CHAPTER 1

The Stereomicroscope

The stereomicroscope is used in most preliminary forensic examinations. This low magnification microscope provides viewing of samples in a manner that is similar to the view of the human eyes. Our eyes function along with our brain to produce what is referred to as stereoscopic or three-dimensional vision. This occurs because of the brain’s ability to interpret two slightly different images received from each eye’s retina. A distance of approximately 64–65 mm separates the human eyes. Because of this separation, each eye perceives an object from a somewhat different viewpoint. When the images are relayed to the brain, they are combined and still retain a high degree of depth perception. This provides spatial, three-dimensional images of the object. The stereomicroscope takes advantage of this ability to perceive depth by transmitting twin images that are inclined by a small angle (usually between 13°) to yield a true stereoscopic effect.

There are two basic types of stereomicroscope: Greenough and Common Main Objective. Greenough stereomicroscopes use two identical optical systems within twin body tubes that are inclined to produce the stereo effect. Common Main Objective (CMO) stereomicroscopes use a single large objective that is shared between a pair of ocular tubes and lens systems.

Stereomicroscopes offer low magnification, generally utilizing oculars and objectives that provide total magnification within the range of 0.7X to 40X. Step-type objective lenses or continuous variable zoom objective lenses are used to increase magnification in both Greenough and CMO stereomicroscopes. Because of the low total magnification, a large field of view and greater depth of field are obtained. Samples can be viewed with either reflected or transmitted light. Many forensic samples are often opaque in that they block visible light and are viewed with reflected light. This allows the stereomicroscope to be mounted on a boom stand, allowing even greater flexibility of viewing large samples.

The stereomicroscope is used to view items and to locate samples. The low-level magnification allows viewing of the initial characteristics of an item or sample. Samples can be collected and examined further with the stereomicroscope or by using additional microscopes and/or instrumentation.
Experiment 1A: Familiarization with the Stereomicroscope

Recommended pre-lab reading assignment:

OBJECTIVE

Upon completion of this practical exercise, the student will have developed a basic understanding of:

1. components of the stereomicroscope
2. magnification
3. field of view
4. depth of field
5. working distance

INTRODUCTION

A microscope is defined as an optical instrument that uses a combination of lenses to produce a magnified image of small objects. To accomplish this, a stereomicroscope uses several components that gather light and redirect the light path so that a magnified image of the viewed object can be focused within a short distance. Figure 1A-1 shows the arrangement of the basic components of a stereomicroscope: light source, sample stage, objective, support and alignment portions and oculars. A stereoscopic microscope is somewhat different in construction from standard light microscopes, in the fact that there is no condenser.

There are two types of stereomicroscopes: the Greenough and the Common Main Objective (CMO). The Greenough uses two identical optical systems within twin body tubes. The CMO uses a single objective that is shared between a pair of ocular tubes and lens assemblies. Most stereomicroscopes are CMO. There are two choices of illumination with the stereomicroscope. Reflected light is used for objects that are opaque (objects impervious to light). If the sample is transparent it can be observed with transmitted light. Some samples are best observed with both reflected and transmitted light. With a CMO stereomicroscope, as shown in Figure 1A-1, the light interacts with the sample and is then collected by the common main objective.

Light entering the objective is divergent light but once it leaves the objective it is parallel light, which is then split by a series of prisms redirecting the light to each of the oculars. The objective produces an image on its back focal plane. The eyepieces or oculars receive this image and re-focus it onto the viewer’s eye. The objective lenses in stereoscopic microscopes are built into the body tube with some mechanism for changing magnifications from the outside. Older model stereomicroscopes and the less expensive newer stereomicroscopes employ a series of fixed objective lenses, which step up the magnification in discrete increments. The newer and better
stereomicroscopes use a continuous zoom lens system, which allows any magnification within the range of the microscope.

Magnification is the process by which lenses are used to make objects appear larger. A simple lens increases the refraction and in turn produces a virtual image that appears larger. Magnification of a simple lens is described by the following equation:

\[ M = \frac{25}{f} + 1 \]  

(1A-1)

where, \( f \) is the focal length (the distance from a lens to its point of focus in cm) and 25 is the normal reading distance in cm.

Magnification of an image of an object produced by a lens can be determined by the following relationship:

\[ \text{Magnification} = \frac{\text{height of image}}{\text{height of object}} = \frac{\text{image distance}}{\text{object distance}} \]  

(1A-2)

The portions of a microscope (e.g., oculars, objectives) that increase magnification have the magnification power engraved on them. To determine the combined magnification of a lens system, all magnification components must be taken into account. Total magnification is determined by
multiplying all factors as shown in the following equation:

\[ \text{Total magnification} = \text{ocular magnification} \times \text{objective magnification} \]  

The microscopist must select the viewing magnification for each sample. There are several factors to consider. To start, it is important that the sample be viewed so that there is sufficient detail. When examining objects, a good microscopist always fills the viewing area to enhance detail and minimize white space. This often requires that the item be viewed under high magnification. However, it is equally important to remember that high magnifications only examine a small portion of a sample. Field of view relates to that portion of the object that one is able to see when using the microscope. Field of view varies with magnification. A low power of magnification will provide the greatest field of view. Likewise, higher magnification restricts the field of view.

Depth of field is another factor to consider when choosing magnification. In photography, if a lens focuses on a subject at a distance, all subjects at that distance are sharply focused. Subjects that are not at the same distance are out of focus and theoretically not sharp. However, since human eyes cannot distinguish very small degrees of ‘unsharpness’, some subjects that are in front of and behind the sharply focused subjects can still appear sharp. The zone of acceptable sharpness is referred to as the depth of field. Thus, increasing the depth of field increases the sharpness of an image. Just as in classical photography, depth of field is determined by the distance from the nearest object plane in focus to that of the farthest plane also simultaneously in focus. In microscopy depth of field is very short and usually measured in units of microns. The term depth of field, which refers to object space, is often used interchangeably with depth of focus, which refers to image space. Once a focus has been obtained on a sample, areas lying slightly above and below will be blurred. The area or thickness of the sample that remains in focus is the depth of field. Depth of field also varies with magnification.

The working distance of a stereomicroscope is another factor to bear in mind. The working distance is the distance between the objective lens and the sample. Stereomicroscopes generally have a large working distance and may also be placed on an adjustable stand allowing for even more flexibility. The distance between the objective and the specimen is determined by the focal length of the objective. To focus the sample the distance is changed using the coarse focus for large increments and the fine focus for small changes in distance.

### EQUIPMENT AND SUPPLIES

- **Stereomicroscope**
- **Micro kit**

**Samples:**
- Artificial Sweetener
- Cigarette Ash
- Glass
- Oregano
- Rosemary
- Sand
- Tea
- Beard Hair
- Cigarette Tobacco
- Graphite
- Pencil Dust
- Rust
- Soap Powder
- Black Pepper
- Coffee
- Nutmeg
- Pencil Eraser Dust
- Salt
- Soil

Petri dish unknowns (various combinations of eight samples from the above list)

### SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor.
PART I: PARTS OF A STEREOMICROSCOPE

Label the parts of the Leica EZ4™ stereomicroscope (see Figure 1A-2) by writing the name next to the appropriate number. A copy of this worksheet can be obtained from http://www.wiley europe.com/college/wheeler.

Figure 1A-2  Photograph of an EZ4™ stereomicroscope. (Reproduced with permission of Leica Microsystems, Inc.)

In the space below write a single sentence explaining the function of each part. Attach additional pages if necessary.
PART II: OPERATION OF A STEREOMICROSCOPE

1. Familiarize yourself with the stereomicroscope. Locate each part of the stereomicroscope. Place a sample on the stage. After turning on the light source, manipulate the oculars of the stereomicroscope to adjust the interpupillary distance so that when viewing an object, the right and left image merges as one.

2. Adjust the focus up and down. Using the non-adjustable ocular, focus on an item to obtain a clear image of an item.

3. Focus the second ocular if necessary.

4. Try viewing the sample with both transmitted and reflected light (if both are available). What is the difference?

5. Adjust the magnification up and down to become familiar with the range of magnifications possible while looking at a metric ruler. Try to keep both eyes open.

6. Look at the side of the oculars or its top. The number designating the magnification power is usually followed by an ‘X’. Record the power here.

Ocular lens power:

7. Look at the side of the low power objective lens. The number designating the magnification power is usually a whole number followed by an ‘X’, but can also be in fractions or may be a range of numbers. Record the magnification of the low power (magnification powers are located on the knob for a zoom objective microscope).

Low power objective lens power:

8. To calculate the magnification of the stereomicroscope, multiply the ocular lens power by the objective lens power according to Equation 1A-3. This will give you the total magnification of the stereomicroscope when using these two lenses.

9. Total magnification of the microscope on low power: ______________________

10. Total magnification of the microscope on high power: ______________________

11. Now, place a ruler on the stage. Using the lowest magnification, look through the oculars (adjust the focus if necessary) and carefully move the ruler so that you are able to count the number of spaces it takes to reach across the field of view. Also count or estimate any partial spaces. This will give you the number of millimeters that equal the diameter of the field of view on low power.

   Diameter of the field of view: _____________________mm on low power

12. Repeat the measurement using the high power objective:

   Diameter of the field of view: _____________________mm on high power

13. Using the lowest magnification, place a small piece of printed paper under the stereomicroscope. Make sure the section of paper has a letter ‘e’ in it.

14. Use the focus adjustment to bring the letters into sharp focus. Adjust the printed section so that the ‘e’ is in the center of the field of view.
15. Using the circle templates located in Appendix F, make a drawing of the letter ‘e’ on low and high power. Determine the magnification each time and record the total magnification. Try to fill the field of view.

16. Now, move the sample to the right, towards you, and away from you. Note the direction in which the ‘e’ appears to move in respect to the original placement.

17. Next, examine samples of tea, cigarette tobacco, and cigarette ash under both low and high power. Draw what you see. Record the magnification.

18. Do you have more ‘depth of field’ at low or high power?

19. Examine a dollar bill under low and high power on the stereomicroscope. Are the fibers intertwined? What color fibers do you see? Draw what you see.

**PART III: TRACE EVIDENCE UNKNOWN**

Now use a stereomicroscope to examine an unknown sample and determine the possible contents.

1. Examine the known samples taking note of color, size, shape, texture, and any other characteristics viewed. Use the following worksheet to describe each sample that might be present in the Petri dish.

   Artificial Sweetener
   Beard Hair
   Black Pepper
   Cigarette Ash
   Cigarette Tobacco
   Coffee
   Glass
   Graphite
   Nutmeg
   Oregano
   Pencil Dust
   Pencil Eraser Dust
   Rosemary
   Rust
   Salt
   Sand
   Soap Powder
   Soil
   Tea

2. Choose a Petri dish containing an ‘unknown’. Each dish contains a combination of eight samples.
3. Using the stereomicroscope, examine the unknown to determine which possible samples might be contained in the Petri dish.

Trace Evidence Unknown Number: ______________________

1. ______________________ 2. ______________________
3. ______________________ 4. ______________________
5. ______________________ 6. ______________________
7. ______________________ 8. ______________________

REPORT REQUIREMENTS

Include all drawings, calculations, or other information obtained during the laboratory procedure. Notes and/or drawings should include the sample identification, magnification, and a complete description.

REPORT QUESTIONS

1. What are the five basic components of a stereomicroscope? What function does each component perform in the stereomicroscope?
2. Explain the optics used in a stereomicroscope.
3. What is the difference between a Common Main Objective and Greenough stereomicroscope?
4. Name three types of evidence that could be examined with a stereomicroscope. Of what would the examination consist?
5. What are the two main benefits of using a stereomicroscope?
6. What are the limitations of a stereomicroscope?
7. What is total magnification? Calculate the magnification of a microscope that has an ocular lens power of 10 and an objective lens power of 4.
8. What was the magnification of the microscope at low and high power? How would you state the magnification range of this microscope?
9. What was the field of view of the microscope in mm at low and high power?
10. Why is the area viewed under high power less than the area viewed on low power?
11. What is meant by depth of field (DOF)? Does a stereomicroscope have more DOF at high or low magnification?
12. What is working distance? What is the approximate working distance of the stereomicroscope?
13. What is the difference between transmitted and reflected light? Give one example of evidence which would be viewed with each.

RECOMMENDED AND FURTHER READING
