MINDS ON MICROSCOPY: A FORENSICS APPROACH

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Introduction

The theory and practice of microscopy is a common lab exercise in introductory biology labs for both majors and non-majors. Typically students are taught basic microscopy skills through a series of traditional activities like examining the letter e and viewing slides of crossed threads. Directions for these activities are usually presented in a set of cookbook-like instructions. The following lab activity demonstrates the use of a hypothetical crime "The Case of the Missing Candy" to teach students the basic skills of microscopy. Details for setting up a crime scene, preparing "student crime kits," conducting the lab, and evaluating student performance are presented. The activity is amenable to use in multi-section courses and can be easily adapted to meet an instructor's particular objectives or curriculum. The basic microscopy skills students will develop in this activity include operation of both the compound and stereomicroscope, preparation of specimens for observation, and drawing specimens. The following concepts are introduced: magnification, resolution, field of view, image orientation, depth of field, and working distance. This investigative approach helps to develop students' ability to solve problems, to work cooperatively in groups, and to apply and synthesize data. It is consistent with science education reform efforts advocating investigative experiences that model how scientists actually learn about the natural world. We have found this forensics approach to be effective in teaching basic microscopy, developing students' analytical skills, and motivating them to explore the microscopic world.

Procedures

Assemble student crime kits. Prepare one student crime kit for each group of four students. Kits may be assembled in an inexpensive plastic container with a lid. Each crime kit should contain the following: newsprint samples from a French magazine, classified ads, and a sports page; several small cardboard boxes; a serrated knife, scissors

and single-edge razor blade; spools of cotton, rayon, and wool thread; a magnifying glass, and samples of sand, mulch, soil, rock salt, gravel, mud, pure hay, grass in small, individual re-sealable bags or containers.

Materials for the crime scene: Supplies needed: yellow police crime tape or yellow crepe paper; candy boxes cut open with a serrated knife (at least one per group); hay and sand; a small shovel and small containers (e.g., weighing boats) for students to scoop their sample, ransom notes made from letters from a French newspaper or magazine (one copy per group); spools of cotton thread (three different colors); articulated skeleton; tomato juice or other red liquid; prepared microscope slides of frog blood with label covered; small piece of something that resembles human skin. The following laminated signs are needed to label crime scene exhibits: Exhibit 1: candy boxes; Exhibit 2: debris; Exhibit 3: ransom note; Exhibit 4: fibers; Exhibit 5: blood; Exhibit 6: human skin.

Sources of crime scene materials: Microscope slides of normal and transformed cultured cells can be prepared from Kit 1, Visualization of Normal and Transformed Cells from CellServ (www.cellserv.kits.com). The cells can be prepared ahead of time or if you prefer, students can stain the cells themselves. Yellow tape saying "caution" can be found in most large discount stores in the paint department. French newspapers are available at newsstands in most metropolitan areas. If this is not available another news source may readily be substituted. They key is to find sources that have subtle differences in font style and size.

General lab supplies: Students will require compound light microscopes, stereomicroscopes, plain microscope slides, cover glasses, and dropper bottles of water.

Directions for setting up crime scene: The crime scene can be set up as follows in a convenient location in the laboratory.

- 1. Use yellow police crime tape or yellow crepe paper to cordon off a section of the lab.
- 2. Place several Exhibit 1 candy boxes in the area (at least one for each group)
- 3. Place debris on the floor: a mixture of hay and sand (enough for each group to take a small sample.) Nearby place the sign: Exhibit 2: debris. Place small shovels and small containers like weighing boats for students to scoop up samples adjacent to the debris.
- 4. Place copies of Exhibit 3 ransom notes made of cut out letters from a French newspaper. Each group should have their own copy.
- 5. Position an articulated skeleton within the crime scene area. Place three colors of cotton thread in the ribs of the skeleton. There should be enough thread so that each group can have one of each color. Nearby place the sign: Exhibit 4: fibers.
- 6. Sprinkle "Blood" drops on the floor. These can be made with any red liquid. Nearby, place the sign: Exhibit 5: blood. Outside the yellow taped crime area, place a box of prepared microscope slides of the frog blood with the labels covered.
- 7. A piece of flesh from the butcher shop or grocery store can represent the human skin tissue. Nearby place the sign: Exhibit 6: human skin. Nearby place a box of prepared

microscope slides showing normal cultured cells prepared from CellServ kit 1. (See note above for source.)

The actual material distributed to students appears on the next page. It includes the crime scenario, directions for handling and examining exhibits, crime lab reports, and list of suspects. The results of the six exhibits are as follows:

Exhibit 1:	Candy box. Cuts were made with serrated knife.		
Exhibit 2:	Debris. The debris consisted of a mixture of sand and hay.		
Exhibit 3:	: Ransom Note. The letters to compose the note were taken from a		
French newspaper.			
Exhibit 4:	Fibers: The fibers at the crime scene are cotton.		
Exhibit 5: Blood. The blood cells are nucleated and therefore are from a non-			
mammalian source.			
Exhibit 6. Tissue Sample. The cultured cells are normal (not cancerous.)			

Student groups can identify the criminal on the basis of their microscopic studies. They can also use their results to justify their decision and to explain how they eliminated other suspects. The criminal is Barbara Styeshand. Ms Styeshand was born in France, a clue that she may use a French newspaper to prepare a ransom note. She lives on a farm and she recently returned from the shore. This provides clues for the debris. Her sunscreen confirms that she did not have skin cancer. Her Hawaiian shirt was cotton, consistent with the fibers found at the scene. She did carry a serrated knife to cut her French bread. Her pet is a bird and would have nucleated cells.

The Crime: The Case of the Missing Candy

Late last night your lab instructor, Dr. Workaholic, was slaving away in the A & P lab writing an impossible and truly evil lab exam. To stay awake and pass away the hours, Dr. Workaholic was indulging in her favorite weakness—chocolate. She loved all kinds of chocolate. She was consuming boxes and boxes of chocolate! The crunchy kind, the chewy kind, the kind with nougat centers, the kind with caramel, the kind that melts in your mouth and not in your hands...you get the picture. In the middle of writing a dastardly question requiring students to name the 600 muscles of the human body in alphabetical order, the fire alarm rang. Dr. Workaholic promptly left the lab and exited the building. In her haste she left the lab door ajar. Alas, it was just a drill. Dr. Workaholic was surprised to see so many people outside the science building-and most of them had animals. Why it looked like one of those TV shows "animals and the people who love them." Must be some conference the university was hosting, she thought. When the fire drill was over, Dr. Workaholic returned to the lab. Catastrophe had struck! The candy boxes looked like they had been cut open and THE CHOCOLATES WERE GONE!!!!!!! (all of them... the crunchy kind, the chewy kind, the kind with nougat centers, the kind with caramel, and even the kind that melts in your mouth and not in your hand.) Dr. Workaholic surveyed the room. Unfortunately, cut candy boxes were everywhere. Fortunately, the thieves did not take the lab exam! The thief left a ransom note saying "if you ever want to see that candy again you'll leave the answers to the lab

exam under the tree by the bookstore." The words in the ransom note were made from letters cut out of a newspaper—just like in the movies. Nearby was a trail of blood! On the floor was some kind of debris. Closer examination revealed that some type of fibers had been caught in a nearby skeleton. Dr. Workaholic immediately called university police who arrived on the scene lickety split. Chief Lawandorder took photos of the crime scene and collected six pieces of evidence marked as Exhibits 1- 6. The crime scene has been recreated for you here in the lab.

Your mission as biology crime lab detectives is to examine Exhibits 1- 6 using your microscopes and other tools available in the lab. Examine each piece of evidence independently and prepare a crime lab report for each exhibit. Compare and discuss your findings with your lab partners. When you have examined all of the evidence, you will work together in your group to figure out who dunnit!

EXHIBIT 1: The Candy Box

- 1. Obtain a candy box from the crime scene.
 - a. Examine the candy box with your naked eye and describe its appearance under "general observations" in the crime lab report for Exhibit 1.
 - b. Examine the candy box with the dissecting microscope. Follow the directions for using the dissecting microscope outline under part F. Pay special attention to the cut edges of the box. Draw the cut edges of the box in the crime lab report. Be sure to record the magnification.
 - c. Based on your examination of the cut edges of the candy box, what type of implement do you think made the cuts? DETECTIVE WORK: Chief Lawandorder has confiscated a number of cutting implements from the suspects. These are available in the lab. If you would like to do some tests, these cutting implements and some extra boxes are available at your table.

	CRIME LAB REPORT	
EXHIBIT 1: CANDY BOX	Forensics Expert	
a. General observations		
b. Drawing of cut edges under the dissecting microscope		
Total Magnification		

c. Conclusions: What type of implement was used to cut the box?

EXHIBIT 2: THE DEBRIS

- 1. Obtain a representative sample of debris from the crime scene.
 - a. Examine the debris with your naked eye and describe its appearance under "general observations" in the crime lab report for Exhibit 2.
 - b. Examine the debris with the dissecting microscope. Follow the directions for using the dissecting microscope that are in your appendix. Draw the debris in the crime lab report. Be sure to indicate the magnification.
 - c. What are the various components of the debris? Record your conclusions in the crime lab report.

EXHIBIT 2: DEBRIS	CRIME LAB REPORT Forensics Expert	
a. General observations		-
b. Drawing of debris under the dissecting microscope.		
Total Magnification		
Conclusions: Debris composition.		-

EXHIBIT 3: THE RANSOM NOTE

- 1. Obtain a copy of the ransom note from the crime scene.
 - a. Examine the note with your naked eye and describe its appearance under "general observations" in the crime lab report for Exhibit 3.
 - b. Cut a word containing a small letter e out of the note and prepare a wet mount.
 - Obtain a clean microscope slide and a cover slip.

- Place a drop of water in the center of the microscope slide.
- Place the word containing the letter e in the drop of water on the slide.
- Place the edge of the cover slip on the slide and gently lower it over the specimen. Examine your wet mount of the letter "e" with your naked eye and draw it in your crime lab report.
- c. Examine your wet mount of the letter "e" under low power magnification. Sketch it in your crime report. Be sure to indicate the total magnification. How has the orientation of the letter "e" changed?
- d. Examine your wet mount of the letter "e" under high power magnification. Sketch it in your crime report. Be sure to indicate the total magnification.
- e. What is the source of the letters used in the ransom note? DETECTIVE WORK: Several of the suspects apprehended by chief Lawandorder were carrying printed material. They are available in the lab. Could any of these samples be the source of the letters used in the ransom note? Why? Record your conclusions in the crime lab report.

EXHIBIT 3: RANSOM NOTE	CRIME LAB REPORT Forensics Expert	
Sketch of letter e with naked eye	Sketch of letter e when viewed at low power magnification	Sketch of letter e when viewed at high power magnification
Conclusions: Source of letters used in ransom note.		

EXHIBIT 4: FIBERS

- 1. Obtain fiber samples from the crime scene. Try to get one of each kind of fiber.
 - a. Examine the fibers with your naked eye and describe their appearance under "general observations" in the crime lab report for Exhibit 4. Note color and texture.

b. Prepare a single wet mount that contains one of each type of fiber found.

- Obtain a clean microscope slide and a cover slip.
- Place a drop of water in the center of the microscope slide.
- Overlap the fibers in the drop of water on the slide.
- Place the edge of the cover slip on the slide and gently lower it over the specimen.
- c. Examine your wet mount of the fibers with the 4X objective. Sketch it in your crime report. Be sure to indicate the total magnification Are all the fibers in focus at the same time when using the 4X objective? _____ Increase the magnification by rotating the 10X objective into place. Are all fibers in focus at the same time now? _____ Repeat your observation with the 40X objective. When using higher magnifications it will become necessary to focus up and down using your fine adjustment knob to see all layers of the specimen in focus. The **depth of field** is the thickness of a specimen that can be seen in sharp focus at the same time. Based on your observations, does the depth of field INCREASE or DECREASE as magnification is increased?
- d. What kind of fiber is present at the crime scene? DETECTIVE WORK: To help you determine the type of fiber, Chief Lawandorder has provided you with some samples of cotton, rayon, and wool fibers for comparison. Which of these fibers most closely resemble the ones found at the crime scene. Why? Record your conclusions in the crime lab report.

	CRIME LAB REPORT	
	Forensics Expert	
EXHIBIT 4: FIBERS		
General Appearances: colors, textu Thickness		
Sketches:	Sketch when viewed with naked	Sketch when viewed with 4X Objective
	Total Magnification:	Total Magnification:
Conclusions: Type of fiber found at crime scene. Does it resemble		

cotton, rayon, or wool?

EXHIBIT 5: THE BLOOD

1. Your lab assistant, Egor, has prepared a microscope slide for you of crime scene blood. It is marked Exhibit 5.

- a. Begin by examining the blood slide under low power magnification. Increase magnification until you are using the 40X objective lens. Sketch the blood cells in your crime report including as much detail as possible. Be sure to include the total magnification.
- b. Egor just completed a zoology course and commented that the red blood cells of mammals lack nuclei but that nuclei are present in amphibia, birds, fish, and reptiles. Do you think the blood from the crime scene is from a mammalian or non-mammalian source? Record your conclusion in the crime lab report. (Hint: For reference, images of blood from humans (mammals) are available in the histology atlases in the resource area in the lab.

	CRIME LAB REPORT
EXHIBIT 5: BLOOD	Forensics Expert
Sketch blood viewed with 40X objective	
Conclusion: Is the blood from a mammal (red blood cells lack a nucleus) or from a non-mammal such as a bird, fish, amphibian or reptile? (red blood cells contain a nucleus).	

EXHIBIT 6: THE TISSUE SAMPLE

A piece of human skin was found at the crime scene. Chief Lawandorder informs you that one of the suspects has skin cancer. You immediately want to know if the skin

sample contains normal or cancer cells. Your lab technician, Egor, reminds you that cells can be grown in an artificial nutrient medium under carefully controlled conditions, a technique called tissue culture. You direct Egor to culture the cells from the crime scene. You then check in your pathology reference book and learn that cancer cells and normal cells growing in culture can be distinguished as follows:

NORMAL CELLS: Normal cells grow in culture until they physically come in contact with each another. Growth then stops, a phenomenon called **contact inhibition**. The cell membrane, cytoplasm, and nucleus can be identified. The cells do not grow randomly, but are oriented in a particular direction.

CANCER CELLS: Cancer cells lack contact inhibition and thus continue to grow forming layers of cells. You can still identify the cell membrane, cytoplasm, and nucleus but they frequently display unusual shapes and sizes. They often have multiple nuclei and an abnormal number of chromosomes. The transformed cells grow randomly in culture. If you look closely, you may observe several extensions of the cell membrane. These projections, referred to as "membrane ruffling," occur as a result of migration during growth.

To practice your ability to distinguish normal and cancer cells, some samples of each from the National Tissue Culture Lab are available. These have been set up in demonstration microscopes in the resource area or may be available at your tables. The slides will be clearly marked normal cells or cancer cells.

- a. Sketch the known samples of normal and cancer cells from the National Tissue Culture Lab in the crime lab report for Exhibit 6.
- b. Obtain from Egor the sample of cells that have been cultured from the crime scene. Examine them with the microscope. Sketch the crime scene sample in the crime report.
- c. Do the cells from the crime scene resemble normal or cancerous cells? Write your conclusions in the crime lab report.

	CRIME LAB REPORT	
	Forensics Expert	
EXHIBIT 6: TISSUE		
Known samples from the National Tissue Culture Lab		

	Total Magnification:	Total Magnification:
Sample from crime scene		
		Total Magnification:
Conclusion: Is the tissue from		
the crime scene normal or		
cancerous?		

SUMMARY: Review your six crime lab reports and then analyze the descriptions of the five suspects in the table.

THE SUSPECTS

Chief Lawandorder apprehended five suspects. All were in the vicinity of the science building on the night in question and were identified by eyewitnesses.

Sp-leen Dion

Born in Canada; spends weekends in Atlantic City baking in the sun; works in the campus Dining Hall as a dishwasher; recently traveled to Paris with the Rotary Club; wearing a fraying, multi-colored, cotton poncho with a new patch on it; had a serrated knife in her purse when apprehended; currently being treated for skin cancer; has a poodle named FiFi with a bandaged leg.

Whitknee Houstongue

Born in Massachusetts; works at a local horse farm; has a prior conviction for shoplifting; wearing a torn rayon, tiedyed body suit when apprehended; has a brand new, smooth, shiny hunting knife in her saddle bag; has a cat named Thyroid; claims she was on campus to answer an advertisement for a new job; was carrying the classified ads.

Polly McCartilage

Born in the Yukon; parent of four children under 6; works in a nearby stone quarry; likes to watch the Flintstones; wears a rainbow, rayon pants suit; has a pair of sharp scissors in her diaper bag; avid NASCAR enthusiast who reads the sports page daily; doesn't go anywhere without her pet frog, Croak, in her pocket; Croak was recently attacked by some feisty lizards and has been seen with a bandaged webbed foot.

Barbara Styeshand

Born in France near the Eiffel tower; currently lives on a farm outside town; recently returned from a week at the shore; always uses sunscreen with an SPF of 30; found with a serrated knife; claimed it was to slice French bread; wearing a cotton Hawaiian shirt with a torn sleeve; never goes anywhere without her bird, Polly, last seen nursing a hurt wing.

Heel Eyemond

Born in Russia; lives on Main Street above the bagel shop; always seen in an oversized, wool ski sweater that is unraveling at the sleeve; found carrying a razor blade in his backpack; said he shaves hourly; has a pet iguana that he recently had to take to the vet; is an avid yard sale shopper; has the classified ads with him at all times; is allergic to hay.

Think About It...

- 1. Based on the evidence from your microscopic studies, who is the thief?
- 2. Explain how you eliminated the other suspects.