Practical Forensic Microscopy

A Laboratory Manual

Barbara P. Wheeler and Lori J. Wilson

Department of Chemistry,
Eastern Kentucky University
Richmond, KY, USA

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West Sussex PO19 8SQ, England

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Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

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John Wiley & Sons Canada Ltd, 6045 Freemont Blvd, Mississauga, Ontario, L5R 4J3, Canada

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Library of Congress Cataloging-in-Publication Data

Wheeler, Barbara P.
Practical forensic microscopy : a laboratory manual / by Barbara P. Wheeler and Lori J. Wilson.
p. cm.
Includes index.
ISBN 978-0-470-03176-6
1. Medical jurisprudence–Laboratory manuals. 2. Microscopy–Laboratory manuals. I. Wilson, Lori. II. Title.
RA1034.W54 2008
614′.1078 – dc22

2008022098

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-470-03176-6

Typeset in 10/12 Times by Laserwords Private Limited, Chennai, India
Printed and bound in Singapore by Markono Ltd
This book is printed on acid-free paper.

Contents

Preface

Forensic science is a discipline that has evolved from the application of science to questions arising from crime or litigation. Since the popularity of forensic science as a career choice has emerged, many colleges and universities have developed criminalistics and forensic science programs. Swelling enrollments have created a market for texts within this field. Because of this heightened interest, there are many texts that concentrate on the general aspects of the field, providing an introduction to forensic science. Due to rising public interest, there are even a few texts that bring attention to separate disciplines within forensic science, for instance, firearms, drugs, and DNA. However, in the past, forensic microscopy has had little distinction, being overshadowed by more visible disciplines. In recent years, some highly publicized criminal cases have brought forensic microscopy into the spotlight.

Practical Forensic Microscopy is a comprehensive lab manual that adapts microscopic procedures used in the forensic laboratory to practical experiments that can be taught in college laboratories. The manual is written by a practitioner and an academician, and so a balanced approach to the topic was able to be reached. This laboratory manual provides a general overview and understanding of the numerous microscopes and microscopic techniques used within the field of forensic science. Each topic covered begins with a list of simple objectives for the experiment. To assist the student in obtaining the objective, an explanation of the topic, selected reading references, and an experiment are used. Worksheets and drawing templates have also been included to compile analytical results. Instructors may find it useful to download the worksheets and templates from http://www.wileyeurope.com/college/wheeler. To test the student's knowledge, report requirements and questions are included.

This manual is unique among other laboratory manuals in the fact that the microscopic techniques commonly used by scientists have been applied to forensic disciplines. However, in some cases it is impractical to use forensic laboratory procedures in an educational setting due to the large number of students or when equipment and supplies such as controlled substances are not available. Every attempt has been made to adapt forensic laboratory procedures to best address these concerns. When significant modifications are made for the educational setting, the scientifically accepted theory and principles of the forensic lab procedures are still covered thoroughly in the introduction. To address these concerns, it was at times necessary to make concessions for accuracy or precision. For example, the absolute measurement of density of glass evidence in a forensic laboratory would use a temperature controlled system, whereas in our procedure students use standard laboratory glassware and balances. However, we felt that the need to cover the topic of measurement of the density of glass far outweighed the concessions made in accuracy and precision.

This manual is an attempt to bring forensic microscopy to the student so that the future of this discipline within forensic science will continue to flourish. Forty laboratory experiments have been developed to cover the variety of evidence disciplines within the forensic science field. The manual starts with the use of simple stereomicroscopes and gradually introduces more complex microscope systems used in a forensic laboratory. Each forensic science discipline, which uses microscopes, is

covered so that the student will obtain a general understanding of the microscopes and microscopic techniques used in examinations. For example, impression evidence such as fingerprints, shoe print patterns, tool marks, and firearms are analyzed using simple stereomicroscopic techniques. Biological, drug, and trace evidence (i.e., paint, glass, hair fiber) are covered by a variety of microscopes and specialized microscopic techniques.

The authors have successfully used a mock case scenario at the end of each semester where students are placed into groups of three and provided with items of evidence. This has been an effective way to solidify the topics covered throughout the semester and in some cases extend the procedures covered. The group divides the tasks and can ask for additional exhibits if available. The group fills out a laboratory report form (Appendix E) and provides individual notes as their lab report. The ability to reach a conclusion and communicate the necessary information in a concise manner is one of the goals of the mock case scenario. Obtaining the knowledge and developing the skills will allow students to bring forensic microscopy once again to the forefront.

Acknowledgements

When we agreed to write this laboratory manual, we couldn't begin to comprehend the time and effort that would be required to complete the manuscript. Thanks to the work and encouragement of many individuals, our thoughts and ideas have been realized.

To begin, we wish to thank Dr Vernon Stubblefield who instilled in us the beginnings of forensic microscopy. Without his initial insight and commitment to the fundamentals of polarized light microscopy, we wouldn't be the microscopists we are today.

Many others have also contributed a significant amount of effort toward the manual. We are most grateful to Melanie Bentley of the College of Arts and Sciences at EKU who spent an extraordinary amount of time and effort assisting with sketches, photographs, and the final layouts of our figures. Because of her skills, many of the experiments are enhanced by visual aids. Her perfectionist quality and willingness to assist in this project greatly improved the lab manual. We also appreciate the work of Forensic Science majors Jesse Meiers, for his assistance with photographs, and Ethan Harlacher, for his assistance with references and glossary items. We are grateful to Marci Adkins, Lara Mosenthin, and Patrick McLaughlin of the Kentucky State Police Central Forensic Laboratory for providing technical assistance in each of their areas of the forensic science discipline. We also appreciate the laboratory experiments contributed by Dr Larry Kaplan, Williams College, Dr Larry Quarino, Cedar Crest College, and Mr Joe Wallace, Kentucky Department of Criminal Justice Training/EKU Forensic Science Program.

We feel it is also important to acknowledge the support of Dr Diane Vance and the other faculty members of the Department of Chemistry at Eastern Kentucky University for their support and encouragement during the project. The support of the Department of Chemistry and Eastern Kentucky University also helped make this project possible.

We wish to acknowledge the government agencies, instrument manufacturers, and private companies cited in the manual for contributing their photographs and illustrations.

Finally we wish to thank Fiona Woods and the staff at John Wiley & Sons, Ltd.

And most of all, special thanks is given to our families for their endless patience, encouragement, and support during the project. We promise not to burn any more dinners until our next project.

Let's take a closer view at the amazing field of forensic microscopy. (Photograph by Chris Radcliffe, reproduced with permission of Eastern Kentucky University)

Laboratory Safety

Laboratory work can be very interesting and exciting, however certain safety concerns should always be taken into consideration. General laboratory safety rules follow. Each laboratory will have its own set of rules, so make sure that you have read those for your specific laboratory, understand them, and comply with them. When there is a question concerning laboratory safety, please ask the instructor.

1. Many materials in a laboratory may cause eye injury. Wear approved safety glasses to protect against chemical splashes and stray impacts.
2. Wear a protective laboratory apron or coat and close-toed shoes.
3. No eating, drinking, smoking, or applying makeup in the lab.
4. Keep your work area clean and free of clutter.
5. Be prepared to work while you are in the laboratory. No horseplay is permitted in the lab.
6. Do not perform any unauthorized experiments.
7. Handle scalpels and razor blades with extreme caution. Never cut materials toward you.
8. Use a fume hood for all substances that produce strong odors or fumes.
9. Do not remove any materials from the laboratory.
10. Dispose of all waste properly. Ask your instructor for directions if you are not sure what to do. To avoid contamination, never return chemicals to their original containers.
11. Do not work alone in the laboratory.
12. Check equipment to be sure that it is in good condition. Don't use chipped or cracked glassware.
13. Never pipette by mouth. Always use suction bulbs or disposable pipettes.
14. Never touch, taste, or smell a chemical. If you are instructed to note the smell, gently wave your hand over the opening to direct fumes toward your nose. Do not inhale the fumes directly from the container.
15. Rinse off any acid or base spills on your skin/clothing. Clean up all spills immediately and notify your instructor. Ask your instructor for assistance if you are not sure what to do.
16. Read labels carefully to be sure that you are using the correct reagent for an experiment.
17. Know the location and operation of the eyewash, safety shower, spill materials, and fire extinguisher in the lab.
18. Know the location of the safety kit.
19. All accidents and injuries should be reported to the instructor immediately.
20. Follow other safety rules as set by your laboratory.

Microscope Maintenance

The microscopes that you will be using in this class work on the same principles but vary greatly in their mechanical design and various operating parts. If possible, make yourself familiar with the microscope's operational manual prior to using the microscope. It is important to inform the instructor of any problems. Most routine maintenance can be performed in the laboratory, however some maintenance would require disassembly of the microscope, requiring a qualified service technician.

Basic Handling/Storage

The most critical step in microscope maintenance is prevention. Proper carrying, handling, use, and storage of the microscope is the greatest single thing that can be done to avoid major microscope repairs.

When microscopes are moved always support them from the bottom. Only use the arm to balance the weight if necessary. When changing objectives hold the nosepiece and not the objectives. Dust is a microscope's worst enemy, so keep it covered when not in use. Plastic bags can be used if microscope covers are not available. Never store a microscope with the eyepiece or objective removed or uncovered. This also applies to the third ocular area if the microscope is equipped for setup with a camera. Such storage allows dust to collect in the body tube and will be very difficult to clean. Microscopes should always be stored clean and covered.

Optical Cleaning

All lenses in a microscope are made of coated, soft glass, which can be easily scratched. Lenses should be treated with care. Never use a hard instrument or abrasive to clean a lens.

For the top of the eyepiece and the ends of the objectives, clean as follows: Use a camel's hair brush and an air aspirator or similar air source to remove all loose dust and dirt. Next try 'fogging' by breath. If the eyepiece or objective is still dirty, use lens paper or moisten the end of a Q-tip™ with lens cleaning solution. Clean the optical surface with the moist end of the Q-tip using a circular motion. Remove any remaining dust and dirt using an air source. To determine which lens surfaces need cleaning, focus the microscope on a clean slide free of all dust. Moving the slide will determine if the visible dust is on the slide. Rotating the eyepiece will establish if dirt is on the eyepiece. If any dirt rotates, the eyepiece needs cleaning. Likewise, rotating objectives will establish if dirt is on a specific objective. Dust on a condenser lens can be detected in a similar fashion. If the dirt still persists, it may be necessary to clean the inside surfaces of the objective. If after cleaning all optical surfaces carefully, dirt is still found in the field of view, it is possible

that dirt is between the lenses of the objective. This dirt cannot be removed without disassembling the compound lens in the objective. Do not attempt this – advise the instructor of the problem so that a microscope repair technician may be called.

Mechanical Maintenance

Most microscopes require periodic cleaning, lubrication, and minor adjustments. *Never over tighten or use force when doing any repair/maintenance of your microscope.* All high quality microscopes are manufactured from brass or other soft metals and are easily damaged with excessive force.

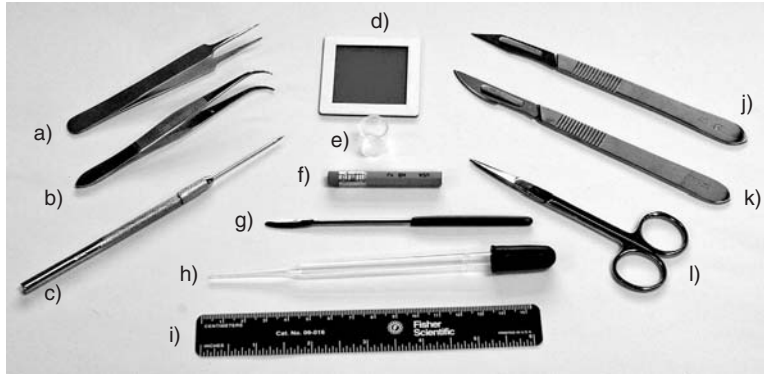
The objective nosepiece can be adjusted if it becomes too tight or loose. The adjustment is often as simple as loosening or tightening the slot-headed screw in the middle of the nosepiece. Sometimes there is a two-hole ring nut. This requires using round nose pliers such as a wrench to loosen or tighten the collar. On some microscopes the stage must be removed to gain access to the nosepiece adjustment. Be sure to check the manual for your specific microscope.

Tension of the coarse and fine adjustment knobs can also be adjusted. Again, various mechanical methods have been designed. Some microscopes are adjusted by simply turning the knobs on each side of the microscope in opposite directions to tighten or loosen as desired. Others have adjustable collars on the shaft and require the use of specially designed collar-wrenches or Allen wrenches to make the adjustments. Moving the collars out usually provides more tension. If your microscope requires unique collar-wrenches, obtain these from your microscope supplier.

Sliding surfaces on the microscope can be cleaned and lubricated. This should be done as needed or on an annual basis. Clean any grease and dirt from all sliding surfaces, using clean paper towels and a solvent such as alcohol. Wipe completely dry. Apply a thin layer of fresh grease to the sliding surfaces. Lithium-based grease or other grease specified by the manufacturer is recommended. Do not oil or grease the teeth of the rack and pinion gears.

Instructions for replacing the bulb in each specific microscope are found in its corresponding user's manual. Always allow a bulb to cool before attempting to replace it. When replacing bulbs, avoid touching the glass with your bare hands. Fingerprints left on the bulb will 'burn into' the glass and reduce the bulb quality and life expectancy.

The Micro Kit



Most experiments in this book will make use of a student micro kit.

The micro kit contains various tools that are used in the experiments included in this manual. The contents of the kit are listed below, and possible sources for the more specialized items are also included.

a) straight end forceps, fine[1]
b) curved end forceps, fine[1]
c) needle probe[2]
d) orange (589 nm) filter slide[1]
e) 1/4 inch (6 mm) glass ring (1/4 inch (6 mm) thick)[1]
f) pencil eraser
g) microspatula
h) glass pipette and bulb
i) 6 inch (15 cm) ruler
j) scalpel with ability to accept rounded edge blade[2]
k) scalpel with ability to accept straight edge blade[2]
l) scissors[2]

[1] Available from McCrone Microscopes & Accessories, Attn: Order Department, 850 Pasquinelli Drive, Westmont, IL 60559-5539. (630) 887–7100. http://www.mccronemicroscopes.com.

[2] These items are part of a standard student classroom dissection kit which may be purchased from companies such as Ward's Natural Science Establishment, LLC, 5100 West Hentrietta Road, Rochester, NY 014692-9012. (800) 962–2660. http://www.wardsci.com.

Experiments

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.

Practical Forensic Microscopy: A Laboratory Manual Barbara P. Wheeler and Lori J. Wilson

Experiment 1A: Familiarization with the Stereomicroscope

Recommended pre-lab reading assignment:

Schlueter GE, Gumpertz WE. The Stereomicroscope, Instrumentation and Techniques. *American Laboratory*. 1976; 8(4): 61–71.

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

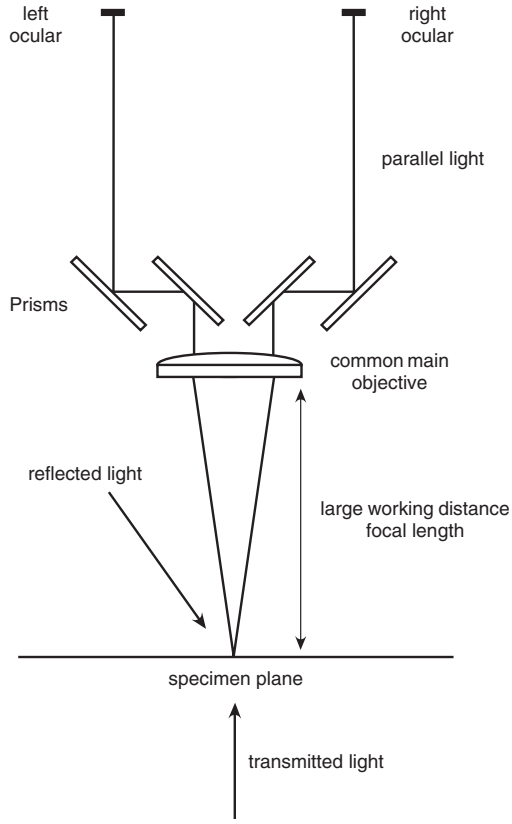


Figure 1A-1 Optical path for a CMO stereomicroscope.

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:

$$M = \frac{25}{f} + 1 \tag{1A-1}$$

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:

$$\text{Magnification} = \frac{\text{height of image}}{\text{height of object}} = \frac{\text{image distance}}{\text{object distance}} \tag{1A-2}$$

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:

$$\text{Total magnification} = \text{ocular magnification} \times \text{objective magnification} \quad \text{(1A-3)}$$

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	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		

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.wileyeurope.com/college/wheeler.

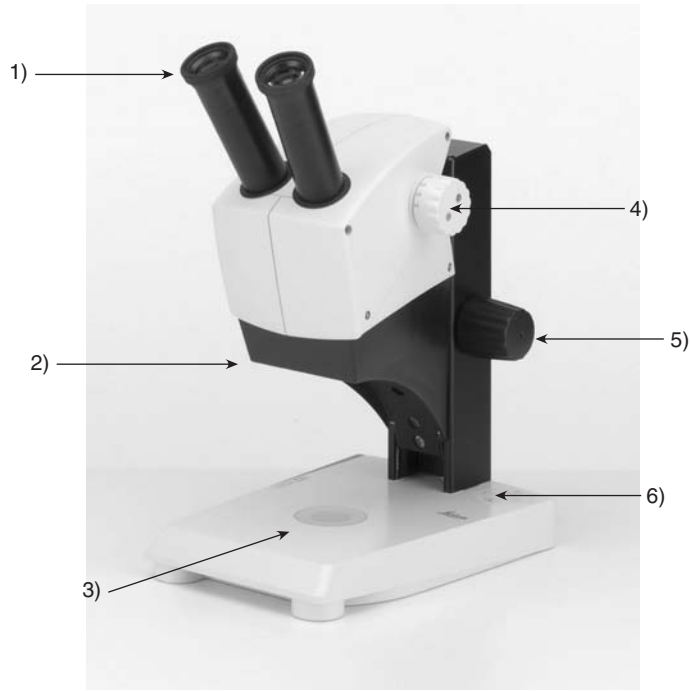


Figure 1A-2 Photograph of an EZ4™ stereomicroscope. (Reproduced with permission of Leica Microsystems, Inc.)

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

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Schlueter GE, Gumpertz WE. The Stereomicroscope, Instrumentation and Techniques. *American Laboratory*. 1976; 8(4): 61–71.

Walz M. Eye on Forensic Microscopy. *R&D Magazine*. 2005; 47(12): 33.

CHAPTER 2

The Compound Light Microscope

Microscopes are used in many types of forensic examinations. The stereomicroscope discussed in Chapter 1 is used for lower magnification examinations and the compound light microscope is generally used to obtain higher magnification.

Like the stereomicroscope, the compound light microscope uses a combination of lenses to produce a magnified image. To accomplish this, the light microscope has several components, which gather light and redirect the light path so that a magnified image of the viewed object can be focused within a very short distance. A light microscope has the following basic parts:

- light source
- condenser
- sample stage
- objective
- support and alignment portions
- oculars

Basically, the light originates from the illuminator and is collimated by the condenser. The light then interacts with the sample on the sample stage, and is then collected by the objective. The objective re-focuses the image onto the back focal plane of the microscope. The oculars receive this image and re-focus it onto the viewer's eye.

Compound light microscopes offer higher magnification, generally using oculars and objectives that provide total magnification within the range of 40X to 400X. Because of the higher total magnification, a smaller field of view and less depth of field are obtained. Samples are viewed with transmitted light.

The compound light microscope is used to identify and characterize samples. The higher level magnification allows viewing of the initial characteristics of an item or sample, with additional comparison and microscopic examinations also possible. Further analysis of samples may also be performed with additional microscopes and/or instrumentation.

Practical Forensic Microscopy: A Laboratory Manual Barbara P. Wheeler and Lori J. Wilson

Experiment 2A: Familiarization with the Compound Light Microscope

Recommended pre-lab reading assignments:

Goldberg O. Köhler Illumination. *The Microscope*. 1980; 28: 15–21.

McCrone WC. Checklist for True Köhler Illumination. *American Laboratory*. 1980; 12(1): 96–8.

McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978; 30–34.

Recommended website:

Parry-Hill MJ, Fellars TJ, Davidson MW. Microscope Alignment for Köhler Illumination. [Java Interactive Tutorial]; 2007 [updated 2007; cited 2007 November 20]; Available from: http://www.microscopyu.com

OBJECTIVE

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

1. components of a compound microscope
2. use of the compound microscope
3. numerical aperture
4. resolving power
5. centering objectives
6. proper techniques for setting up Köhler illumination

INTRODUCTION

As you learned with the stereomicroscope, a microscope is an optical instrument that uses a combination of lenses to produce a magnified image of small objects. To accomplish this, a compound microscope uses several components, which gather light and redirect the light path so that a magnified image of the viewed object can be focused within a short distance. A compound microscope is shown in Figure 2A-1 and has the following basic components: light source, condenser, sample stage, objective, support and alignment portions, and oculars.

Basically, the light originates from the illuminator and is collimated by the condenser. The light then interacts with the sample and is then collected by the objective. The objective produces a real intermediate image onto the ocular front focal plane of the microscope. The oculars receive this image, magnify it, and then re-focus it onto the viewer's eye. The four focal points are: the field diaphragm, the specimen plane, the ocular front focal plane, and the retina of the eye. These optical components are mounted in a well designed base that lends itself to precision centering and alignment.

The compound microscope is used in various forensic applications. Generally, thin samples are prepared and the light is transmitted through the sample, focused on the objective, and then passed

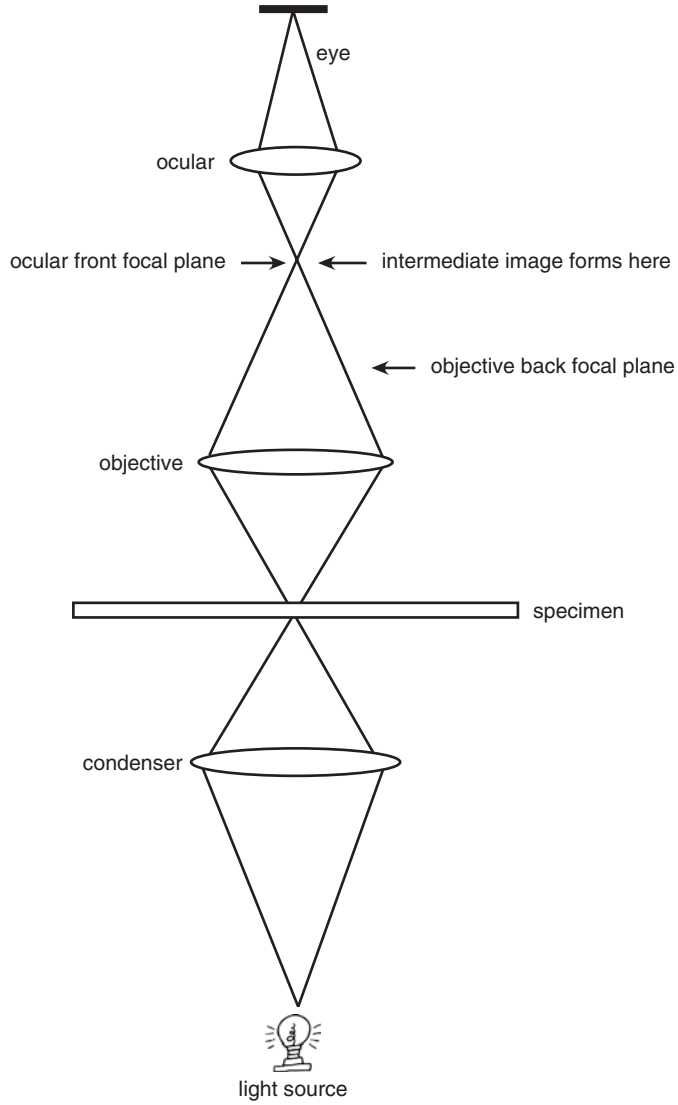

Figure 2A-1 Optical path for a compound microscope.

to the oculars. This microscope is generally used with total magnifications in the range of 40X to 400X the object. This microscope allows an examiner to learn morphological information about a sample. The visual appearance of the sample and its construction can be examined because of the higher magnifications possible with the microscope. Of similar importance is the ability to obtain analytical information. Classifying characteristics, such as color and thickness, aids in the identification of unknown samples.

Several factors come into play when using a compound microscope. Numerical aperture is a numerical measure of the ability of a lens or an objective to gather light and resolve fine sample detail at a fixed distance. Numerical aperture is related to the angular aperture by the following

formula:

$$NA = n \sin\frac{AA}{2} \quad (2A\text{-}1)$$

where NA is the numerical aperture; n is the refractive index of the space between the cover slip and objective; and AA is the angular aperture (the angle formed by the outermost rays of light that can be collected by the lens).

Lenses of short focal length (higher magnification) have greater angular aperture, which allows for the greatest angle for image forming rays. This, in turn, relates to the microscope's resolution therefore, the higher the NA, the greater the resolving power. Resolution is the smallest distance between two points on a sample that can still be distinguished as two separate entities. Resolution is a somewhat subjective value in microscopy, because at high magnification an image may appear unsharp but still be resolved to the maximum ability of the objective. Numerical aperture determines the resolving power of an objective, but the total resolution of a microscope system is also dependent upon the numerical aperture of the condenser. The higher the numerical aperture of the total system, the better the resolution.

Magnification and good resolving power are important for good microscopy. Although the optics on a microscope may be suitable, correct illumination is critical. Illumination should be evenly distributed over the entire viewing field, but also allow control of intensity, size of the illuminated field, and the angular aperture of the illuminating cone. The substage aperture diaphragm is used to control the light intensity and obtain the best compromise between resolution and contract. Neutral density filters and a variable voltage transformer on the light source can also control the light intensity; however, the later method also affects the color of the light. The field diaphragm can control the size of the light field, and the substage iris can control the angular aperture.

Illumination is generally accomplished using three techniques: Nelsonian, Köhler, and Diffuse. Most forensic laboratories use Köhler or modified Köhler illumination. This technique is based upon the positioning and alignment of various optical components in the microscope such as the lamp condenser, substage condenser, objective, ocular, and light source, to produce two sets of conjugate images. One image is observed orthoscopically (no Bertrand lens) and the other conoscopically (with the Bertrand lens). In the first, the field diaphragm, sample, and ocular front focal plane are in good focus and centered on the microscope axis. In the conoscopic view, the lamp filament, substage aperture diaphragm, objective back focal plane, and ocular focal plane are in good focus and centered on the microscope axis. Many modern microscopes are equipped with ground glass diffusers so that true Köhler illumination cannot be obtained. Köhler and modified Köhler illumination produces illumination that is uniformly bright and free from glare, which allows the examiner to use the microscope's full potential.

EQUIPMENT AND SUPPLIES

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X).

McCrone™ Particle Reference Set (or something comparable). This is a general reference set, including 100 prepared slides of the most commonly found particles, each mounted in Meltmount™ 1.662.

SAFETY

Use standard laboratory safety procedures as described in rules set by your instructor. Be cautious of microscope light levels to avoid eye damage.

PART I: PARTS OF THE COMPOUND MICROSCOPE

Label the parts of the Leica DME™ compound light microscope (see Figure 2A-2) by writing the name next to the appropriate number. A copy of this worksheet can be obtained from http://www.wileyeurope.com/college/wheeler.

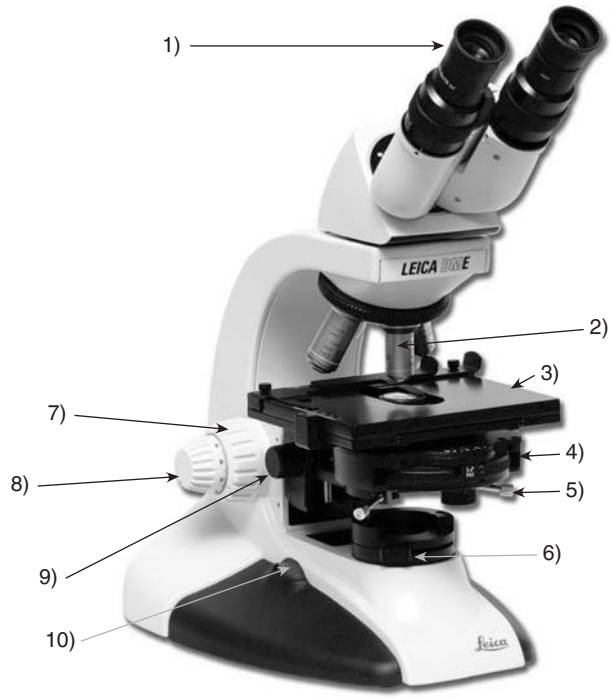

Figure 2A-2 Photograph of a DME™ compound light microscope. (Reproduced with permission of Leica Microsystems, Inc.)

In the space below write a single sentence explaining the function of each part. Attach additional pages if necessary.

PART II: OPERATION OF THE COMPOUND MICROSCOPE

Procedure

1. Familiarize yourself with the compound microscope. Locate each part of the microscope. Place a prepared microscope slide on the stage. After turning on the light source, manipulate the oculars of the microscope 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. Adjust the magnification up and down to become familiar with the range of magnifications possible while looking at the prepared microscope slide. Try to keep both eyes open.
5. Calculate the total magnification for the microscope at low and high power.
   Total magnification at low power_____
   Total magnification at high power_____

PART III: CENTERING THE OBJECTIVES

Procedure

1. Place a previously mounted sample (one with granular particles is best) on the microscope stage and focus using the lowest powered objective.
2. Place the mounted sample so that one particle is located in the center of the field of view (on the crosshairs).
3. Rotate the stage. The particle should stay essentially in the same spot. If it moves greatly or completely out of view, the objective requires centering.
4. To center the objective, rotate the particle again to pay special attention to the 'path' of the particle as the stage is rotated. Note when the particle is at the greatest distance from the center of the field of view. Rotate the sample so the particle is now in that location.
5. Move the location of the viewed particle, by adjusting the centering screws located on the objective, until the particle is about half way from its original location to the center of the field of view (on the crosshairs).
6. To check the newly aligned objective centering, move the mounted sample so that the particle is in the center of the field of view (on the crosshairs). Rotate the stage and note the 'path' of the particle. Continue with steps 4 and 5 until the particle stays centered when rotating the stage.
7. Continue to center all the objectives on the microscope.

PART IV: SETTING UP KÖHLER ILLUMINATION

Procedure

1. Place a previously mounted sample on the microscope stage and focus using the 10X objective.
2. Close down the field diaphragm. The edges of the diaphragm will become multi-sided. Adjust the substage condenser, using the focus knob, so that the edges are crisp and in focus.

3. Center the field diaphragm by adjusting the centering screws of the substage condenser.
4. Open the field diaphragm until it is just out of the field of view.
5. If the microscope has a ground glass diffuser, you are finished setting up modified Köhler, proceed to step 6. If there is no diffuser or it can be removed, continue with the following steps.
   a) Insert the Bertrand lens. If there is no Bertrand lens, remove one ocular. This allows for viewing the image of the lamp filament.
   b) Focus and center the lamp filament by using the adjustment knobs and moving it back and forth.
   c) Remove the Bertrand lens (or replace the ocular).
6. Adjust the contrast and resolution by setting the substage condenser aperture to optimum appearance. Normally, this is approximately 70–80 % open.
7. Köhler illumination should be checked with each magnification.

PART V: VIEWING SAMPLES WITH THE COMPOUND LIGHT MICROSCOPE

Procedure

1. From the prepared slide box, view and draw five of the following: diatoms, moth scales, seed hairs, insect parts, straw, cotton, table salt, and sawdust. Use various magnifications to become familiar with the microscope. Select magnifications which minimize white space but allow you to see a significant portion of the sample.

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 six basic components of a compound microscope? What function does each component perform in a compound microscope?
2. Explain the optics used in a compound light microscope.
3. What are the four foci for light in a compound microscope?
4. Name three types of evidence that could be examined with a compound microscope. Of what would an examination consist?
5. What types of evidence would not be examined with the compound microscope? Why?
6. What are the two main benefits of using a compound microscope?
7. What is numerical aperture?
8. What is the most important factor in determining the resolving power of a microscope?
9. Describe Köhler Illumination. Why is this technique used in most microscopy work over Nelsonian or Diffuse Illumination?

Experiment 2B: Measurements Using the Ocular Micrometer

Recommended pre-lab reading assignments:

McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978; 96–99.

Recommended website:

Parry-Hill MJ, Fellars TJ, Davidson MW. Eyepiece Reticle Calibration. [Java Interactive Tutorial]; 2007 [updated 2007; cited 2007 November 20]; Available from: http://www.microscopyu.com.

OBJECTIVE

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

1. calibration of the ocular micrometer scale
2. measurements with the ocular micrometer scale

INTRODUCTION

The measurement of a sample size can also be an important portion of an examination. Very small linear distances can be measured accurately with the microscope. To determine the sample size, micrometer scales within the ocular of the microscope are used. Specialized oculars have a transparent scale graticule in the ocular that is superimposed onto the image being observed. This scale is arbitrary and so must be calibrated for each objective. Calibration requires a stage scale micrometer. Although there are many types of stage scale micrometers, the most common is one that reads 0.01 mm per stage scale division (ssd). The unit of length used for measurements obtained on the microscope is the micrometer (μm), so in this case each stage scale division equals 10 μm.

Remember:

$1\ \mu m = 10^{-6}$ meters
$1\ mm = 10^{-3}$ meters
$1\ mm = 1000\ \mu m$

To calibrate the ocular scale, the stage scale is placed on the stage in a manner so that the scales are slightly offset from one another, as illustrated in Figure 2B-1. Align the scales so that there are two division lines on the scales that line up. The number of divisions between these two lines