

FORENSIC MICROSCOPY

General Directions:

1. You will be working independently in this lab.
2. Materials will be available at your desk station and on the side counter.
3. Handle your microscopes *carefully!*
4. Clean and return your microscope to the cabinet at the end of class



Introduction

Some people see more clearly when objects are close while others see more clearly when an object is far away. None of us, however, can see objects that are smaller than the head of a pin, for instance, without the aid of a microscope. Scientists have been able to view very, very small organisms since the 1600s when Antwon van Leeuwenhoek produced the first single lens microscope.

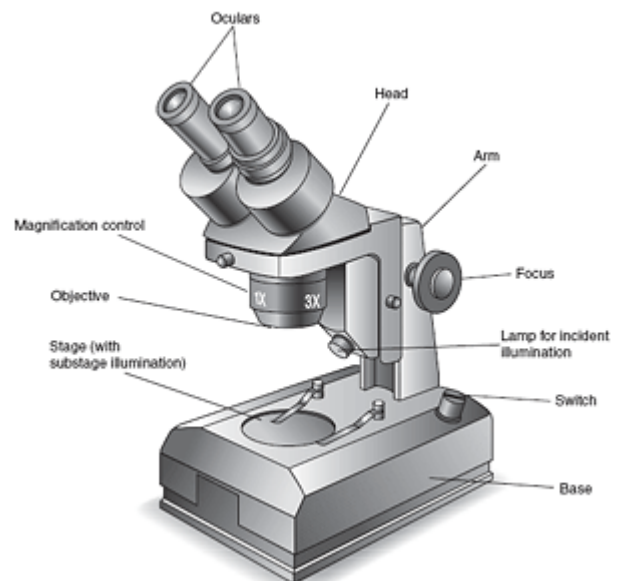
There are many different kinds of microscopes used for a variety of purposes from designing and manufacturing computer chips to doing microsurgery. During the semester, you will be using both the compound light microscope and the stereoscope or dissecting microscope.

Compound microscopes are used to magnify a transparent object (light passes through it). A typical student compound microscope can usually magnify an image anywhere from 40X to 1500X. The dissecting microscope is used to see larger objects or living specimens that can be either transparent or opaque (light doesn't pass through it).

THE DISSECTING (STEREO) MICROSCOPE

A dissecting light microscope typically has a much lower magnification than the compound microscope. The dissecting microscope is used to view large or living organisms. The dissecting scope allows the observer to magnify the image of solid specimens. You could for example examine the external characteristics of a pill bug. You could not use a compound microscope to examine a pill bug because in order to observe a specimen with a compound microscope light must pass through it.

Notice that there are two **oculars** or eyepieces; microscopes with two oculars are called **binocular** (meaning two oculars) scopes. You can move the oculars on the scope together or further apart to accommodate your interpupillary distance. If you check the side of the ocular you will see 10X written there. The oculars will magnify the image of the specimen 10 fold or 10X. The long, moveable lens located directly above the stage is the **objective lens**. On the side of the microscope body is a round knob with numbers 1-4 written on it. This knob controls the magnification of the objective lens. The numbers on the knob reflect the magnifying ability of the objective lens. Total magnification of the specimen is calculated by multiplying the magnification of the objective lens times the magnification of the ocular lens. When the knob is turned to the number 1, the total magnification is calculated as 10 (magnification of the ocular) X 1 (magnification of the objective) = 10X total magnification. When the knob is turned to the number 4 the total achievable magnification is 10 X 4 = 40X total magnification.



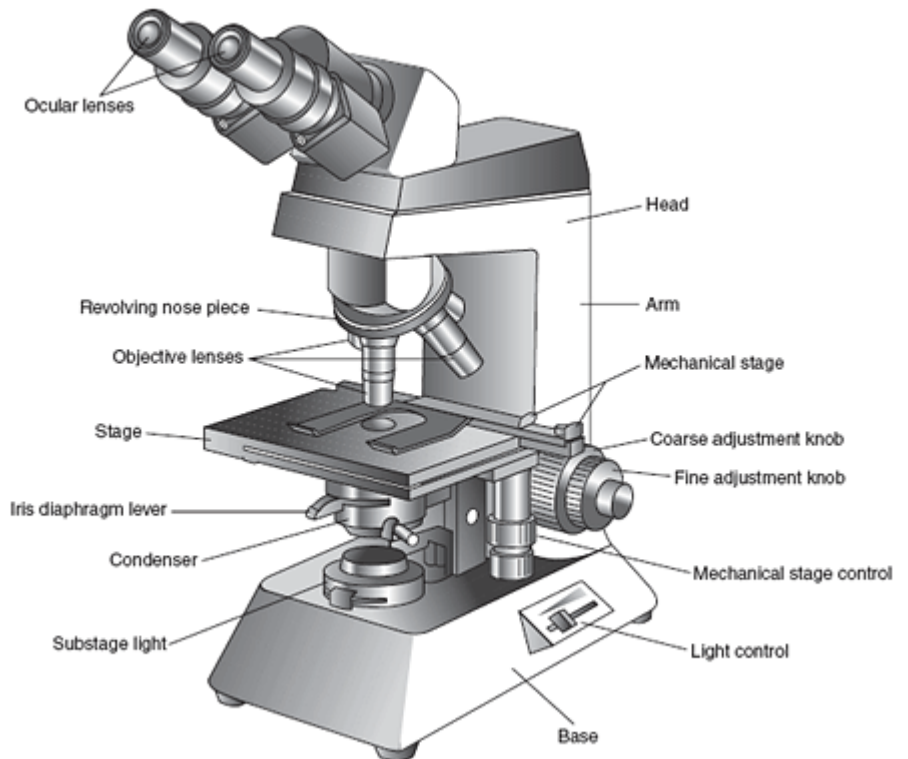
Turn on the light switch. The on/off switch is the toggle switch on the left side of the stage. On the right side of the microscope, on the base you will find a black wheel. If you roll the wheel you will notice the light getting brighter or dimmer. It is possible to control the amount of light and the direction of the light hitting the specimen. Also on the left side of the microscope, toward the back of the stage you will see 2 more toggle switches. These switches control the direction of the light. Place your hand on the stage and toggle the two switches back and forth. Observe the direction of illumination. Toggling one switch turns on the light above the stage. Toggling the other switch turns on the light below the stage. You can have the light hit the specimen from above the **stage** (platform on which the specimen rests), or from below the stage or from both above and below at the same time. The setting you use depends on the type of specimen you are viewing.

The Compound Microscope

A compound microscope uses two different lens systems to form an image. One lens system is contained in the ocular lens close to your eye. The objective lens is the lens closest to the specimen and attached to the revolving nosepiece. You can achieve different magnifications by selecting different objective lenses. Compound microscopes can have either one (monocular) or two oculars (binocular).

USING THE MICROSCOPE

Review the diagram to the right. You are responsible for knowing the parts of the microscope and what they do. Everyone will do the first activity individually. The crime scene work you can do in groups of two.



Let's take some time to look at how your microscope works. Look at the ocular(s).

Does the ocular indicate its magnification? _____

What is it? _____

Notice how the objective lenses rotate on the revolving nosepiece. Turn the nosepiece and examine each objective starting with the shortest. Their magnification will be indicated on their sides. Fill in this information below.

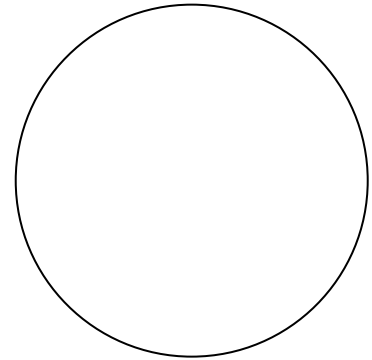
Magnification of the Ocular(s): _____ X

Examine your microscope and fill in the table below:

Length	Description	Magnification of Objective	Magnification of Ocular	Total Magnification
shortest ↓ longest	scanning			
	high dry			
	oil immersion			

Use these steps to focus the microscope; following these steps will ensure that you will be able to focus your microscope with ease. You will practice your microscope skills using a slide of a common flea.

1. Pick up a flea slide from the supply table.
2. Plug in the microscope
3. Switch on the light to make sure it works; on most of the microscopes there is a dial on the left side of the base to control light levels. Your light should be set to almost maximum brightness.
4. Use the large black knob (coarse adjustment knob) on the base to lower the stage as far as it will go.
5. Rotate the lowest power objective over the stage.
6. **Before** placing the slide on the stage, hold it up to the light. Draw what you see in the circle below. It doesn't have to be perfect; just include the direction in which the head and feet are pointing.



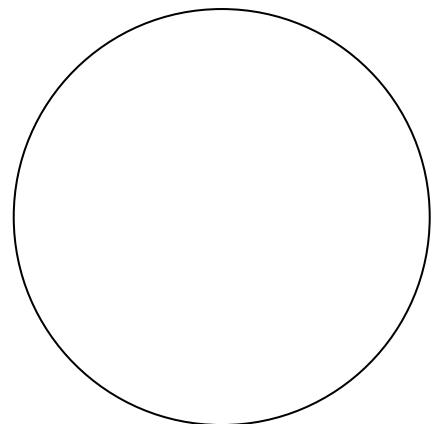
Flea – naked eye

7. Place the flea slide on stage making sure it is not upside down or backwards
8. Use the mechanical stage controls to move the slide over the circle of light passing through the stage.
9. Use the coarse adjustment knobs to raise stage up as high as it will go.
10. Adjust the light passing through the specimen by adjusting the lever on the condenser/iris diaphragm below the stage. Look at the surface of the stage and then move the lever all the way to the right and then all the way to the left. You should see the light go from very bright to black. Move the lever about halfway between these extremes. The condenser/iris diaphragm complex functions to adjust the amount and to concentrate the light passing through the specimen.
11. Look into the oculars. Adjust the interpupillary distance to fit your eyes. **Keep both eyes open.** This is difficult for most new students so practice. If you close one eye you strain the muscles in your neck and will get headaches when doing microscope work. The circle of light that you see is called the **field of view**.
12. Grab the coarse adjustment knobs and turn them until the image starts to come into focus. You always start with the slide and the objective close to each other. Then turn the focus knobs so that the objective moves further from the slide. Focusing in this manner prevents the objective from crashing into and destroying the slide.
13. Once you see the flea you can use the fine adjustment knobs (smaller black knobs on base) to fine tune the image.
14. Draw the image of the flea that you now observe in the circle to the right.

___40X___

Is the image of the flea facing in the same direction as in your earlier drawing? _____

Are the feet of the flea facing in the same direction? _____



15. Use fine focus to get the clearest image.
16. Once you have seen the flea clearly, grab and rotate the nosepiece (what the objectives are attached to) so that the 10X objective is in place over the stage.
17. Look into the oculars. The image should still be in place, but a little fuzzy. Grab the fine adjustment knob and turn it slowly until the image is sharp. These microscopes are **parfocal** which means that if an image is in focus at one magnification it will be nearly in focus at all powers. So you can change from one power to another without using the coarse adjustment knobs. You may need to adjust the light to achieve the best image and contrast using either the iris diaphragm lever or the side mounted light knob. The rule of thumb is, the higher the magnification the more light you need.
18. Using the mechanical stage knobs (below the stage) move the flea so that the flea's head is in your field of view. Grab the fine adjustment knobs and turn. As you turn the fine adjustment knobs, notice how some of the flea spines go out of focus and others come into focus. You are actually focusing through the flea's body. This phenomenon is called **depth of field**. Specimens can be composed of hundreds of layers of cells. With a slight turn of the focusing knob you can scan through them. The higher the magnification the fewer the layers of cells that can be clearly seen.
19. Remove the slide and return it to the tray.

You and your partner are going to play crime scene investigators. You will use both a compound and dissecting microscope to examine evidence from a crime scene and hopefully solve a heinous crime. Below is a description of the events leading up to the crime, the crime scene itself, and instructions for how to solve the crime.

The Scene of the Crime

On a late night in December just prior to final exams your lab instructor, Dr. Procrastinator, was slaving away in the lab writing a truly evil lab exam. Surrounding the doctor were several unopened boxes containing computers and computer equipment that were supposed to have been set up several weeks ago. In the middle of writing the ultimate trick question the fire alarm went off. Upon hearing the unbearably loud alarm, Dr. Procrastinator promptly left the lab and exited the building. In his haste, however, he left the lab room door ajar. After being told by fire officials that it was just a drill and that the building was safe, Dr. Procrastinator returned to the lab. Upon entering the room, the good doctor discovered catastrophe had struck! The computer boxes were all cut opened, upended, and empty! The room was a mess. Dr. Procrastinator speculated that the thieves must have been looking for the exam, but he had taken the disk with him. After surveying the room, Dr. Procrastinator observed the following: there was what looked like a trail of blood near several of the boxes; there was debris of some kind on the floor; there were several fibers caught on a chair; and there was a ransom note. The ransom note said "if you ever want to see your computers again you will leave the answers to the exam under the large tree next to Lakeside Café." The words in the ransom note were made from letters cut out of a newspaper, just like one you would see in a movie. Dr. Procrastinator, against his nature, immediately called the university police who arrived on the scene within minutes. Chief Lawandorder took photos of the crime scene and collected five pieces of evidence marked Exhibits 1 through 5. The crime scene has been recreated for you here in the lab.

Your mission as crime lab detectives is to examine Exhibits 1-5 using your microscopes and other tools available in the lab. Examine each piece of evidence independently and prepare a lab report for each exhibit. Compare and discuss your findings with your lab partners. When you have examined all of the evidence you and your lab partners will figure out who the culprit of this heinous crime was.

The Crime Report

You will need to prepare a crime report using the sheet located at the end of this lab exercise. You will be determining the origin of five different exhibits from the crime scene in an effort to find the culprit. For each exhibit you will record the following:

1. General observations using your naked eye
2. Drawing(s) of the exhibit as seen using the microscope
3. Conclusions as to where the exhibit originated from

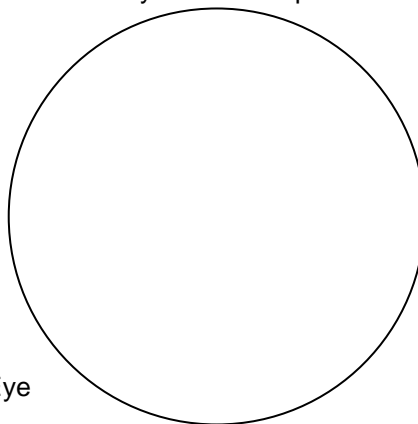
Exhibit 1: The Boxes

Obtain a box or a piece of a box from the crime scene.

a. Examine the box first with your naked eye and record your observations in your crime report and in the circle below.

Description of cut edge and box material:

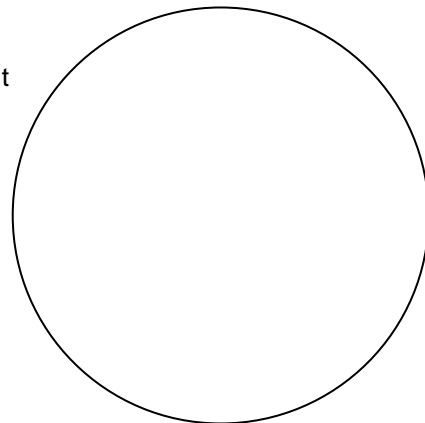
Naked Eye



b. Examine the box with a dissecting microscope. 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 your magnification.

Draw the edges as they appear under the microscope.

Magnification _____ X



c. Based on your examination of the cut edges of the box from the crime scene, what type of implement do you think made the cuts? (Compare to the known examples) _____

DETECTIVE WORK: Chief Lawandorder of the University Police has confiscated a number of cutting implements from the suspects. For testing purposes, these cutting implements and some extra boxes are available at your table or from your instructor.

Exhibit 2: The Debris

Obtain a representative sample of debris from the crime scene.

a. Examine the debris with your naked eye describe its appearance in the crime report. Label this section "General observations."

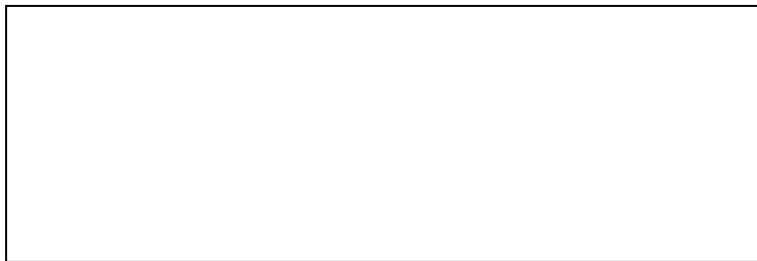


and

Description: _____

Draw what you observe in the debris.

b. Examine the debris with a dissecting microscope and what you see in the crime report. Be sure to record your magnification.



Description: _____

Draw what you observe in the debris.

Magnification _____X_____

c. What are the various components of your debris? Record your conclusions in the crime lab report.

Exhibit 3: The Ransom Note

In order to examine exhibit 3 you will need to use the compound microscope.

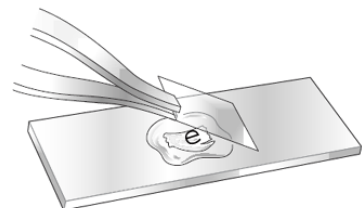
1. Obtain a copy of the ransom note from the crime scene.
2. Examine the note with your naked eye and describe its appearance under "general observations" in the crime report .

Reproduce the note in the box to the left.



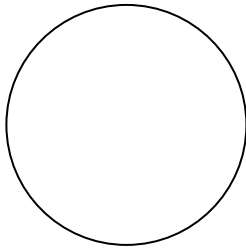
3. Cut a word containing a small letter "e" out of the note and prepare a wet mount

- a.obtain a clean microscope slide and a cover slip
- b.place a **small** drop of water in the center of the microscope slide
- c.place the word containing the letter "e" in the drop of water on the slide

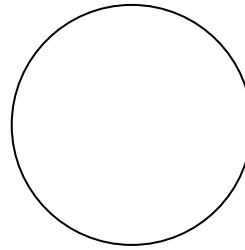


d. place the edge of the cover slip on the slide and gently lower it over the specimen

c. Examine your wet mount with your naked eye and draw it in your crime scene report and below.



Naked eye

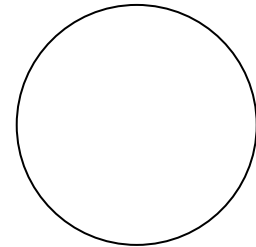


__40X__

d. Examine your wet mount of the letter "e" under low power magnification using a compound microscope. Sketch it in your crime scene report and in the circle above. Be sure to record your magnification.

e. Examine the slide under high power magnification. Sketch it in your crime report and below. Be sure to record the magnification.

4. Remove the slide. Wash the slide; throw away the cover slip.



Compare your observations with the known print types.

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 scene report.

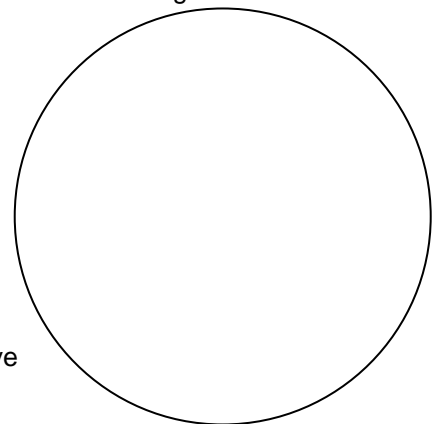
Exhibit 4: Fibers

Obtain fiber samples from the crime scene. Try to get one of each kind of fiber.

1. Examine the fibers with your naked eye and describe their appearance under "general observations" in the crime scene report and below. Note color and texture.

Description of debris:

Naked eye



2. Prepare a single wet mount that contains one of each type of fiber found from the fiber samples in your crime scene kit:

a. obtain a clean microscope slide and covers slip

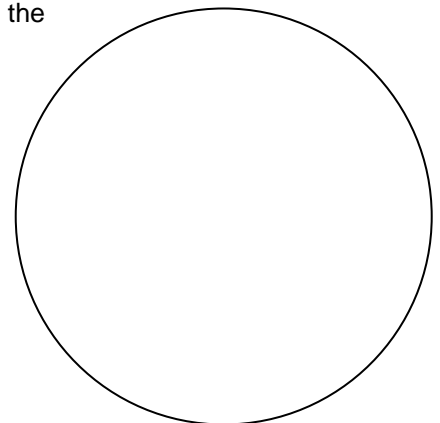
b. place a drop of water on the center of the slide

c. overlap the fibers in the drop of water

d. place the edge of the cover slip on the slide and gently lower it over the specimen

3. Examine your wet mount of the fibers with the 4X objective. Sketch it in your crime report and in the circle to the right. Be sure to indicate total magnification.

_____ X



Are all the fibers in focus at the same time when using the 4X objective? _____

4. Increase the magnification by rotating the 10X objective into place.

Are all fibers in focus at the same time now? _____

5. 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.

6. Remove the slide. Wash the slide and throw away the cover slip.

The depth of field is the thickness of a specimen that can be seen in sharp focus at the same time. Based on your observation, does the depth of field INCREASE or DECREASE as magnification is increased? _____

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 pictures of samples of cotton, rayon, and wool fibers for comparison.

Which of these fibers most closely resembles the ones found at the crime scene? _____

Why? _____ Record your conclusions in the crime lab report.

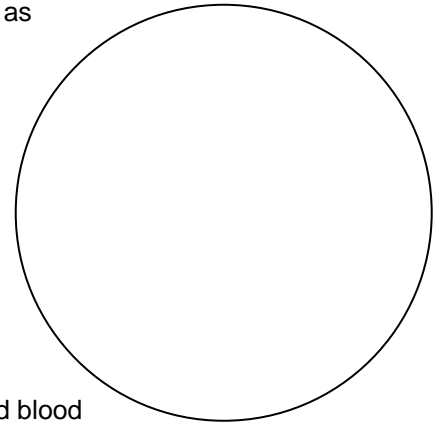
Exhibit 5: The Blood

Your lab assistant has prepared a microscope slide for you of crime scene blood marked exhibit 5.

1. Add a very, very small drop of blood to a slide.
2. Apply the cover slip.
3. Place a small drop of dye on the slide next to the cover slip.
4. Place a thin piece of paper on the opposite side of the cover slip and draw the dye under the cover slip.
5. The dye will stain the nuclei if present.

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 and below including as much detail as possible. Be sure to include total magnification.

_____ X



2. Your instructor has just completed a zoology course and commented that the red blood cells of mammals lack nuclei. Nuclei are present in the red blood cells of amphibians, 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.

3. After you have made your observations, remove the microscope slide and toss it and the cover slip in the glass/biohazard box.

CLEANING THE MICROSCOPE

You should clean your microscope before and after each use. Follow these simple steps for cleaning:

1. Get a piece of lens tissue paper. Wet it with the lens cleaning solution found on your bench.
2. Gently rub the wetted tissue paper over all the lenses (both ocular and objective lenses) and the condenser lens found in the center of the stage as well as the lens above the light. Do NOT use anything other than lens tissue paper to clean the glass parts of your microscope. Other materials such as facial tissue, paper towel, or Kimwipes will scratch the lenses. You are now ready to use or store your microscope.

3. Wipe off the stage with a damp paper towel. Dyes, salts, blood and other chemicals damage the microscope.

The Suspects

Five suspects were apprehended by Chief Lawandorder. All were identified by eyewitnesses as being in the vicinity of the science building on the night in question.

Suspect #1:

Born in southern California; lives on Main Street close to Midas Muffler; always seen during winter months in an oversized, wool sweater that is unraveling at the sleeves; 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.

Suspect #2:

Born in Daytona; parent of four children under 6; works in a nearby stone quarry; likes to watch the Flinstones; 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; often eats raw onions; doesn't go anywhere without her pet frog, Croak, in her pocket; Croak was recently attacked by some feisty lizards and has been seen sporting a bandaged webbed foot.

Suspect #3:

Born in Massachusetts; works at a local horse farm; has a prior conviction for shoplifting; wearing a torn rayon, tie-dyed 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 classifieds.

Suspect #4:

Born in Canada; works at the recycling center; recently visited the Georgia Southern Botanical Gardens; wearing a fraying, multi-colored, cotton poncho with a new patch on it; had a serrated knife in her purse when apprehended; has a poodle named Manhattan with a bandaged leg.

Suspect #5:

Born in Montana; currently lives on a farm outside of town; amateur day trader -never seen without the Wall Street Journal; found with a serrated knife - claimed it was to slice French bread; loves the smell of onions; wears a cotton Hawaiian shirt with a torn sleeve; never goes anywhere without her bird, Polly, last seen nursing a hurt wing.

CRIME LAB REPORT

Date: _____

Forensic Experts: _____

EXHIBIT 1: THE BOXES

a. General observations:

b. Drawing of cut edges under the dissecting microscope

Total Magnification:

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

EXHIBIT 2: DEBRIS

a. General observations:

b. Drawing of debris under the dissecting microscope

Total Magnification:

Conclusions: Debris composition

EXHIBIT 3: THE RANSOM NOTE

a. General observations:

Sketch of letter "e" with naked eye

Sketch of letter "e" using low magnification

Sketch of letter "e" using high magnification

Conclusions: Source of letters used in the ransom note.

EXHIBIT 4: FIBERS

a. General appearance: colors, texture, thickness

Sketch viewed with naked eye

Sketch viewed with 4X

Sketch viewed with 10X

Conclusions: Type of fiber found at crime scene - cotton, rayon, wool?

EXHIBIT 5: THE BLOOD

Sketch the blood viewed with 40X objective:

Conclusion: Is the blood from a mammal or a non-mammal? How do you know?

Based on the evidence, who is the thief?

Explain how you eliminated the other suspects.