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The Fluorescence Files: Solving Fictional Crimes with Spectroscopy

Experiment

Secret Message

- Go Direct SpectroVis Plus Spectrophotometer

Workshop Presenter

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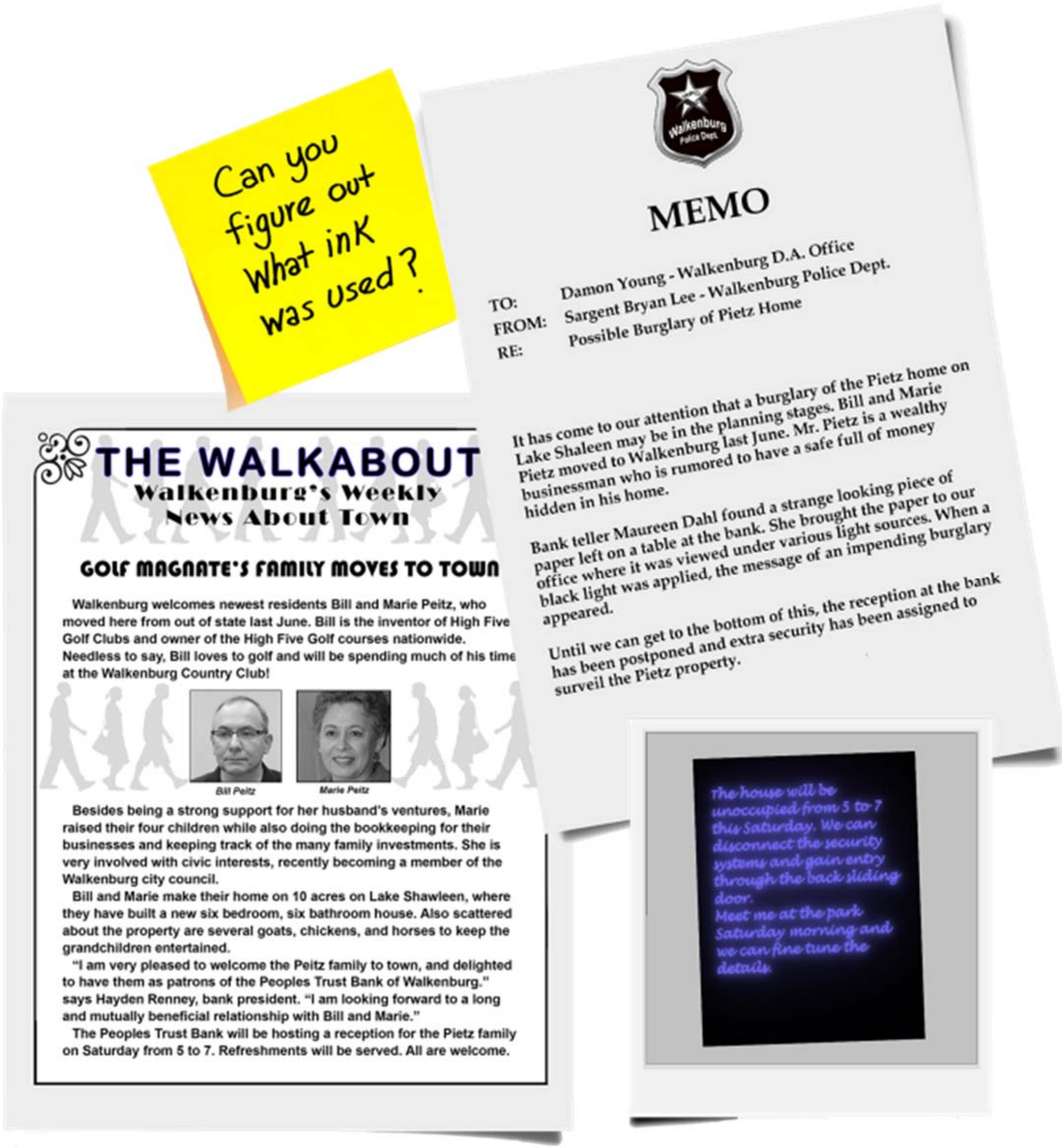
Secret Message



Your lab has been asked to help the prosecution explain the note that was discovered and introduced into evidence. How does it work? Why is a special light required to read the message?

For some chemicals, when light of one wavelength (color) is shined on them, they will absorb some of the energy and become excited as they reach a higher level of energy. These higher energy levels are frequently unstable, so the chemical will re-emit some light at a different wavelength to return to a lower energy state. This process is called *fluorescence*.

excitation light + chemical ⟶ excited chemical ⟶ less excited chemical + light of different wavelength

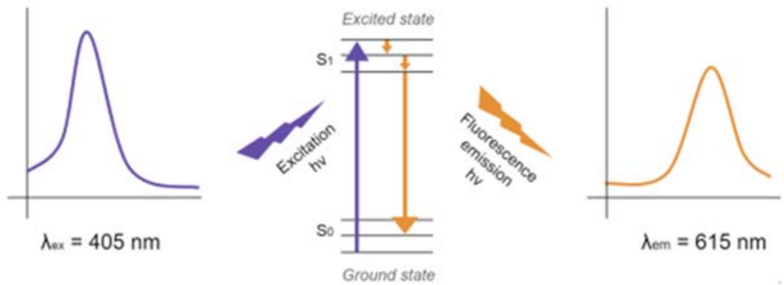


Figure 1

This experiment has two parts. In the first part, you measure the absorbance spectrum of samples of invisible marker ink. From the absorbance spectrum, you will determine the wavelength of maximum absorbance.

In Part II, you will use the wavelengths you found in Part I as a guide to determine which wavelength to use to excite the compounds in the ink to emit fluorescent light.

The Go Direct SpectroVis Plus Spectrophotometer has two LEDs that produce excitation wavelengths. In the software, you will need to set the wavelength to the value closest to that wavelength determined in Part I.

Photons of light carry different amounts of energy depending on the wavelength and frequency. You will use the wave equation and energy equation for photons, sometimes called the Planck-Einstein equation, to determine the energy carried by the excitation photons and the energy emitted during the fluorescence.

The wave equation provides a mathematical relationship between wavelength and frequency.

$$c = \lambda v$$

where c = speed of light (3.00×10^8 m/s), λ = wavelength in meters, and v is the frequency in 1/s or Hz.

The photon energy is given by this expression

$$E = hv$$

where E = energy in Joules, h = Planck's constant, 6.63×10^{-34} J-s, and v is frequency in 1/s or Hz.

OBJECTIVES

- Conduct an investigation to capture absorbance and fluorescence spectra of invisible inks in solution.
- Analyze the spectra to identify the wavelengths of maximum absorbance and fluorescence for each ink.
- Use mathematical representations to calculate the frequency and energy of the photons producing the peaks in the spectra.
- Interpret data to explain the difference in energy of the photons producing the spectra for each ink.

MATERIALS

Chromebook, computer, **or** mobile device
Vernier Spectral Analysis app
Go Direct SpectroVis Plus
4 cuvettes + lids
cuvette rack
beral pipets
Secret Message
black light flashlight
solutions of invisible inks of different colors
distilled water
lint free wipes

PRE-LAB ACTIVITY

Use the black light to look at the Secret Message.

1. What is the wavelength of light emitted by the black light flashlight? This may require looking at the packaging or doing some online research.
2. What do you see when you look at the Secret Message while shining the blacklight? Specifically, what color does the message appear to your eye? Record this observation in the Evidence Record.

PROCEDURE

Part I Measuring Absorbance Spectrum

1. Launch Spectral Analysis. Connect the Go Direct SpectroVis Plus Spectrophotometer to your Chromebook, computer, or mobile device. Click or tap Advanced Full Spectrum.
2. Click or tap Collect Absorbance. Follow the instructions to calibrate the spectrophotometer for reading absorbance.
    a. Prepare a blank by filling the cuvette 3/4 full with water.
    b. Place the cuvette in the spectrophotometer being certain to align the clear sides of the cuvette with the light path.
    c. Select Finish Calibration.

3. Collect absorbance *vs*. wavelength data.

   a. Remove the blank cuvette from the spectrophotometer. Empty the cuvette.

   b. Fill a cuvette with 3/4 with a solution of an invisible ink sample.

   c. Observe the color of the liquid in the cuvette. Record the color in the Evidence Record.

   d. Place the sample in the spectrophotometer taking care to align the cuvette correctly.

   e. Click or tap Collect Absorbance to start data collection. Once the absorbance spectrum is displayed, stop data collection. The data is automatically stored. **Note**: Click the y-axis label to display or hide data sets.

4. Click or tap Graph Options, , and choose View Statistics. Record the wavelength of maximum absorbance in the Evidence Record.

Part II Measuring Fluorescence Spectrum

5. Click or tap to switch to Fluorescence mode. Set the Excitation Wavelength to match, as close as possible, to the wavelength of maximum absorbance from Part I. Set the Integration Time to 200 ms.

6. Collect fluorescence *vs*. wavelength data.

   a. Click or tap Collect Fluorescence. The fluorescence graph is automatically plotted on the right y-axis. If the fluorescence spectrum is producing low peaks, you can stop data collection and adjust the Integration Time to improve the spectrum.

   b. Observe the color of the solution in the cuvette in the spectrophotometer. Record the color in the Evidence Record.

   c. Once the fluorescence spectrum is displayed, stop data collection. Look for any peak wavelength that is at least 50 nm higher than the excitation wavelength. Record this wavelength in the Evidence Record.

7. Repeat Part I and Part II with the other invisible ink solution samples if available.

EVIDENCE RECORD

Wavelength of black light flashlight: ________________________

Color of secret message writing to the eye: ________________________

Table 1

Sample number	Color of ink solution sample before reading absorbance	Wavelength of maximum absorbance (nm)	Excitation wavelength set on spectrophotometer (nm)	Wavelength of maximum fluorescence (nm)	Color of ink sample while reading fluorescence

Table 2				
Sample number	Frequency of excitation photons (Hz)	Energy of excitation photons (J)	Frequency of emitted photons during fluorescence (Hz)	Energy of emitted photons during fluorescence (J)

CASE ANALYSIS

1. Calculate the frequency, ν, that corresponds to the excitation wavelength of the photons used for each ink sample. Be careful with units. Record your answer in Table 2.

2. Calculate the energy of the excitation photons used with each ink sample. Record your answer in Table 2.

3. Calculate the frequency, ν, that corresponds to the wavelengths of the fluorescence peaks for each ink sample. Show your calculations for your first ink sample. Be careful with units. Record your answers in Table 2.

4. Calculate the energy of the photons emitted by fluorescence from each ink sample. Show your calculations for your first ink sample. Record your answers in Table 2.

5. Using an electromagnetic spectrum and your data, what is the color of the light used to excite the ink samples? Explain your answer.

6. Using an electromagnetic spectrum and your data, what is the color of the light emitted by each ink sample while it fluoresces? Explain your answer.

7. How is the energy of the emitted light during fluorescence related to the energy of photons used to excite the samples? Explain your answer.

8. What wavelength of light were you seeing when you looked at the secret message with the black light flashlight? Explain your answer.

9. There was a peak in the fluorescence spectra that was really close to the excitation wavelength. Why were you instructed to ignore that peak?

CASE REPORT

When you write your case report, make sure to include graphs, supporting data, and

- How did you measure the spectra of the ink sample solutions?
- How are the absorbance and fluorescence spectra related?
- Why are fluorescence spectra peaks always lower in energy than the energy of the excitation source?