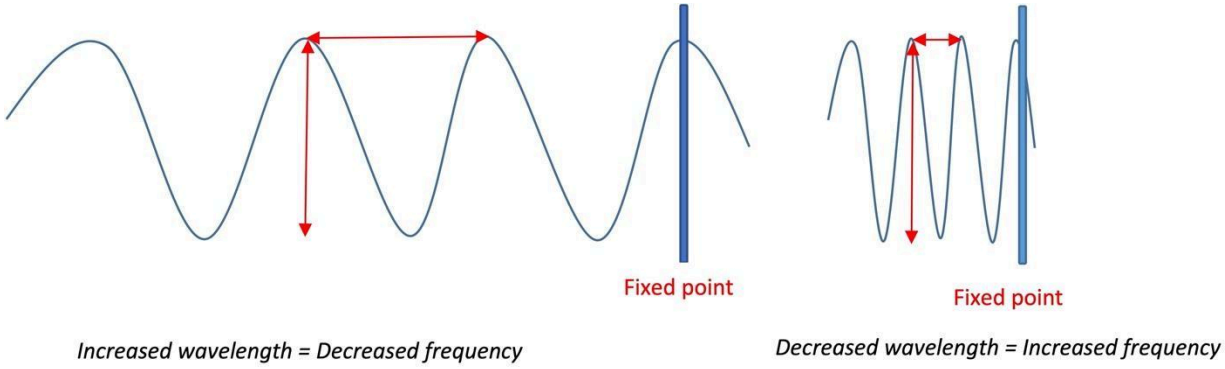

Figure 3. Frequency is inversely proportional to wavelength.

[3] "Alternate Light Source Imaging | ScienceDirect," 4.
[4] "Alternate Light Source Imaging | ScienceDirect," 4.
[5] "Alternate Light Source Imaging | ScienceDirect," 5.

Wavelength, wave speed and frequency are related by the formula $c = \lambda\nu$, where c is the speed of the wave, λ is the wavelength and ν is the frequency.[6] The amplitude of a wave is the height of the wave from the trough to the crest. The amplitude is a measure of the energy of the wave. Notice in Figure 3 although the wavelength changes quite a bit, the amplitude, or energy, of the wave does not change at all. Amplitude is independent of changes to wavelength, speed or frequency.[7]

Ultraviolet light falls on the EMS just above x-ray radiation beginning around 10 nm and extending to the visible portion of the spectrum at approximately 380 nm. UV light is invisible to the human eye but has many uses including disinfecting food.[8] It is used extensively in forensics to detect and analyze evidence. Visible light falls between 380 nm and approximately 750 nm and can be broken down into the basic color range remembered as ROY G BIV. The figure below gives approximate wavelengths for each visible color.

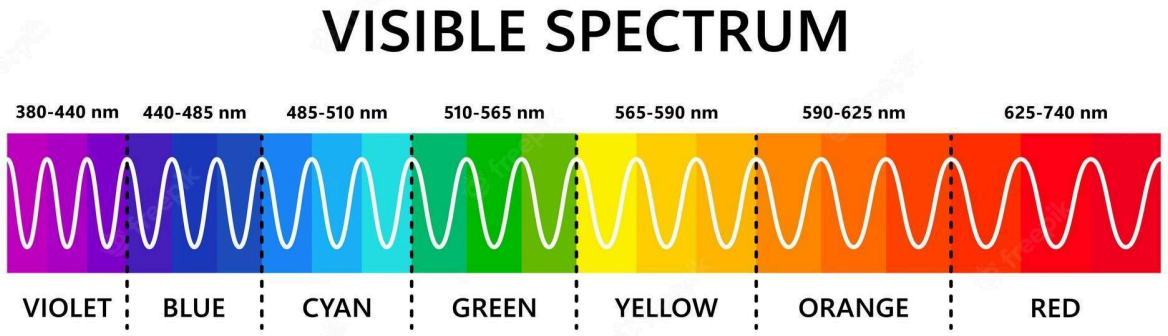

Figure 4. Wavelengths of Visible Light in nanometers.
Image in the Public Domain.

Adjacent to visible light on the EMS in the longer wavelengths is infrared light. It runs from about 700 nm to 1 mm. The part most useful for detecting and analyzing evidence in forensics is called near-IR and goes up to about 2500 nm.

While light travels like a wave, it also acts like a particle in the way it transfers energy to electrons. Every element has a defining number of electrons. Electrons gain and lose energy as photons, which are packets of light energy.[9] As these electrons gain energy, they move to a higher orbital. When they lose energy, the electron drops back down to its original orbital and the lost energy is released as a photon of light. Different elements emit this light at different energy levels causing each element to have its own unique emission spectrum. This principle is one basis for identifying unknown substances in forensics laboratories. As seen in Figure 5, when the electron in a hydrogen atom loses energy and drops down to its original orbital, it releases photons at very discrete wavelengths of light that produce a characteristic spectrum for hydrogen.

[6] "Alternate Light Source Imaging | ScienceDirect," 6.
[7] Forensic Use of Light, Texas Education Agency.
[8] Forensic Use of Light, Texas Education Agency.
[9] Forensic Use of Light, Texas Education Agency, 4.

Figure 5. Emission Spectrum of Hydrogen
Image in the Public Domain.

Use of Visible Light

UV/Vis Spectrometry uses light in the ultraviolet and visible ranges. The amount of absorbance of the light as it is passed through the substance is measured producing a unique spectrum, or graph, for the material. The concentration of a substance can also be determined using the same technique and applying Beer's Law which states that there is a linear relationship between the concentration of a substance and its absorption. A more concentrated solution will absorb more light and a more dilute solution will absorb less light.[10] A plot of absorbance versus concentration of a solution, called a Beer's Law plot, will be a straight line with a y-intercept of zero. The slope of the line is equal to the molar extinction coefficient times the distance the light traveled through the substance. The distance is just the width of the cuvette used in the spectrometer. The molar extinction coefficient is a measure of how strongly a substance absorbs light at a particular wavelength and is a property of each substance. The formula is written:

$$A = \varepsilon Lc$$

where A is absorbance (which is unitless), ε is the molar extinction coefficient (in $M^{-1}cm^{-1}$), L is the length the light traveled (in cm) and c is the concentration of the substance (M).

In the toxicology lab, this principle is used to determine the identity and concentration of unknown substances recovered from crime scenes. First the spectrum of the unknown sample is taken and digitally compared to a library of knowns to determine the makeup of the substance. The absorbance of a solution increases with the concentration of the absorbing dye. A graph of absorbance versus concentration of dye is a straight line. (See Figure 6.) Here, the maximum absorbance of a substance was determined to occur at 531 nm, so four dilutions of the substance (20%, 40%, 60% and 80%) were run through the spectrometer at 531 nm. The data is below.

Concentration	Absorbance (at 520 nm)
0.20	0.106
0.40	0.214
0.60	0.326
0.80	0.431

Table 1. Concentration vs. Absorbance data for a substance at 531 nm.

A plot of concentration vs. absorbance was obtained and is below. This is called a calibration curve.

[10] Illustrated Glossary of Organic Chemistry. Chem.ucla.edu/`harding/IGOC/B/beers_law.html#:1text=Beer's%20Law%20(Beer-Lambert%20Law)%3A%20The%20amount%20of%20energy,a%20more%20dilute%20solution%20does. Accessed 9/9/2022.

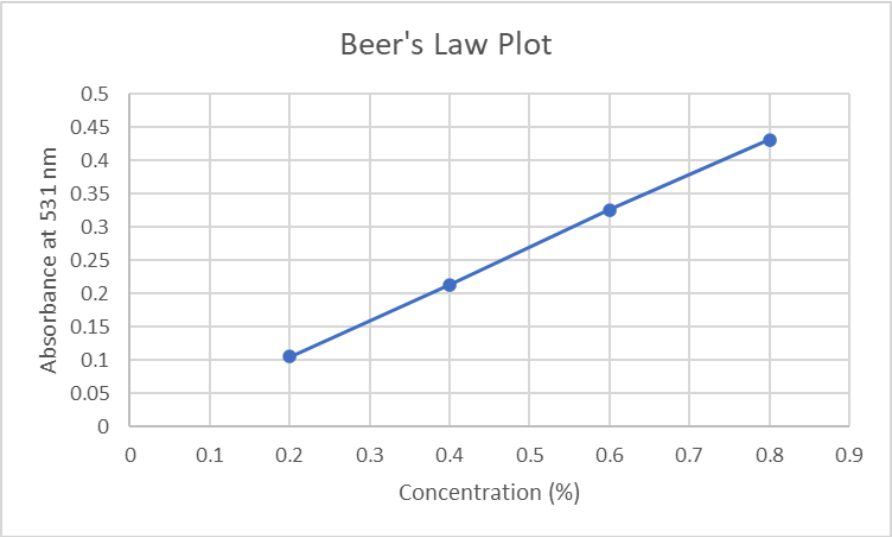

Figure 6. Beer's Law plot of test substance data.

The slope of this straight line was calculated using the formula rise/run or $(y_2 - y_1)/(x_2 - x_1)$ or (0.8-0.2)/(0.431-0.106) = 0.6/.325 = 1.085. The slope divided by the width of the cuvette used (L = 1 cm) gives the molar extinction coefficient, ε. The absorption of the solution of unknown concentration is determined (at the same wavelength) and Beer's Law can then be used to calculate the concentration of the unknown using the formula: A = εLc and solving for concentration, c:

$$A(\text{unknown}) = \varepsilon Lc$$
$$c = A/\varepsilon L$$
$$c = .262/(1.085*1)$$
$$c = 0.262/1.085$$
$$c = .241$$

Notice in Figure 6, that the absorption values used in the calibration curve were obtained at a wavelength of 531 nm, which was the maximum absorbance wavelength for the sample. For optimum accuracy, calibration data are obtained at a wavelength where the absorbance is at a maximum.

Use of Infrared Light

Infrared light is energy on the electromagnetic spectrum with wavelengths from 750 nm to 1 mm. Figure 7 shows the breakdown of IR light into the near-IR, thermal and far-IR ranges. Near-IR is the most useful in forensic detection and analysis although thermal imaging has been used to locate suspects hiding from police, including the Boston Marathon bomber.

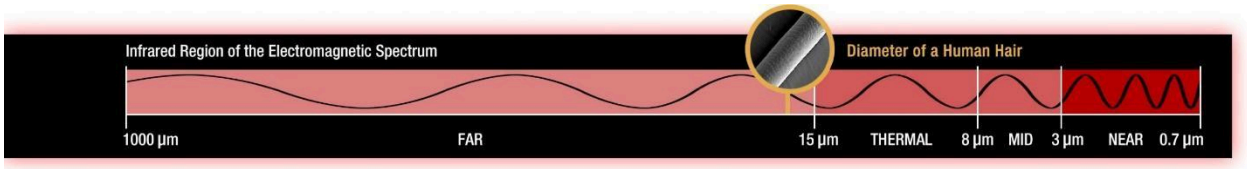

Figure 7. The infrared part of the EM spectrum.
Image from Science Mission Directorate. "Infrared Waves" NASA Science. 2010. National Aeronautics and Space Administration. (13 Sept 2022) http://science.nasa.gov/ems/07_infraredwaves.

IR light is so useful because it penetrates materials further than other types of electromagnetic radiation with less scattering and absorption of the light. This allows scientists to "see" through objects. One current use of IR technology is on the James Webb Space Telescope which is sending back amazing pictures of distant galaxies as IR images. A more common use of IR light that everyone has experience with is pointing the television remote at the TV and changing the channel. What often appears as a red light at the end of the remote is a beam of IR radiation sending a signal to the receptor in the television to change frequencies.

When a molecule absorbs infrared light, its chemical bonds vibrate. Different molecules vibrate at different frequencies because their structures are different. This is how materials are distinguished using IR spectroscopy. Every molecule has a characteristic spectrum often referred to as the "fingerprint" of the substance.[11] This technique is valuable in forensics because it requires a very small sample size and is non-destructive of the evidence.

Another growing application of light in the infrared range is the use of IR photography at crime scenes. Substances such as blood and gunshot residue (GSR) are very difficult to visualize and document on dark-colored backgrounds. When illuminated with an IR light source, the contrast with the background becomes much more obvious and the evidence can be photographed *in situ* without having to move or alter any of the sample. Bloodstains absorb light with a wavelength greater than 830 nm, while many dark fabrics used in clothing reflect this light. This enhances the contrast between the blood and the background making it available to photograph.

When illuminating a potential area of GSR with IR light, the GSR also absorbs the light causing it to appear darker and the background to appear lighter. This allows the crime scene technician to photograph the GSR at the crime scene allowing for very quick analysis of the events of the crime. This in turn allows detectives to follow up on leads much more quickly, possibly while the suspect is still in the area. The patterns that can be visualized can tell investigators the distance between the discharged firearm and the target as well as the direction and sometimes sequence of the shots fired. Figure 8 shows pictures taken in normal light and then with IR light. The difference in detail is amazing. The dark fabric was shot with a 9mm pistol at a distance of three inches. In the second picture, the fabric was visualized with an infrared light source and photographed with a camera altered to detect IR light. The GSR pattern

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11 https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Vibrational_Spectroscopy/Infrared_Spectroscopy/Infrared%3A_Interpretation. Accessed 9/14/2022.

is much more visible and a ballistics expert could use this information to determine the distance the shooter was from the victim at the time of the shot from the dispersion of the GSR.

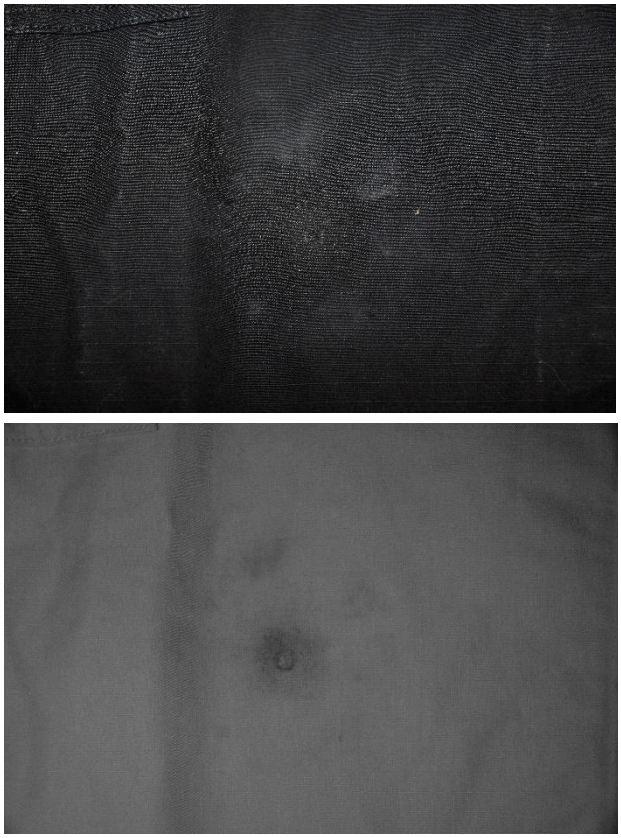


Figure 8. At left a visible light image showing GSR on dark fabric. At right, an infrared image of the same GSR.
Images by Allison Spiegel, Charlotte Mecklenburg Police Department. Used with permission.

Infrared photography can also be used to determine whether someone has altered the ink on documents such as checks. If the numbers on a check were written in two different inks, it is possible that one ink will reflect IR light more than the other and this difference will show up in photographs making alterations obvious.

One limitation of IR photography is that visible light will interfere with the images, so it must be done in complete darkness unless one has a camera that has been altered to only "see" IR light. There are companies that will alter your camera to do this, but it can be expensive and your camera can no longer be used to take regular visible light pictures. There are IR filters that can be attached to the front of a regular digital camera, but because the camera already has an internal filter that works to block most IR light, and the IR filter blocks visible light, the camera must be used on a tripod because the exposure time will be very long. Also, because the eye cannot see through an IR filter, the camera has to be focused before the filter is attached.[12]

[12] Vishneski. Introduction to Infrared Photography. photographylife.com/introduction-to-infrared-photography. 4/12/20. Accessed 10/9/22.

Many types of cell phones, such as the Galaxy A32, have the ability to photograph infrared wavelengths of light. You can test your phone by pointing the end of a remote control at the phone and viewing it through the camera app. If you can see the red light, your phone can photograph IR light. You will need to purchase an IR light source. Simple IR flashlights can be obtained on Amazon for less than fifteen dollars. To be able to take IR pictures without a tripod and complete darkness, purchase an IR camera filter. Mine is a 52 mm filter at 720 nm. You can obtain one on Amazon for as little as twenty dollars. I also purchased a filter holder that clips on to my phone to hold the IR filter in place.

Instructional Implementation

These lessons on the use of light in forensics can be brought to the classroom over the course of several days. It may even be better to keep them as mini-lessons in several different units. For example, the use of visible wavelengths of light to identify substances and their concentrations could be discussed during a unit on Toxicology. Mass spectrometry is the gold standard for identifying suspected drugs, but is beyond the reach of most high school laboratories. On the other hand, UV/Vis spectrometry is a realistic method that can be taught in many schools. After an introduction to drugs and perhaps a white powder lab to identify cutting agents, students can be introduced to spectrometry and the way visible light is projected through a solution at discrete wavelengths and the absorbance recorded. This data is graphed and a unique spectrum is obtained for the unknown solution which can then be compared to known spectra to identify the substance. The absorbance at known concentrations of the substance can be obtained and Beer's Law used to calculate the concentration of the unknown solution at its wavelength of maximum absorbance. (See Appendix 2.)

Infrared light can be incorporated into units on blood, document analysis and firearms. Since blood does absorb infrared light, it appears darker than its background when illuminated with IR light. The IR Blood Lab (see Appendix 3) can be done to determine on which fabrics and at what dilutions blood is most visible under infrared light. Different inks can be tested when talking about document analysis and potential forgeries. (See Appendix 4.) Gunshot residue can be visualized on dark clothing with IR light. All three of these phenomena can be documented with a camera that is sensitive to IR light.

Content Lessons - Toxicology

To prepare for this lab, students should be introduced to the electromagnetic spectrum. Visible light should be shown as a small part of the spectrum. Students should become familiar with the properties of light such as wavelength, amplitude and frequency and the relationship between them. Students should then be taught that every substance has a characteristic wavelength to absorbance ratio which can be determined, graphed and compared. Students should understand the property of absorbance to be the amount of light at a particular wavelength that does not pass through a substance and that this absorbance is an identifying property of the substance. Students should then be taught to use a UV/Vis spectrometer. It must be properly calibrated each time the

wavelength is adjusted on the machine. The teacher should prepare two different colors of Kool-Aid, without the sugar. Lemon-lime (green) and grape (purple) will give very distinctive spectra. The teacher will also need to prepare several dilutions of each color of Kool-Aid for the construction of a Beer's Law plot. Dilutions of 10%, 25%, 40% and 60% are suggested. Also prepare a dilution unknown to the students for them to determine concentration from their Beer's Law data. Fifty-five percent is a good target for the unknown. Have students work in groups of four with the spectrometer. Thirty students can use the machine in one 90 minute class if they are properly trained and supervised. Students should graph wavelength versus absorbance for their 100% Kool-Aid sample. This will give them the spectrum of their Kool-Aid. They should also prepare a Beer's Law plot using the dilution data. They should be able to explain the color of their Kool-Aid in terms of absorbance using their spectrum. They should also be able to explain the concentration of the unknown sample using their Beer's Law data.

Content Lesson - Bloodstain Analysis

In this lab, students will learn how to perform a serial dilution. They will then determine which fabric is the best for visualizing latent blood stains with infrared light. Infrared flashlights can be obtained on Amazon relatively inexpensively. The WayLLSHine (E6) 850 nm IT Illuminator flashlight is $14.99 or you can buy a 2-pack for $23.98. The drawback of this light is that in addition to the IR radiation, it shines a red light to let you know the light is turned on. This visible light can interfere with the results obtained. This lab will also work with students using their cell phones that can see IR light. Test for this by pressing the button on a remote control and looking at the end of the remote through the camera lens. If you can see the red light, your phone can "see" IR light.

In addition to an IR light source or an IR camera, the teacher will need to obtain animal blood from the butcher shop or grocery store as well as approximately 0.5 yard samples of 10 different types of fabrics. See Appendix 3 for the fabrics used. The fabrics should all be cut up into one inch by one inch squares. Students will staple five samples of each of the ten types of fabric to a piece of cardboard to form a grid pattern as described in the lab directions. Students will use the animal blood to perform a serial dilution from 100% down to 6.25%, giving them 5 dilutions of blood with which to work. They will use this blood to stain the appropriately labeled fabric samples. The samples should be dried overnight so this lab will take approximately 1.5 class periods to complete. Students will judge the intensity of the bloodstains under IR light on a scale of 0-4, where 0 is not visible and 4 is extremely visible. This can be fairly subjective so student data will vary but they should all have certain fabrics that are easier to visualize blood on than others.

Content Lesson - Document Analysis

A similar lab can be performed using different types of inks. (See Appendix 4) A collection of permanent markers, fountain pens and ballpoint pens should be gathered giving students twelve different inks to test. The ink is tested on three different fabrics: 100% cotton, 100% polyester and a 35/65 cotton/poly blend. The rating scale is much the same as for the blood lab in Appendix 3. Again, students should be able to identify a fabric which allows for the best visualization of the inks.

Content Lesson - Gunshot Residue Analysis

A separate lab is not included for gunshot residue, however if the teacher has access to a pistol and firing range, they can prepare gunshot residue samples to be tested with infrared light and a lab like the Document Analysis lab with the different inks could be set up. Three different black fabric samples should be obtained. A 100% cotton, a poly/cotton blend and one other fabric are suggested. Arrange the fabrics on cardboard backings to give them some rigidity. Shoot each sample with the same weapon from three different distances in three different areas of the fabric far enough apart that the residue patterns won't overlap. Suggested distances are 0-6 inches, 12-18 inches, and 24+ inches. That will leave you with nine samples. Create a code to label each of the shots so the distances are known to the teacher but not to the students.

Students with cell phone cameras that can "see" IR light should form groups with other students. Students can use the phones along with an IR filter over the camera lens to photograph the different areas of gunshot residue, attempting to visualize the residue as best as possible. It should appear darker on a lighter background. This can be done in normal room light conditions or in sunlight. Students should be able to examine the GSR patterns and determine the distance at which the gun was fired based on the residue pattern and amount of residue present.

Appendix 1: Teaching Standards

HS-FS-T-3c Students will be able to compare and contrast chromatography, UV/Vis/IR spectrophotometry and mass spectrometry.

This standard is from the Honors Forensic Science course in the Toxicology unit. Students will learn about the properties of light and how it is used in spectrometers to identify and quantify drug and poison samples. Students will work with a UV/Vis spectrometer to identify a substance and determine its concentration.

HS-AFS-FUL-1 Students understand the properties of light.

- HS-AFS-FUL-1a Students can explain the Dual Theory of Light.
- HS-AFS-FUL-1b Students can explain the electromagnetic spectrum.

This standard is from the Advanced Forensic Science course. It can be used in any of several units. Understanding the basic properties of light is essential to then understanding how to use light in a forensic setting to visualize latent evidence. This material should be review for students at this level.

HS-AFS-FUL-2 Students can describe how light interacts with matter including absorbance, reflection, refraction, and transmission.

The way light and matter interact is the basis of how light is used in forensic investigations. Absorbance and reflection are the basis of all color observations. Each material has a characteristic refractive index by which it can be identified.

HS-AFS-FUL-4 Students understand how light's interactions and reactions aid in forensic science.

- HS-AFS-FUL-4b Students can conduct simple UV/Vis Spectrometry testing.
- HS-AFS-FUL-4e Students can differentiate between different inks used on a document.

Students should be able to synthesize all the material on light to become proficient in the use of light techniques in a forensic setting. Objective 4 touches on some of the many ways light can be used to locate, observe, identify, and quantify evidence from crime scenes.

Appendix 2: Kool-Aid Spectrometry Lab

KOOL AID SPECTROMETRY LAB

There's A Dye In Your Drink

Light

White light, such as sunlight, is composed of light of several different colors. This is observed by passing white light through a spectroscope, Figure 1. The white light is decomposed into its component colors which are displayed as a continuous spectrum.

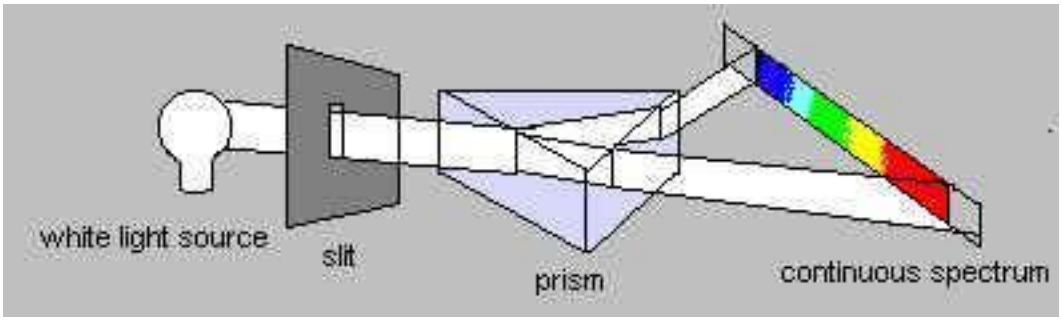


Figure 1
A schematic drawing of a spectroscope, showing the spectrum of white light.

According to the wave model of light, light is energy which travels through space as a wave. The wavelength, λ, of a wave is the distance between two identical points on the wave, Figure 2. The unit of wavelength is the **nanometer, nm.**

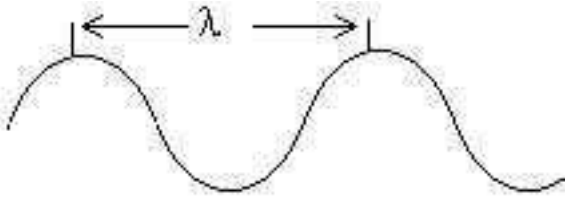


Figure 2

Light of different colors has different wavelengths, as shown in Figure 3.

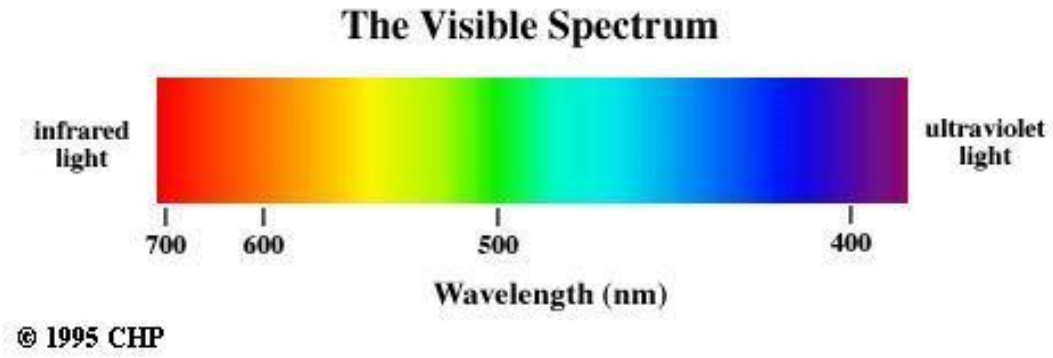


Figure 3.
Continuous spectrum of white light

Color

A colored solution appears colored because molecules in the solution absorb only light of certain colors and not others. Lemon-lime KOOL- AID, for example, appears yellow-green because dye molecules in the solution absorb light of all colors except green and yellow.

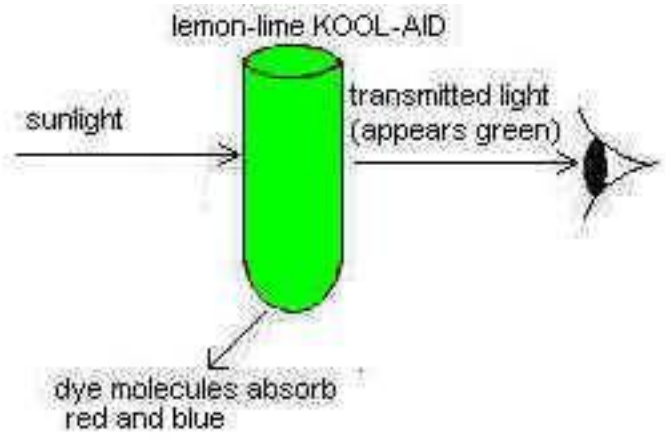


Figure 4.

The amount of light absorbed by the sample is expressed in terms of the **absorbance (A)**. The absorbance increases with the amount of light absorbed. The absorbance depends upon: the wavelength of the incident light, the concentration of the absorbing molecules, and on the sample thickness.

Absorbance depends on wavelength: The dependence of A on 入 for lemon-lime KOOL- AID is shown in Figure 5. A graph of absorbance versus wavelength such as Figure 5, is an **absorption spectrum**. The absorption spectrum explains the color of a substance.

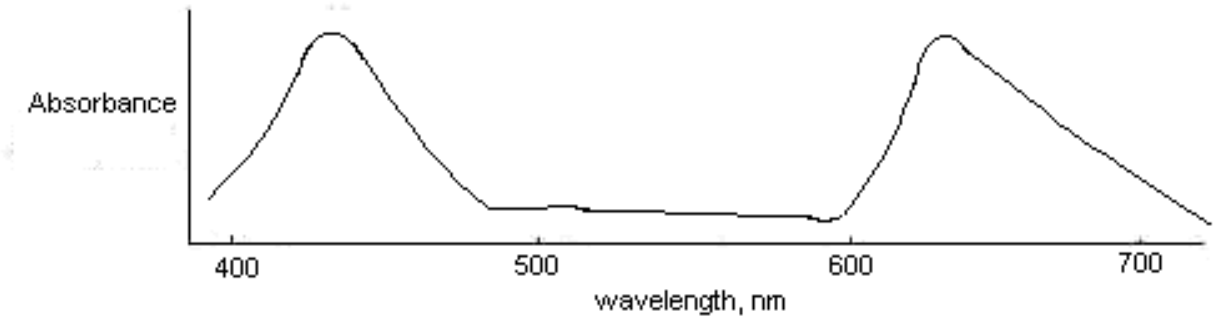


Figure 5.
The absorption spectrum of lemon-lime KOOL AID

In the case of lemon-lime KOOL-AID, maximum absorbance occurs at 440 nm and 630 nm, corresponding to blue and red light, respectively. The absorbance is very low at wavelengths between 500 nm and 600 nm, corresponding to green and yellow light, respectively. Thus the dye molecules in the KOOL- AID absorb red and blue light, but not green and yellow and the drink appears green.

Absorbance and sample thickness: The absorbance of a solution increases with the sample thickness. This is evident if we compare the intensity of transmitted light along the long axis of a pitcher of KOOL- AID with the intensity viewed perpendicular to the pitcher axis.

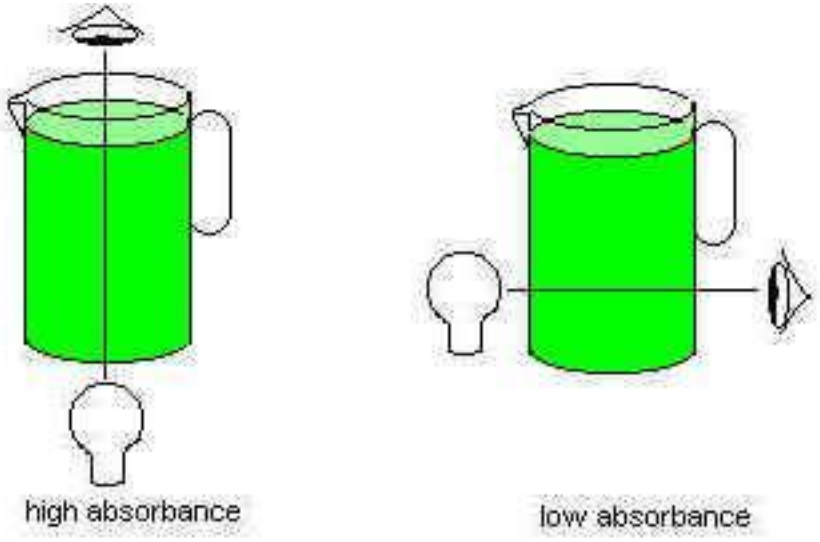


Figure 6.

Absorbance depends on concentration: The absorbance of a solution increases with the concentration of the absorbing dye. A graph of absorbance versus concentration of dye is a straight line, Figure 7. Here, the maximum absorbance of lemon-lime Kool-Aid was determined to occur at 630 nm (see Figure 5), so four dilutions of lemon-lime Kool-Aid (10%, 20%, 30% and 40%) were run through the spectrometer and a plot of concentration vs. absorbance was obtained. This is called a **calibration curve**. The slope of this straight line was calculated using the formula rise/run or $(y_2 - y_1)/(x_2 - x_1)$. The slope divided by the width of the cuvette used (L = 1 cm) gives the molar extinction coefficient, ε. The absorption of the solution of unknown concentration is determined (at the same wavelength) and Beer's Law can then be used to calculate the concentration of the unknown using the formula: A = εLc and solving for concentration, c.

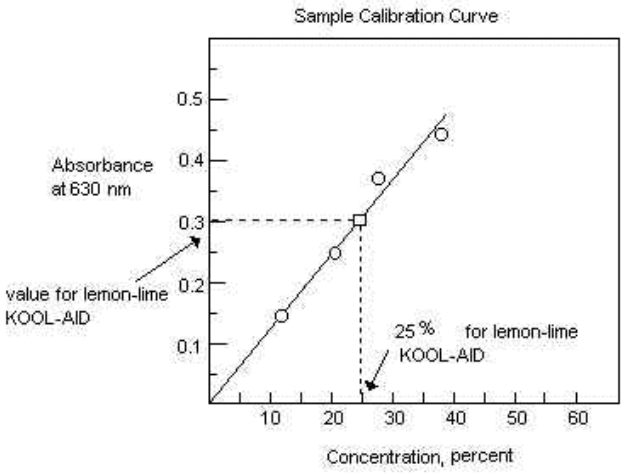


Figure 7

Notice in Figure 7, that the absorption values used in the calibration curve were obtained at a wavelength of 630 nm. Reference to Figure 5 shows that 630 nm corresponds to a maximum absorbance value. For optimum accuracy calibration data are obtained at a wavelength where the absorbance is a maximum. A wavelength of 440 nm would also have been suitable in this case.

Kool Aid Spectroscopy Lab

Purpose: The purpose of this experiment is to obtain the absorption spectrum of a KOOL-AID sample and to use it to explain the color of the KOOL-AID and then to determine if a sample of Kool-Aid has been diluted and to what degree.

Materials:
Spectrometer
2 cuvettes
distilled water
Kool-Aid prepared without the sugar
graph paper
disposable pipettes

Procedure:
Part 1. Measuring the spectrum
Samples of several different flavors of KOOL-AID, prepared according to the instructions on the package, will be available in the lab. Select one. You will only do this experiment with one color of Kool-Aid.

1. Be sure the spectrophotometer is turned on.
2. Set the wavelength knob to 660 nm.
3. Fill one cuvette with distilled water and insert it in the sample compartment with the line facing the front. Close the top.
4. Use the red button to set the transmittance to 100%. The absorption should read 0% with the water-containing cuvette in the holder. Remove the cuvette and set it aside without emptying it.
5. Fill the other cuvette with your KOOL-AID solution.

6. Insert it in the instrument and close the cover. Read the absorbance. Record, in the data table, the wavelength and absorbance readings. Remove the cuvette, close the top and change the wavelength to a setting which is 20 nm lower.
7. Insert the cuvette of distilled water and reset the 0%A.
8. Replace the water cuvette with your sample-containing cuvette and read the absorbance, and again record your results.
9. Repeat steps 3 through 6 until you reach 400 nm.
10. Plot the absorption spectrum of your KOOL-AID sample on graph paper. Your graph should fill the entire page. All graphs should contain a title and appropriate labels on the axes. Include the spectrum in the results section of your lab report.

Kool-Aid Absorbance Data

Wavelength (nm)	Absorbance (%)
660	
640	
620	
600	
580	
560	
540	
520	
500	
480	
460	
440	
420	
400	

Part II. Quantitative Analysis of your KOOL-AID:
calibration curve:

Materials
4 beakers with 10%, 25%, 40% and 60% Kool-Aid solutions
3 beakers with an unknown concentration of Kool-Aid

1. From your graph in Part I, determine the wavelength of maximum absorbance, and set the spectrophotometer to that value.
2. Zero the machine as in Part I.
3. Measure the absorbance of each of the four standard solutions of KOOL-AID. Record the absorbance and the solution concentration (in percent) in your data table.
4. Measure and record the absorbance of an unknown sample of KOOL-AID obtained from the front desk. Be sure to record the unknown identification letter as well. Be sure to use the same color Kool-Aid that you have been using. These unknowns were confiscated in a raid on a KOOL-AID bar in Davidson.

Kool-Aid Absorption Data

Sample	Absorbance (%)
10%	
25%	
40%	
60%	
Unknown (Letter _____)	

5. Graph the absorption at the wavelength you used versus the concentration of your solutions as in Figure 7.
6. Use Beer's Law to determine the concentration of your unknown sample. Be sure to record the letter of your sample. Show your work.

Conclusion

1. **Discuss** the color of your KOOL-AID sample in terms of its absorption spectrum. What colors are absorbed by the sample. What is/are the wavelength(s) of maximum absorbance?
2. **Report** the concentration and identification number of the unknown KOOL-AID sample.
3. **Comment** on the honesty of the proprietors of the KOOL-AID bar.

Appendix 3: Using IR Light to Detect Latent Blood Evidence

Name(s): _____ Date: _____ Block: _____

Using IR Light to Detect Latent Blood Evidence

Purpose In this lab, you will learn how to perform a serial dilution of blood. You will then use the properties of infrared light to determine what fabric is best for detection of latent bloodstains.

Materials Part 1 -
20 mL animal blood in a test tube
4 additional clean test tubes
Test tube holder
5 disposable pipettes
10 mL graduated cylinder
Labeling tape and pen
50 mL distilled water
Gloves
Wooden stirrers

Materials Part 2 -
Cardboard sheet, labeled
White gel pen
5 samples of each of 10 types of fabric
Stapler

Materials Part 3 -
Cardboard with dried blood samples from Day 1
IR light source
IR protective goggles

<u>Day One</u>
Part 1 - Serial Dilution
Procedure:

1. Collect all materials for the blood section of this lab at your workstation.
2. The test tube with the undiluted blood in it should already be labeled 100%. If not, do it now.
3. Using small pieces of labeling tape, label the other test tubes “50%”, “25%”, “12.5%” and “6.25%”.
4. Using a clean disposable pipette and a 10 mL graduated cylinder, remove 10 mL of 100% blood and place in the 50% test tube.
5. Label the pipette 100% and set it aside. Rinse the graduated cylinder.
6. Add 10 mL of distilled water to the 50% test tube. Use a clean stirrer to stir the solution. Discard the stirrer.
7. With a clean pipette, remove 10 mL of the 50% blood and place it in the 25% test tube.
8. Label the pipette 50% and set it aside. Rinse the cylinder.

9. Add 10 mL of distilled water to the 25% test tube. Stir the solution with a clean stirrer. Discard the stirrer.
10. With a clean pipette, remove 10 mL of 25% blood and place it in the 12.5% test tube.
11. Label the pipette 25% and set it aside. Rinse the cylinder.
12. Add 10 mL distilled water to the 12.5% test tube. Use a clean stirrer to stir the solution, then discard the stirrer.
13. With a clean pipette, remove 10 mL of 12.5% blood and place it in the 6.25% test tube.
14. Label the pipette 12.5% and set it aside. Rinse the cylinder.
15. Add 10 mL distilled water to the 6.25% test tube. Use a clean stirrer to stir the solution, then discard the stirrer.
16. Label one additional pipette 6.25% and set aside.

Part 2 - Preparing Blood Evidence (Fabric Samples)

Procedure

1. Obtain 5 pieces of each of the different types of black cloth and a cardboard sheet.
2. Using the white gel pen, as you collect each sample, label each piece with its number below:

Sample Composition	#	**Sample Composition**	#
35% rayon/65% polyester	1	50% acrylic/50%wool	6
35% cotton/65% polyester	2	5% lycra/95% cotton	7
100% cotton	3	5% spandex/95% polyester	8
35% polyester/65% cotton	4	30% polyester/70% rayon	9
100% velvet	5	30% acrylic/70% wool	10

3. Still using the white gel pen, label each of the 5 pieces of #1 with the dilutions 100%, 50%, 25%, 12.5% and 6.25%. Repeat this procedure for each of the 10 types of cloth. When you are finished, your pieces should look like this:

Cloth #	100%	50%	25%	12.5%	6.25%
1	1-100%	1-50%	1-25%	1-12.5%	1-6.25%
2	2-100%	2-50%	2-25%	2-12.5%	2-6.25%
3	3-100%	3-50%	3-25%	3-12.5%	3-6.25%
4	4-100%	4-50%	4-25%	4-12.5%	4-6.25%
5	5-100%	5-50%	5-25%	5-12.5%	5-6.25%

6	6-100%	6-50%	6-25%	6-12.5%	6-6.25%
7	7-100%	7-50%	7-25%	7-12.5%	7-6.25%
8	8-100%	8-50%	8-25%	8-12.5%	8-6.25%
9	9-100%	9-50%	9-25%	9-12.5%	9-6.25%
10	10-100%	10-50%	10-25%	10-12.5%	10-6.25%

4. Staple each of the 50 pieces of cloth to the piece of cardboard in a grid pattern as shown in the table above. Label the grid as in the example.
5. Using the 100% pipette from Part 1, place 2-3 drops of 100% blood on each of the 10 100% pieces of cloth.
6. Using the appropriate pipette from Part 1, repeat this step for each of the dilutions of blood until all 50 pieces of cloth are stained with blood.
7. Wash out all test tubes and pipettes. Return all materials to where you got them.
8. Let the fabric sit until the next class so the bloodstains can dry.

Day 2

Part 3 - Detecting Blood Evidence with IR Radiation

Procedure

1. Obtain an IR light source.
2. Shine the IR light source on each of the pieces of fabric one at a time. Observe the result through the camera app of a phone that can view IR light. In the table below, note the intensity of any blood made visible by the IR light. Use the scale: 0 = not visible, 1 = barely visible, 2 = visible, 3 = good visibility or 4 = excellent visibility.
3. When you are finished collecting your data, deconstruct the cardboard grids without damaging them. Throw away the pieces of cloth. Return all materials to where you got them.

Data Table 1. Intensity of IR Light on Different Dilutions of Blood on Different Types of Fabric.

Fabric #	100%	50%	25%	12.5%	6.25%
1					
2					
3					
4					
5					
6					
7					

8					
9					
10					

Analysis and Conclusions
Answer all questions in narrative form (complete sentences making up coherent paragraphs!).

1. What is IR radiation? How does it work in terms of latent evidence detection?

2. Which fabric(s) best showed the presence of blood at 100% strength? To what dilution could blood still be detected on this fabric? On which fabric(s) was the weakest dilution able to be visualized? What about that fabric made this possible? On the fabrics where you were not able to visualize blood with IR radiation, what possible explanation(s) can there be?

Appendix 4: Using IR Light to Detect Altered Ink Evidence

Name(s): _____ Date: _____ Block: _____

Using IR Light to Detect Altered Ink Evidence

Purpose In this lab, you will test the fluorescence of various types of ink under IR light to determine whether a document was altered.

Materials
Cardboard sheet, labeled
White gel pen
12 samples each of 3 types of fabric (35% polyester/65% cotton, 100% cotton and 100% polyester)
Stapler
4 different permanent markers labeled P-1 through P-4
4 different fountain pens labeled F-1 through F-4
4 different ballpoint pens labeled B-1 through B-4
IR light source
IR protective goggles

Preparing Ink Samples

Procedure

1. Obtain 12 samples of 35% polyester/65% cotton and with the white gel pen, label them "A".
2. Obtain 12 samples of 100% cotton and with the white gel pen, label them "B".
3. Obtain 12 samples of 100% polyester and with the white gel pen, label them "C".
4. Staple the pieces of cloth to a piece of cardboard in a grid pattern as follows:

Table 1. Layout of Ink Sample Cloths.

Pen Type	**Cloth A**	**Cloth B**	**Cloth C**
P-1			
P-2			
P-3			
P-4			
F-1			
F-2			
F-3			
F-4			

B-1			
B-2			
B-3			
B-4			

5. Obtain 4 different permanent markers. They are labeled P-1 through P-4.
6. Obtain 4 different fountain pens. They are labeled F-1 through F-4.
7. Obtain 4 different ballpoint pens. They are labeled B-1 through B-4.
8. With each different pen, write a word or short phrase of your choosing on the appropriate piece of cloth following the grid above.
9. Obtain an IR light source.
10. Shine the light on each of the pieces of fabric individually. View the illuminated fabric through the camera app on a phone that can "see" IR light. Using the following scale, label the intensity of the ink made visible by the IR light in the table above: 0 = not visible, 1 = barely visible, 2 = visible, 3 = good visibility or 4 = excellent visibility.
11. Add up the number of pieces of each type of fabric that had each level of intensity for each of the pen types and record below. For example, if on Fabric A, the permanent markers had intensities of 0, 0, 1, and 2, you would enter under column "Fabric A" a 2 on the Permanent Marker 0 line, a 1 on the 1 line, a 1 on the 2 line and a 0 on the 3 and 4 lines.

Data Table 2. Summary of Ink Intensity Data.

		Fabric A	Fabric B	Fabric C
Intensity of Permanent Markers	0			
	1			
	2			
	3			
	4			
Intensity of Fountain Pen Ink	0			
	1			
	2			
	3			
	4			

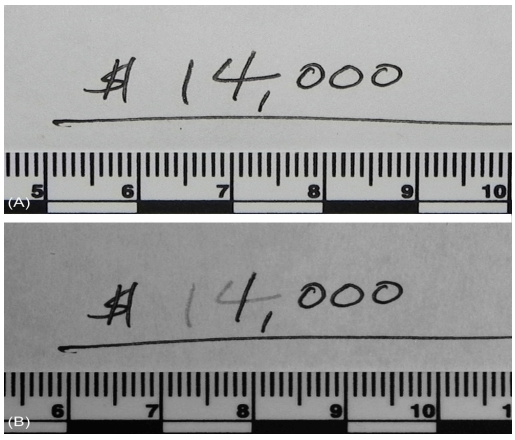
Intensity of Ball-Point Pen Ink	0			
	1			
	2			
	3			
	4			

12. Deconstruct the cardboard grid without damaging it. Throw away the pieces of cloth. Return all materials to where you got them.

Analysis and Conclusions
Answer all questions in narrative form (complete sentences making up coherent paragraphs!).

1. Which of the three types of pens (permanent, fountain or ballpoint) showed the strongest results? The weakest? How can you explain the difference?

2. You are working in a forensics lab as a latent evidence detection specialist. The police bring you a check that their suspect attempted to cash. The face amount of the check appears to be $14000, but the police are convinced that the suspect altered the check. You conduct an IR analysis of the ink on the check. Below (A) is the check photographed with white light and (B) is the check photographed with IR radiation.



What is your conclusion about the original value of the check? Explain to the police what is happening in these photos in terms of IR radiation that supports your conclusion.

Student Resources

https://www.phet.colorado.edu/en/simulations/waves-intro

Online simulation of water, sound and light waves helpful in introducing the concepts of wavelength, frequency and amplitude to students. Students can manipulate settings to produce different effects and study the relationships between the variables.

https://www.phet.colorado.edu/en/simulations/bending-light

Online simulation introducing reflection and refraction of light waves. Students can alter materials the wave passes through to examine the impact of the index of refraction of a material on the refracted rays. They can also use online tools to measure intensity of the light.

Teacher Resources

"TX CTE Resource Center." TX CTE Resource Center Home. https://www.txcte.org/resource/lesson-plan-forensic-use-of-light.

Excellent outline of dual-theory of light, light reactions and interactions and the forensic applications of those properties and reactions. The article includes several activities, labs, written assessments and practical exercises.

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"TX CTE Resource Center." TX CTE Resource Center Home. https://www.txcte.org/resource/lesson-plan-forensic-use-of-light.