

Practical Forensic Microscopy

A Laboratory Manual

Barbara P. Wheeler Lori J. Wilson

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



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Contents

Preface	ix
Acknowledgements	xi
Laboratory Safety	xiii
Microscope Maintenance	xv
The Micro Kit	xvii
Experiments	1
Chapter 1 The Stereomicroscope	3
Experiment 1A: Familiarization with the Stereomicroscope	4
Chapter 2 The Compound Light Microscope	13
Experiment 2A: Familiarization with the Compound Light Microscope	14
Experiment 2B: Measurements Using the Ocular Micrometer	20
Experiment 2C: Microscopic Mounting Techniques	25
Experiment 2D: Determining Refractive Index	29
Chapter 3 The Polarized Light Microscope	39
Experiment 3A: Familiarization with the Polarized Light Microscope	41
Experiment 3B: Determining Refractive Index of Anisotropic Materials	48
Experiment 3C: Determining Birefringence and Sign of Elongation	53
Chapter 4 The Fluorescence Microscope	59
Experiment 4A: Familiarization with the Fluorescence Microscope	61
Chapter 5 The Phase Contrast Microscope	67
Experiment 5A: Familiarization with the Phase Contrast Microscope	69
Application Experiments	75
Chapter 6 Experiment 6: Physical Match Examinations	77
Chapter 7 Experiment 7: Construction Examinations of Evidence	83
Chapter 8 Experiment 8: Lamp Filament Examinations	93
Chapter 9 Experiment 9: Fingerprint Examinations and Comparison	103
Chapter 10 Experiment 10: Tool Mark Examinations	111

Chapter 11 Firearms Examinations	115
Experiment 11: Firearms Examinations	115
Experiment 11A: Gunshot Residue Examinations	122
Chapter 12 Experiment 12: Shoe and Tire Print/Impression Examinations	131
Chapter 13 Experiment 13: Botanical Examinations	137
Chapter 14 Experiment 14: Paint Examinations	143
Chapter 15 Hair Examinations	149
Experiment 15: Hair Examinations	149
Experiment 15A: Animal Hair Examinations	160
Experiment 15B: Determination of Racial and Somatic Origin Characteristics of Human Hair	169
Experiment 15C: Human Hair Examinations and Comparisons	178
Experiment 15D: Evaluation of Human Hair for DNA	186
Chapter 16 Glass Examinations	193
Experiment 16: Glass Examinations	193
Experiment 16A: Glass Breakage Determinations	201
Chapter 17 Fiber Examinations	209
Experiment 17: Textile Fibers Examinations	209
Experiment 17A: Natural Fiber Examinations	215
Experiment 17B: Man-made Fiber Examinations	227
Experiment 17C: Fiber Comparisons	237
Chapter 18 Soil Examination	243
Experiment 18: Soil Examinations	243
Experiment 18A: Identification of Minerals in Soil	251
Chapter 19 Experiment 19: Microchemical Testing – Inorganic Ions	265
Chapter 20 Experiment 20: Microscopic Analysis of Controlled Substances	277
Chapter 21 Experiment 21: Semen Examinations	289
Instrumental Microscopy	295
Chapter 22 Experiment 22: Fourier Transform Infrared Microspectrometry	297
Chapter 23 Experiment 23: UV-Visible-NIR Microspectrophotometry	307
Chapter 24 Experiment 24: Thermal Microscopy	315
Chapter 25 Experiment 25: Scanning Electron Microscopy	323
Appendices	331
A Optical Properties of Natural Fibers	331
B Optical Properties of Man-Made Fibers	333

C	Michel-Lévy Chart	337
D	Dispersion-Staining Graph	339
E	Mock Case Scenario	341
F	Circle Template	345
	Glossary of Microscopy Terms	347
	Index	357

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.

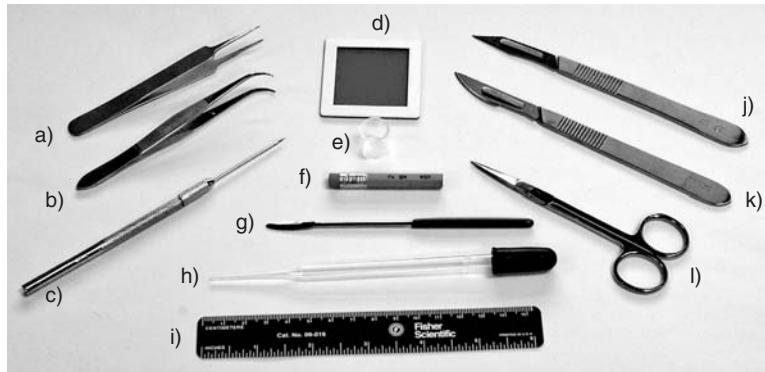
The objective nosepiece can be adjusted if it becomes too tight or loose. The adjustment is often as simple as loosening or tightening the slot-headed screw in the middle of the nosepiece. Sometimes there is a two-hole ring nut. This requires using round nose pliers such as a wrench to loosen or tighten 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, fine¹
- b) curved end forceps, fine¹
- c) needle probe²
- d) orange (589 nm) filter slide¹
- 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 blade²
- k) scalpel with ability to accept straight edge blade²
- l) scissors²

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 Henrietta Road, Rochester, NY 014692-9012. (800) 962-2660. <http://www.wardsci.com>.

Experiments

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:

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

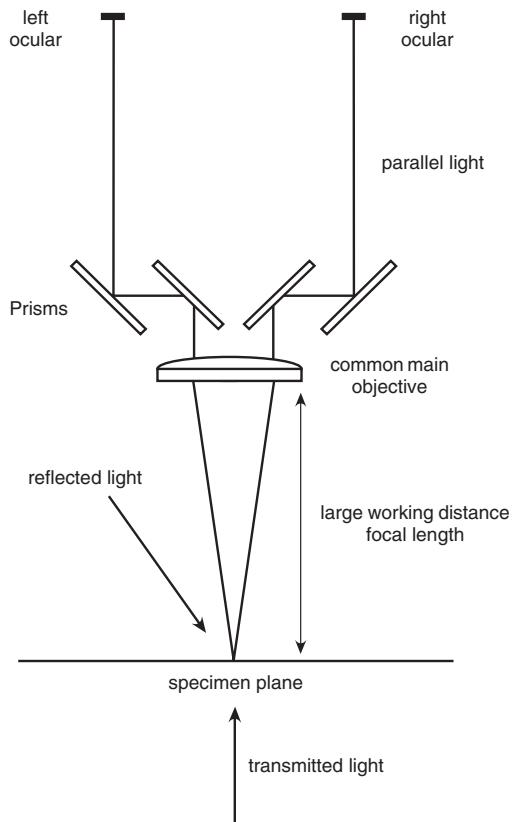


Figure 1A-1 Optical path for a CMO stereomicroscope.

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:

$$M = \frac{25}{f} + 1 \quad (1A-1)$$

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:

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

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:

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

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>.

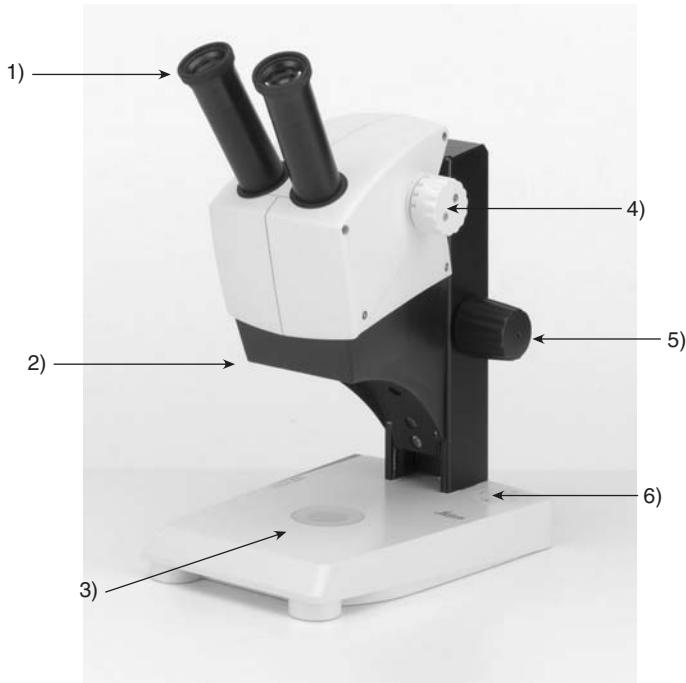


Figure 1A-2 Photograph of an EZ4™ stereomicroscope. (Reproduced with permission of Leica Microsystems, Inc.)

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

Bradbury S. *An Introduction to the Optical Microscope*. Rev. ed. Oxford: Oxford University Press; Royal Microscopical Society, 1989.

Chambers B. Today's Optical Techniques for Stereomicroscopes. *American Laboratory*. 2001; 33(8): 15–21.

- De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002: 301–5.
- Heath JP. *Dictionary of Microscopy*. Chichester, UK: John Wiley & Sons, Ltd, 2005.
- Houck MM. *Mute Witnesses: Trace Evidence Analysis*. San Diego, CA: Academic Press, 2001.
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- Schlüter 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.

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.

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 eyepieces.

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 eyepieces 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

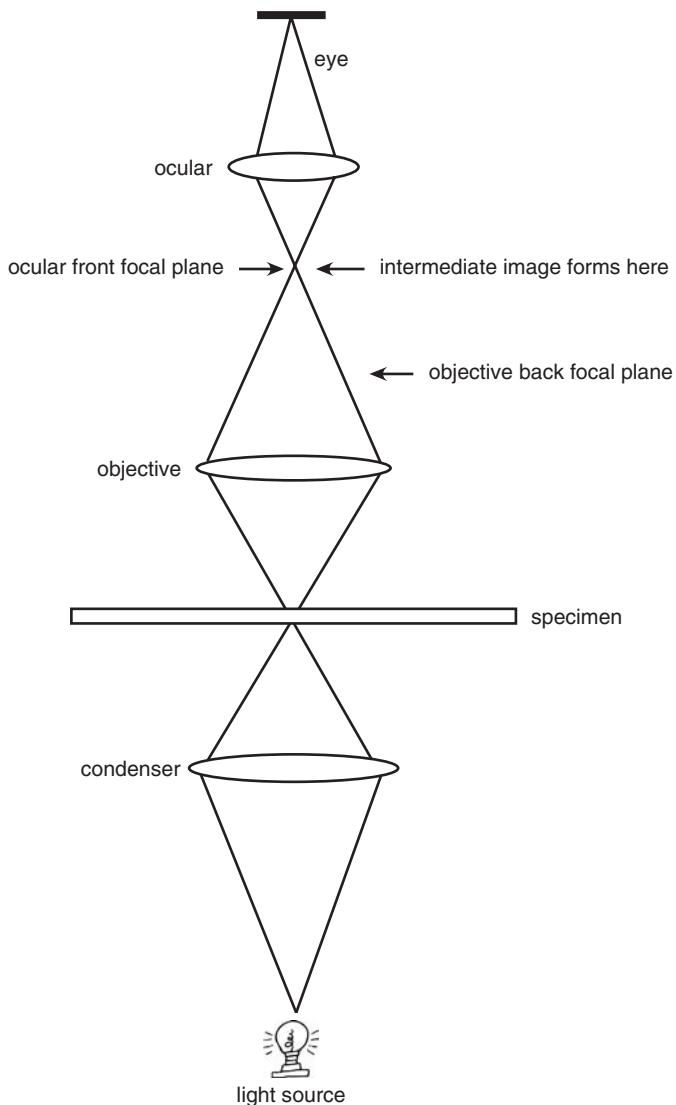


Figure 2A-1 Optical path for a compound microscope.

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:

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

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 contrast. 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).

McCroneTM 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 MeltmountTM 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>.

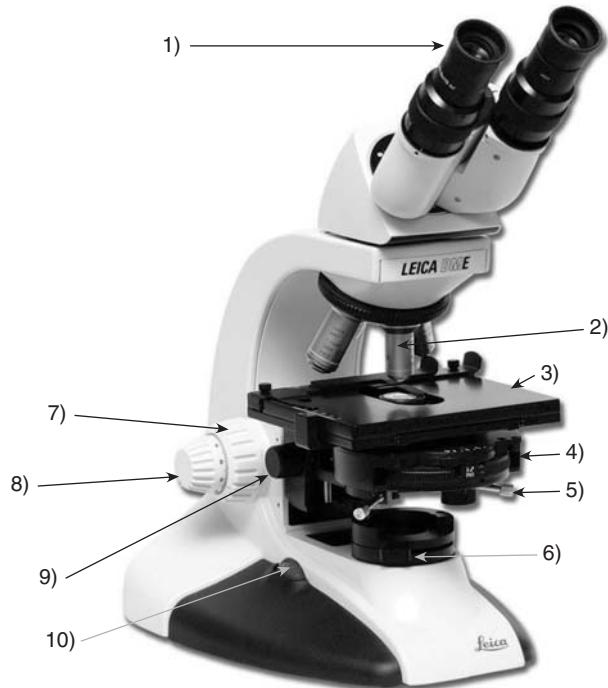


Figure 2A-2 Photograph of a DME™ compound light microscope. (Reproduced with permission of Leica Microsystems, Inc.)

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 Nelsorian 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:

$$\begin{aligned}1 \mu\text{m} &= 10^{-6} \text{ meters} \\1 \text{ mm} &= 10^{-3} \text{ meters} \\1 \text{ mm} &= 1000 \mu\text{m}\end{aligned}$$

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

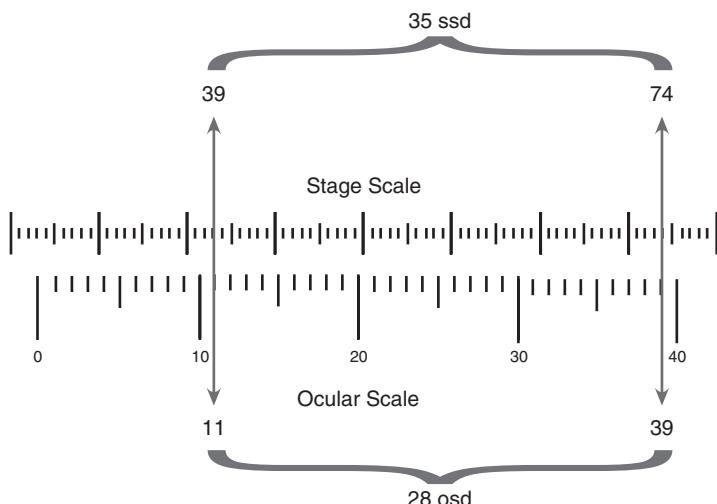


Figure 2B-1 Calibration of the ocular scale using a stage scale with each division equal to 0.01 mm. Scales vary and sometimes do not have numbers.

is then counted for both the stage scale and ocular scale. For accuracy purposes it is best to choose two positions far apart from one another. Since the stage scale is measured in mm, this value must be converted to micrometers. This is done by multiplying the number of counted divisions by the factor as marked on the stage scale. In Figure 2B-1 there are 35 ssd between the lines 39 and 74. The number of micrometers is then calculated as shown in the following equation:

$$35 \text{ ssd} \times \frac{10 \mu\text{m}}{1 \text{ ssd}} = 350 \mu\text{m} \quad (2B-1)$$

The calibration factor for the ocular scale is then determined by dividing the number of stage scale micrometers by the number of ocular scale divisions (osd) according to the following equation:

$$\text{calibration factor} = \frac{350 \mu\text{m}}{28 \text{ osd}} = 12.5 \frac{\mu\text{m}}{\text{osd}} \quad (2B-2)$$

This means that when that micrometer ocular scale is used with this objective, each division of the ocular scale is equivalent to 12.5 μm . Accurate measurements of samples can now be obtained. For example, a particle observed with that objective and measuring 8.5 osd is $8.5 \times 12.5 \text{ mm/osd}$ or 106.3 μm in diameter.

This calibration only applies to this calibrated objective. If there is a change in objective magnification, tube length, or any other part of the magnification system of the microscope the ocular micrometer scale must be recalibrated.

The accuracy of the measurement depends on recognition of the edge of the particle when observing an image that may be slightly out of focus and certainly involves errors introduced by the kind and quality of the illumination as well as errors involved in the optical system itself. Even the refractive index of the mounting medium relative to the index of the particle being measured

has an effect on the apparent size. In all cases, the error will be minimized if the magnification of the optical system is sufficient to image the particle over at least ten ocular scale divisions. At worst, under these conditions, the edge of the particle can be measured accurately to within ± 0.25 division. Hence the overall error in measurement considering two sides of the particle could be ± 0.5 division, or 10 %, for a ten-division particle. The error depends, of course, on the size and increases rapidly as the size decreases to the 1 μm range. The practical lower limit of accurate particle size measurement with the light microscope is about 0.5 μm .

EQUIPMENT AND SUPPLIES

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X and focusing ocular with micrometer scale)

Stage micrometer; marked with 0.01 mm scale

McCroneTM Particle Reference Set

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: CALIBRATION OF THE OCULAR MICROMETER

Procedure

1. After turning on the light source, adjust the microscope to obtain Köhler illumination.
2. Using the lowest magnification, place a stage micrometer on the microscope stage and bring the scale into focus.
3. Rotate to the objective you have been assigned to calibrate.
4. Adjust the focus of the ocular micrometer so that both scales can now be visualized.
5. Line up the stage and ocular scales so that they are slightly offset. This makes it easier to read the values.
6. Select two locations on each scale that line up. It is best to choose one location on the east side of the field of view and one on the west side of the field of view.
7. Count the number of large divisions of the stage scale (ssd) between the two lined up divisions.
8. Count the number of ocular scale divisions (osd), which equals the above stage scale divisions.
9. Each stage micrometer has a 1 mm long scale subdivided into 100 divisions, which makes each division 0.01 mm or 10 μm . Calculate the calibration factor for the ocular scale divisions for that objective.
10. Repeat steps 2–5 to calibrate the ocular micrometer for the second objective you have been assigned. Record the objective magnification and the calibration factors in Table 2B-1.

Table 2B-1 Calibration of two objectives and measurement of fiber diameter.

	Calibration factor μm/osd	Ocular scale readings	Average × calibration factor = μ m
Example		3.1 osd 3.3 osd 3.4 osd 3.2 osd	
4X	12.5 μm/osd		$3.25 \times 12.5 \text{ } \mu\text{m/osd}$ $= 40.6 \text{ } \mu\text{m}$
			Avg = 3.25 osd

Objective 1:**Objective 2:****PART II: MEASUREMENTS USING THE CALIBRATED OCULAR MICROMETER SCALE****Procedure**

You will now use the calibrated ocular micrometer to determine the diameter of a fiber.

1. You should now have an ocular micrometer calibration for two objectives. Measurements will be taken of the fiber diameter of a prepared slide of Dacron Polyester, from the McCrone Particle Reference Sets, using each of the objectives.
2. Using the first calibrated objective, measure the diameter of the fiber in osd. Take four readings to the nearest 0.1 osd along the length of the fiber. Sharpen the focus to get good edge definition. Convert the osd to micrometers by multiplying by the appropriate calibration factor for the first objective. Complete this portion of Table 2B-1.
3. Repeat this measurement with the second objective you calibrated. Complete Table 2B-1. Did you obtain the same diameter?
4. Select an objective and magnification that provides maximum detail. This does not have to be an objective you have calibrated. Draw what you see.

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 is a stage micrometer? How is it used?
2. What is an ocular micrometer? How is it used?
3. Why is it necessary to determine the calibration factor for each objective?
4. If a calibration factor was found to be $17.3 \mu\text{m}/\text{osd}$ for a $10X$ objective, what would you estimate the calibration factor to be for a $20X$ objective?
5. If the stage scale had a label that said 0.05 mm per stage scale divisions and there were 17 ssd equivalent to 41 osd, what would the calibration be in $\mu\text{m}/\text{osd}$? If a pollen particle had a diameter of 2 osd what would this be in micrometers? Show calculations.
6. Report your calibration factor for each objective from Part I. Make a label for the microscope you used to be placed on the base of the microscope. You will continue to use these calibration factors for future measurements.

Experiment 2C: Microscopic Mounting Techniques

Recommended pre-lab reading assignments:

- Cook R, Norton D. An Evaluation of Mounting Media for Use in Forensic Textile Fibre Examinations. *Journal of Forensic Sciences Society*. 1982; 22(1): 57–63.
- Grieve MC, Deck S. A New Mounting Medium for the Forensic Microscopy of Textile Fibers. *Science & Justice*. 1995; 35(2): 109–112.
- Loveland RP, Centifano YM. Mounting Media for Microscopy. *The Microscope*. 1986; 34: 181–242.
- Roe GM, Cook R, North C. An Evaluation of Mountants for Use in Forensic Hair Examinations. *Journal of Forensic Sciences Society*. 1991; 31: 59–65.

OBJECTIVE

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

1. preparing dry mounts
2. preparing wet mounts (oils, semi-permanent, and permanent mediums)
3. preparing scale cast

INTRODUCTION

In order to view samples with a compound microscope, samples must be mounted on a microscope slide. Wet mounts require the use of a cover slip. The cover slip is an important component of the mount and serves three purposes: (1) it protects the microscope's objective lens from contacting the specimen; (2) it creates an even thickness in wet mounts; and 3) it confines the specimen to a single plane and thereby reduces the amount of focusing necessary. Glass cover slips should be handled carefully as they are very fragile and break easily. Cover slips can be round, square, or rectangular. The glass variety is available in two thicknesses: No. 1 and No. 2. No. 1 cover slips are 0.13–0.17 mm thick and are recommended for oil immersion. No. 2 cover slips are 0.17–0.25 mm thick and are used for general applications.

In a forensic laboratory, there are three general techniques used for mounting samples that are to be examined: 1) dry mounts; 2) wet mounts; and 3) scale casts. The choice of the mounting technique is dependent on the sample. Dry mounting is the oldest mounting method and as its name suggests the sample is dry and mounted in air with a cover slip applied. Because dry mounted samples are temporary, do not preserve the sample and do not allow control of the refractive index of the mounting medium; they are not commonly used in forensic settings. Occasionally they are used to observe the external features of the specimen such as the color of fibers and hair.

Many forensic samples are viewed with a microscope to determine their internal features or microscopic characteristics. This is performed on a compound microscope using transmitted light

and wet microscope mounts. A variety of mounting mediums are available for microscopic use (see Table 2C-1).

Table 2C-1 Common mounting mediums used for wet mounts.

Mounting medium	Refractive index
water	1.33
glycerol	1.46
Protex™	1.478
XAM™, neutral medium, improved white	1.491
Flo-texx™	1.495
Permount™	1.52
DPX™	1.5240
Canada balsam	1.5250
Cargille™ oils (numerous oils)	1.470–1.700
Norland™ (numerous mediums)	
65	1.524
68	1.54
MeltMount™ (numerous mediums)	
1.539	1.539
1.582	1.582
1.605	1.605
1.680	1.680

Samples are placed in a liquid mounting medium, which has been positioned on a microscope slide. They are then covered with a cover slip. This helps to reduce the refraction of the light as it passes from air through the sample and back through air to reach the lenses of the microscope. This also allows for the best viewing of the internal features or microscopic characteristics of a sample. The degree to which the microscopic characteristics of a sample may be viewed depends on the actual mounting medium. A colorless transparent specimen is only visible because the light rays are refracted and reflected at the interface between the specimen and the medium. If the specimen and medium are the same refractive index (RI) then the item is invisible. The degree of visibility of a transparent particle is called the relief. The greater the difference in the refractive index the greater the relief.

Relief can be assigned to a mount in terms of zero, low, medium, or high relief. Samples that are placed in a mounting medium that is the same refractive index as the sample will show zero relief, and will be invisible. Likewise, samples that are placed in a mounting medium that has a large difference in refractive indices from the sample will show high relief. So, to view external features it is best to use a mounting medium that has a different RI than that of the sample. However, excessive relief or contrast may obscure the internal features or microscopic characteristics. To view internal features use a mounting medium with a refractive index near, but not the same, as the RI of the sample.

Some forensic samples are viewed with a microscope to determine their external or surface features. This can be performed with a stereomicroscope on large samples (as performed in earlier labs) or with a compound microscope on smaller samples. Since a compound microscope uses transmitted light, the viewed sample must be transparent enough to allow light to pass when

mounted on a microscope slide. This allows the external or surface features to be viewed. Samples can either be placed directly on a microscope slide or a cast can be made.

EQUIPMENT AND SUPPLIES

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X, and focusing ocular with micrometer scale)

Micro kit

Fiber samples for mounting such as acetate, nylon, rayon, silk, polyester, or cotton

Hair samples for mounting such as various animals and human hairs

Various mounting mediums

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by you instructor. Dispose of glass in an appropriate container.

PART I: MAKING DRY MOUNTS

Procedure

1. Label a microscope slide with identifying marks such as analyst initials, sample information, and date.
2. Using tweezers position the sample on the microscope slide.
3. Place a cover slip over the sample. If the sample is large the cover slip will not remain horizontal and a wet mount will have to be made.
4. Repeat steps 1–3 for the assigned samples.

PART II: MAKING LIQUID MOUNTS: SEMI-PERMANENT AND PERMANENT MOUNTING MEDIUMS

Procedure

1. Label a microscope slide with identifying marks such as analyst initials, sample information, and date.
2. Using an appropriate mounting medium from Table 2C-1, apply sufficient mounting medium to slide (see Figure 2C-1).
3. Using tweezers, position the sample on the microscope slide in mounting medium. For some samples, it may be easier to first position the sample and then add the mounting medium according to method illustrated in Figure 2C-1.

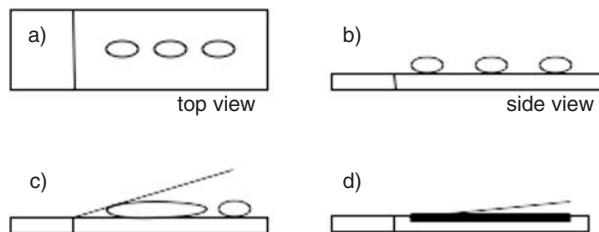


Figure 2C-1 Procedure for preparing liquid mounts. a) Drops of mounting medium are distributed on slide. b) Sample is added to mounting medium. c) A cover slip is positioned above slide so that it is only touching on one side; and d) the cover slip is allowed to fall gently over the mounting medium and sample.

4. If additional mounting medium is needed, it may be added now (or along the edge of the cover slip after the cover slip is applied).
5. Gently apply the cover slip to even out the mounting medium. The procedure that best prevents the formation of bubbles is to position the cover slip so that it touches on one end and then to allow it to fall gently over the mounting medium and sample according to Figure 2C-1 c and d.
6. If bubbles form in the preparation it may be possible to work them out by applying gentle pressure to the cover slip with a pencil eraser. Additionally, slight bumping of the slide may encourage bubbles to move to the edge. A preparation may still be usable if the bubbles occur away from the sample. It is essential that the sample be emerged in the mounting media at all locations. If the bubbles prevent this, the sample may need to be mounted again.
7. Repeat steps 1–6 for the assigned samples, using different mounting mediums.

PART III: MAKING SCALE CASTS

Procedure

1. Label a microscope slide with identifying marks such as analyst initials, sample information, and date.
2. Apply a thin coating of clear nail polish.
3. While still wet, position a hair in the nail polish.
4. Let the nail polish dry completely.
5. Gently remove the hair to obtain the scale cast.
6. Repeat steps 1–5 for the assigned samples.

Experiment 2D: Determining Refractive Index

Recommended pre-lab reading assignments:

- Koons RD, Buscaglia J, Bottrell M, Miller ET. Forensic Glass Comparisons in Forensic Science Handbook. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 188–9.
- De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 301–5.
- McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science; 1978; 169–196.

Recommended websites:

- Parry-Hill MJ, Sutter RT, Fellers TJ, Davidson MW. Refraction of Light. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited 2007 October 25]; Available from: <http://www.olympusmicro.com/>.
- Davidson MW. Refractive Index. [Java Interactive Tutorial]: Nikon Microscopy U; [2007; cited 2007 October 25]; Available from: <http://www.microscopyu.com>.

OBJECTIVE

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

1. refractive index
2. immersion oils
3. Abbe refractometer
4. dispersion staining
5. use of the compound light microscope to determine refractive index

INTRODUCTION

Several interactions can occur when light falls upon matter. The first is that the light may simply bounce or reflect off of the matter with no other interaction. The second is that light may also interact with the matter.

If a material is transparent, the interaction of light will also be dependent on the specific nature of the material. When light strikes a transparent object, the light will continue to move through the material. An example of this is when light traveling through air strikes water (Figure 2D-1). Light will continue to pass through the water, however the direction of the light will change. This change in direction is called refraction. Refraction results from a change in the speed of light as it passes from one medium to another.

Materials will refract light in differing degrees. The angle of refracted light is dependent upon both the angle of incidence and the material into which it is entering. We can define the normal

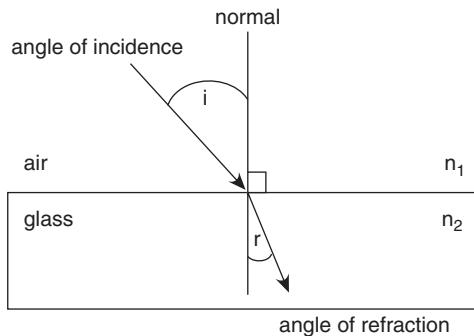


Figure 2D-1 Refraction occurs when light enters a medium with a different refractive index.

as a line perpendicular to the boundary between two substances. Light will pass into the boundary at an angle to the normal and will be refracted according to Snell's Law:

$$\frac{\sin i}{\sin r} = \frac{n_2}{n_1} \quad (2D-1)$$

where n represents the refractive indices of material 1 and material 2, i is the angle of incident light with respect to the normal, and r is the angle of refracted light with respect to normal.

When light moves from one material to another with a slower speed of light, the light will be refracted or bent toward the normal. Likewise, when light moves from one material to another with a faster speed of light, the light will be refracted or bent away from the normal. When the two refractive indices are equal ($n_1 = n_2$), then the light is passed through without refraction.

As previously discussed, refraction occurs as light passes from one medium to another when there is a difference in the speed of light between the two materials. Refractive index is defined as the relative speed at which light moves through material with respect to its speed in a vacuum. The refractive index of a vacuum is defined as having a value of 1.0. The index of refraction, n , of other transparent materials is defined through the following equation:

$$n = \frac{c}{V_m} \quad (2D-2)$$

where c is the speed of light, and V_m is the velocity of light in that material.

Because the refractive index of a vacuum is defined as 1.0 and a vacuum is devoid of any material, the refractive indices of all transparent materials are therefore greater than 1.0. For most practical purposes, the refractive index of light through air (1.0003) can be used to calculate refractive indices of unknown materials. Since light always travels faster in air than in any other medium (except a vacuum), n is always greater than one.

These principles can be applied to forensic examinations. For instance, determining the refractive index for glass particles is one characteristic used in the comparison of glass samples. When a transparent object, such as a glass chip, is immersed in a liquid, under a microscope, it is viewed as having a light or colored boundary, a sort of 'halo'. This 'halo' appears because of the internal reflection created at the boundary of the two media (glass and liquid). It is called the Becke Line. The intensity of this visible boundary around the glass depends on the difference in refractive index between the glass and the liquid. The greater the difference between the refractive indices of a glass and that of the liquid, the more distinct the Becke Line will appear. As the refractive indices of the

glass and liquid approach equality, the Becke Line and the glass particle will begin to disappear. If the refractive indices of a colorless glass and the liquid are equal, the glass may become invisible in monochromatic light. In this experiment the glass fragments rarely disappear because of a principle known as dispersion of refractive index. The dispersion of refractive index is a measure of the variation in refractive index with the wavelength of light. Refractive index measurements are typically recorded at sodium's *D* line (589 nm, yellow), and hydrogen's *C* (656 nm, red) and *F* lines (486 nm, blue). The dispersion of refractive index, ν , can be calculated using:

$$\nu = \frac{n_D - 1}{n_F - n_C} \quad (2D-3)$$

where n_D is the refractive index at 589 nm, n_F is the refractive index at 486 nm and n_C is the refractive index at 656 nm. The dispersion of refractive index can also be analyzed graphically as described below. The dispersion of a particle's refractive indices has evidentiary value since it is possible for particles with different compositions to have matching refractive indices at one wavelength but different refractive indices at other wavelengths. In this experiment, we are using white light which contains all wavelengths so the glass particles rarely disappear. Experiment 16 explains the measurement of refractive index in monochromatic light to calculate dispersion of refractive index.

An important advantage of the Becke Line is not merely the fact that it indicates a difference between the refractive indices of the glass and liquid, but that it also indicates which medium possesses the higher value. Because of this phenomenon, it can be used with immersion oils to determine the refractive indices of samples. As the working distance is increased on a compound microscope, the Becke Line will move toward the medium with the higher refractive indices. Likewise, as the working distance is decreased, the Becke Line will move toward the lower refractive indices. This allows an examiner to select a series of liquids to determine the refractive indices of a sample. There are a variety of immersion liquids that can be used for refractive index determinations. In forensic examinations, Cargille™ oils are commonly used because of their low volatility and chemically stable nature. They are commercially available with a variety of oils offered so that a wide range of refractive indices is possible. At times, it may become necessary to mix oils. To determine the actual refractive index of the oil mixture, a refractometer can be used.

Another method of determining refractive indices is through the use of a focal screening. This method takes advantage of the same phenomenon as the Becke Line. When using immersion oils, a forensic scientist examines the entire cone of light. With the dispersion-staining objective (see Figure 2D-2) only the outer (annular stop) and inner (central stop) portions of the light cone are examined.

When the refractive indices of mounting media and sample are close, the edge of the sample acts as a dispersion prism and separates the white light into its color components. By viewing the light from the outer and inner parts of the sample separately, it is possible to determine the degree of dispersion of a given sample and also to determine the wavelength of light at which the particle and mounting medium refractive index is the same. Table 2D-1 contains colors used to determine the matching wavelength for the annular and central stops.

After acquiring data using several oils, data points can be plotted on a Hartmann dispersion graph. Dispersion graphs, which plot refractive index versus wavelength, for many compounds have been plotted and exist in book form¹. The Becke Line immersion method used with white light gives only a relative refractive index, whereas the dispersion-staining technique can give a true refractive index for a given wavelength of light (i.e., n_D , n_F , n_C). The dispersion-staining

¹ McCrone WC, Delly JG. *The Particle Atlas*. Ann Arbor, MI: Ann Arbor Science, 1978.

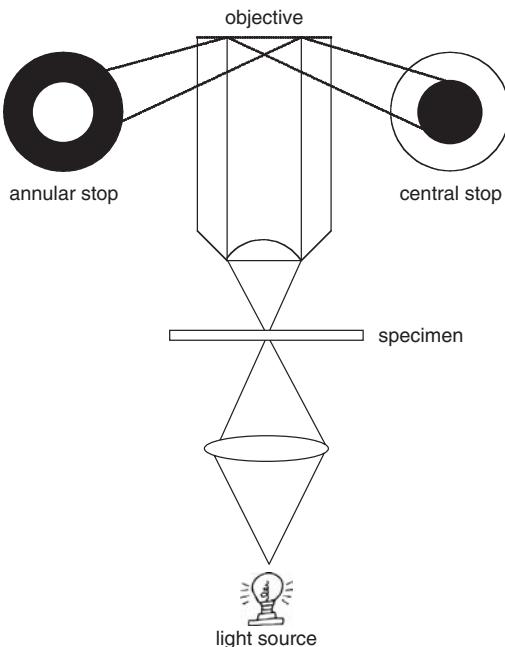


Figure 2D-2 A dispersion-staining objective contains an outer stop called the annular stop and an inner stop called the central stop.

Table 2D-1 Dispersion-staining colors².

Matching λ nm	Annular position	Central position	
		in focus	Becke line
<420	blue-black	light yellow	faint gold + violet
430	blue-violet	yellow	faint gold + violet
455	blue	golden yellow	faint gold + violet
485	blue-green	golden magenta	yellow + violet
520	green	red-magenta	violet + orange
560	yellow-green	magenta	blue-violet + red-orange
595	yellow	blue-magenta	blue + red
625	orange	blue	blue
660	orange-red	blue-green	green
>680	brown-red	pale blue	pale green

objective consists of a standard objective that has been modified with several positions. This allows a forensic scientist to choose a position, called annular, which permits viewing of light coming from the center of the particle or another position, called central, which permits viewing of light from the outer edge of the particle. A final position is also included that contains a full aperture so that it can be used as an ordinary objective.

² Reproduced from McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978, with permission of College of Microscopy.

Because density changes in condensed materials with changes in temperature, refractive index varies with temperature. If temperature control is not possible, a temperature correction must be applied. The change per degree centigrade, $-dn/dT$, is called the temperature coefficient of refractive index. The commercially available Cargille™ refractive index liquids have their $-dn/dT$ marked on each bottle. Refractive index is commonly reported at 25°C whereas the immersion measurements take place at room temperature which is about 23°C. To perform a temperature correction, the following formula would be applied:

$$n^{25} = n^{23} - (25 - 23) \frac{dn}{dT} \quad (2D-4)$$

where n^{25} is the index of refraction at 25°C, n^{23} is the index of refraction obtained at the measurement temperature of 23°C, and $-dn/dT$ is the positive value of the temperature coefficient of refractive index for the liquid that is found on the immersion oil bottle.

Since temperature and refractive index are inversely related it is important to check your answer using the rule that as temperature increases refractive index decreases and vice versa.

EQUIPMENT AND SUPPLIES

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X, and a dispersion-staining objective)

Micro kit

Glass particles of known refractive indices

Unknown glass particles

Cargille™ refractive index liquid mounting set (which would contain oils in the range of glass samples, 1.45–1.80)

Clove and olive oil mixtures; fifteen solutions of various refractive index have been made by mixing clove oil and olive oil, in the ratios listed below. Place these liquids in small, labeled dropper bottles.

Solution #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Parts olive oil	all	13	12	11	10	9	8	7	6	5	4	3	2	1	—
Parts clove oil	—	1	2	3	4	5	6	7	8	9	10	11	12	13	all

Microscope slides

Cover slips

Abbe refractometer, with recirculating water bath (optional)

A copy of *The Particle Atlas*, second edition, Volume 3

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: REFRACTIVE INDEX DETERMINATION OF GLASS SAMPLES BY BECKE LINE OIL IMMERSION METHOD³

Procedure

1. Choose a glass particle of known refractive index (RI) and mount it on a microscope slide with cover slip using:
 - a Cargille™ oil of *higher* RI
 - b) a Cargille™ oil of *lower* RI

For example, if you chose a glass with RI of 1.52, put it in 1.500 and 1.540 oils.

 - 2. Once the microscope is adjusted properly, use the 10X objective to focus on the edges of the glass particle. Use the orange filter in the Micro kit to obtain monochromatic light. This allows you to determine the n_D .
 - 3. Close the substage condenser to the minimum setting. Using the glass particle in the lower RI oil, observe the Becke Line and then focus ‘upward’ and ‘downward’ to determine whether the glass fragment or the solution has the higher refractive index. Remember that as you focus up (raise the microscope lens away from the specimen), the Becke Line will move toward the medium with the higher index. When the refractive index of the glass and the immersion liquid are the same, the chip will become almost invisible, and it may be difficult to find or bring the edges of the chip into focus. What is your conclusion?
 - 4. Repeat the steps above with a glass particle in a higher RI. What did you observe? What are your conclusions?
 - 5. Choose two of the unknown glasses. Write down their numbers.
 - 6. Following the flow chart below, choose clove/olive oil mixtures (which mimic what can also be accomplished with Cargille™ oils) to find an oil that provides minimum relief otherwise known as a ‘match.’ Remember that the Becke Line moves to the medium with the higher refractive index. Note that even though there are 15 different immersion liquids, only 4 of them need be used. Follow the diagram in Figure 2D-3.

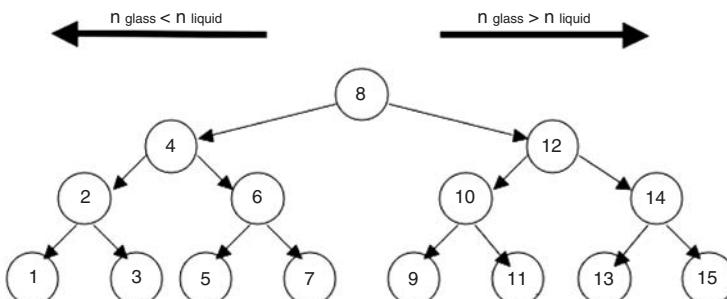


Figure 2D-3 Refractive index flow chart. Start with solution #8. If the index of refraction of the glass is greater than the liquid's, move on to solution #12. If the glass's index is greater still, try solution #14 next. If the index of the glass is less than that of solution #14, try solution #13. After this trial the index of refraction for the fragment may be determined to be between the indices of solutions #13 and #14.

³ This procedure is reproduced with permission of Lawrence J. Kaplan, Department of Chemistry, Williams College, Williamstown, MA 01267.

PART II: DETERMINATION OF THE REFRACTIVE INDEX OF SAMPLES BY IDENTIFYING THE REFRACTIVE INDEX OF THE OIL

Procedure

Note: The most common problem encountered with the refractometer is contamination of one liquid with another. Clean the prisms carefully between samples by washing with 95 % ethyl alcohol and wiping with absorbent lens paper.

CAUTION: THE PRISMS ARE VERY DELICATE AND MUST NOT BE SCRATCHED. DO NOT TOUCH THEM WITH FINGERS OR GLASS MEDICINE DROPPERS!

1. Turn on the Abbe Refractometer and the water bath so that the temperature equilibrates to 25°C.
2. Using the clove oil that you were assigned, apply a small drop of oil between the prisms (but do not touch the prisms with the dropper). Allow it to spread so that a uniform layer is obtained when the prisms are closed.
3. Looking through the eyepiece, find the general light/dark area by rotating the small knob located on the right side of the refractometer. Until it is properly adjusted, this light/dark area will probably show highly colored fringes.
4. The color-compensating prism is adjusted by turning the knurled wheel on the front of the refractometer. When this is done you will see a fairly sharp dividing line between the light and dark fields.
5. Rotate the small knob on the right side of the refractometer so that the crosshairs are exactly aligned on the light/dark dividing line as illustrated in Figure 2D-4.

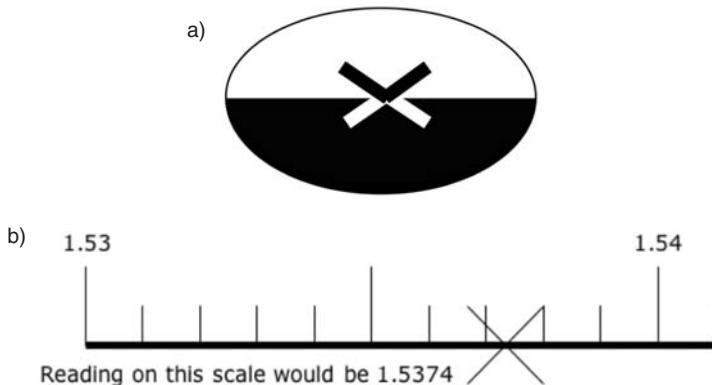


Figure 2D-4 a) The dark and light field are sharpened and centered in the field of view of the refractometer. b) A scale is illuminated by pushing a button, usually located on the left, rear side of the refractometer. This scale can be read to five significant figures.

6. Illuminate the scale by pressing the button on the left, rear side at the base of the refractometer. The numerical reading at the point where the crosshairs intersect the scale is the refractive index. Only each tenth division is numbered. Estimate the position of the crosshairs to the nearest one-tenth division. Read and record the refractive index to four decimal places, which is five significant figures (see Figure 2D-4).

7. If you do not have a circulating water bath, use a thermometer to measure the room temperature. Correct the refractive index measured with the refractometer to report the refractive index at 25°C using Equation (2D-4). The temperature coefficient of refractive index can be found on the Cargille™ oil that was found to be a match to your glass fragment's refractive index.

PART III: REFRACTIVE INDEX DETERMINATIONS OF PARTICLES USING DISPERSION STAINING

Procedure

1. After turning on the light source, rotate the objective turret until the dispersion-staining objective is in place.
2. Prepare a microscope slide using quartz and a Cargille™ oil with a refractive index value of 1.544.
3. Place the sample on the microscope stage and focus using the full aperture position. Adjust the microscope to obtain Köhler illumination.
4. Insert the Bertrand Lens (or remove one eyepiece). Adjust the dispersion-staining objective by rotating the selector disk until the central stop is selected (position marked by a dark center).
5. Close down the substage diaphragm until the field of view is just inside the central stop (background is dark).
6. Remove the Bertrand Lens (or replace the eyepiece).
7. Insert the analyzer to establish a point of extinction for the quartz particle (the point at which the particle disappears).
8. Remove the analyzer.
9. Observe the colors visible in and slightly out of focus (Becke Line) around the edges of the sample.
10. Insert the analyzer and rotate the sample to the next point of extinction. Remove the analyzer. Observe the colors visible in and slightly out of focus around the edges of the sample.
11. Insert the analyzer and return the sample to its original position (the first extinction point viewed). Remove the analyzer.
12. Insert the Bertrand Lens (or remove one eyepiece). Adjust the dispersion-staining objective by rotating the selector disc until the annular stop is selected (position marked by a bright center).
13. Close down the substage diaphragm until the field of view is just inside the annular stop (background is bright).
14. Remove the Bertrand Lens (or replace the eyepiece). Observe the colors visible in and slightly out of focus (Becke Line) around the edges of the particle.
15. Insert the analyzer to establish the next point of extinction for the quartz particle (the point at which the particle disappears).
16. Remove the analyzer.
17. Observe the colors visible in and slightly out of focus (Becke Line) around the edges of the sample.
18. Repeat this procedure (steps 3–17) with a Cargille™ oil with a refractive index of 1.554.
19. Repeat this procedure (steps 3–17) with a Cargille™ oil with a refractive index of 1.560.
20. Repeat this procedure (steps 3–17) with a Cargille™ oil with a refractive index of 1.568.
21. To determine the matching wavelength, compare the color observations to the dispersion-staining colors in Table 2D-1.

22. Plot your data on the Dispersion-Staining Graph found in the Appendix D. Compare these results to the known dispersion-staining curve data for quartz found in *The Particle Atlas*.
23. Using a thermometer measure the room temperature. Correct the refractive index value of the bottle to report the refractive index at room temperature. The temperature coefficient of refractive index can be found on the Cargille™ oil that was found to be a match to your glass fragment's refractive index.

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 is refractive index?
2. Define Becke Line.
3. Which direction does the Becke Line move when the particle is 1.49 and the oil used is 1.46? Why?
4. What are Cargille™ oils? How are they used in forensics?
5. What would you do if you wanted to test for a refractive index between two oils? How would identify its refractive index value?
6. Describe how an Abbe refractometer works.
7. Why was the refractometer temperature controlled?
8. A liquid has a refractive index of 1.532 at 25°C. It has a temperature coefficient of refractive index, $-dn/dT$, of 0.0004. What is the refractive index of the liquid at 20°C?
The following questions should also be answered if Part III was done.
9. Describe how a dispersion-staining objective works.
10. If you were given a sample, how would you go about analyzing it using the dispersion-staining method to prove that it is calcite?

RECOMMENDED AND FURTHER READING

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The Polarized Light Microscope

Microscopes are used in many types of forensic examinations. Whereas the stereomicroscope and compound light microscope are used for many applications in a forensic laboratory, the polarized light microscope is generally used for specialized trace evidence examinations. Using higher magnification and the additional components found in a polarized light microscope, an analyst can identify important characteristics for a particular sample.

Like the compound light microscope, the polarized light microscope uses a combination of lenses to produce a magnified image. In addition to the basic components, several other parts are added to enhance the analytical ability of the microscope. A polarized light microscope has the following basic parts:

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

In addition, some or all of the following components are added:

- polarizer
- analyzer
- compensators (first-order red, quarter-wave, quartz wedge)
- Bertrand Lens

Basically, the light originates from the illuminator and is collimated by the condenser. It then passes through the polarizer, which only allows light vibrating in a single plane. The now polarized light interacts with the sample on the microscope stage, and is then collected by the objective. As the light exits the objective, it passes through the analyzer (if allowed) and onto the oculars. The analyzer is similar to the polarizer in that it only allows light vibrating in a single plane to pass. The oculars receive this image and re-focus it onto the viewer's eye. Various compensators and a Bertrand Lens can also be inserted for specific applications.

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

with crossed polars, an arrangement with the polarizer and analyzer oriented perpendicular to each other, to obtain their microscopic characteristics. The addition of compensators and a Bertrand Lens also provides additional microscopic characteristics.

The polarized light microscope is used to identify and characterize samples. The higher level of magnification allows viewing of the initial characteristics of a sample, with additional comparison and microscopic examinations also possible.

Experiment 3A: Familiarization with the Polarized Light Microscope

Recommended pre-lab reading assignments:

Brenner M. Understanding the Polarizing Microscope. *American Laboratory*. 1980; 42(4): 71.
De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Prentice Hall; 2002; 282–5.

Recommended website:

Abramowitz M, Davidson MW. Polarization of Light. [Java Interactive Tutorial]: Olympus American Inc.; 2007 [updated 2007; cited 2007 October 23]; Available from: <http://www.olympusmicro.com/>.

OBJECTIVE

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

1. components of the polarizing light microscope
2. use of the polarizing light microscope
3. refractive index
4. birefringence
5. isotropic and anisotropic
6. color and pleochroism
7. observance of extinction points and birefringence
8. observance of isotropic and anisotropic materials
9. observance of pleochroism
10. inherent color and interference color

INTRODUCTION

As you learned with use of the stereomicroscope and compound microscope, a microscope is an optical instrument that uses a combination of lenses to produce a magnified image of small objects. To accomplish this aim, like a compound microscope, the polarized light microscope (PLM) 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. However, in a PLM, additional parts enhance the microscope's analytical ability. It contains the basic components: light source, condenser, sample stage, objective, support and alignment portions, and eyepieces, with the addition of a polarizer and analyzer. A polarizer is placed below the sample, usually in a carrier that is capable of rotating. An analyzer is placed above the sample typically at the back of the objective lens. The only difference between the polarizer and analyzer is the location and orientation; both are composed of the same type of specialized filters.

The theory behind a PLM is fairly simple. A polarizer is a polymer film that completely absorbs the light vibrating in all but one direction. In a PLM, basically, the light originates from the illuminator then passes through the polarizer and is collimated by the condenser (Figure 3A-1).

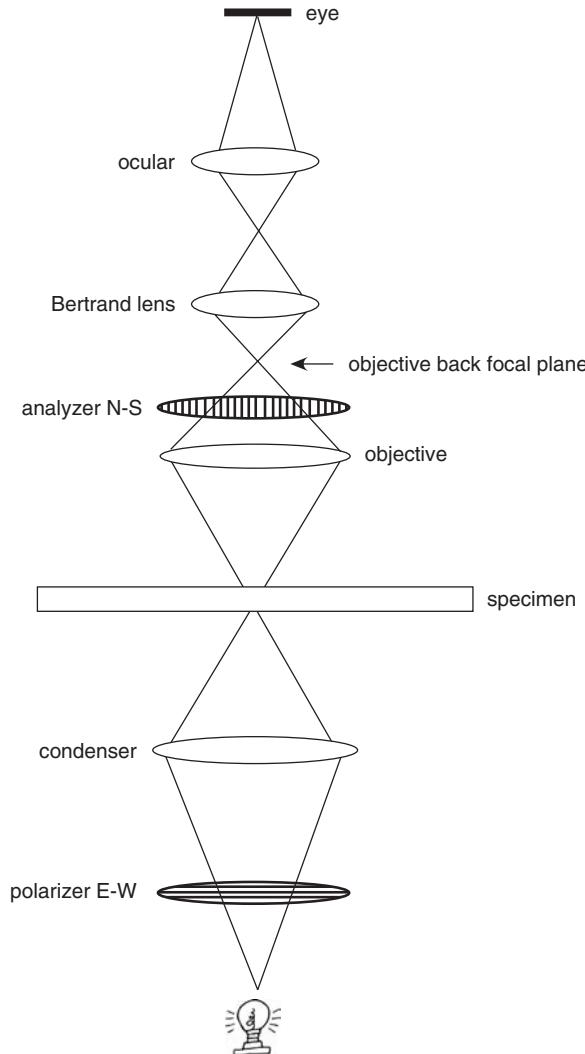


Figure 3A-1 Optical path for a polarizing microscope.

When the light passes through the polarizer, it is oriented in a specific vibrational direction, usually east–west (E–W). The plane-polarized light then interacts with the sample and is collected by the objective. The objective re-focuses the image through the analyzer (when used), which is oriented in the opposite direction from the polarizer, usually north–south (N–S) onto the back focal plane of the objective. The oculars receive this image and re-focus it onto the viewer's eye. The polarizer and analyzer are considered to be in the ‘crossed’ position when their vibrational

directions are perpendicular to one another. When this occurs, no light gets through so the field of view is black. However, when samples are placed on the stage, in the light path, they will react in a variety of manners. Some particles remain dark while others appear brightly colored or seem to ‘glow.’ The samples that glow under crossed polars are called anisotropic, which means they have more than one refractive index. All crystalline materials except for cubic crystals produce two polarized light components corresponding to the extraordinary and ordinary rays. Each of these resulting rays will have a different refractive index.

The numerical difference in these refractive indices is called birefringence or B and is shown in the following equation:

$$B = n_2 - n_1 \quad (3A-1)$$

where n_1 and n_2 correspond to the refractive index of extraordinary and ordinary rays.

Samples that have no effect on a polarized light beam regardless of their orientation are said be considered ‘isotropic’, because they have only one refractive index. Isotropic samples remain dark under crossed polars.

Anisotropic samples produce interference colors that correspond to the retardation or slowing down of one ray with respect to the other. Retardation, Δ , depends on both the thickness, t , and the birefringence, B , according to

$$\Delta = B \times t \quad (3A-2)$$

Since retardation or interference colors is reported in nm and thickness is measured in mm, it is often convenient to use the following equation:

$$\Delta = B \times t \times 1000 \frac{nm}{\mu m} \quad (3A-3)$$

Remember:

$$1 \text{ nm} = 10^{-9} \text{ meters}$$

$$1 \mu\text{m} = 10^{-6} \text{ meters}$$

$$1 \mu\text{m} = 1000 \text{ nm}$$

Interference colors should not be confused with natural or inherent color. Interference colors are observed under crossed polars while inherent color is observed with the analyzer out of the optical path. Both are important properties for identification and comparison of evidence samples.

The orientation of samples with respect to the polarizing and analyzing filters may also cause samples to appear differently. Samples that are aligned with the polarizer’s orientation will appear black. This is because the vibration direction of the light passing through the sample is aligned perpendicular to the analyzer’s vibration direction. These positions are called ‘extinction points.’ Extinction points occur every 90° whereas points of maximum brightness every 45° (see Figure 3A-2).

The extinction characteristics of samples can be useful in identification of materials. For example, cotton fibers never go completely dark upon rotation of the stage under crossed polars. This is due to the convolutions of the fibers produced during the growth of the cotton plant. Lack of extinction is considered a confirmatory test for cotton fibers.

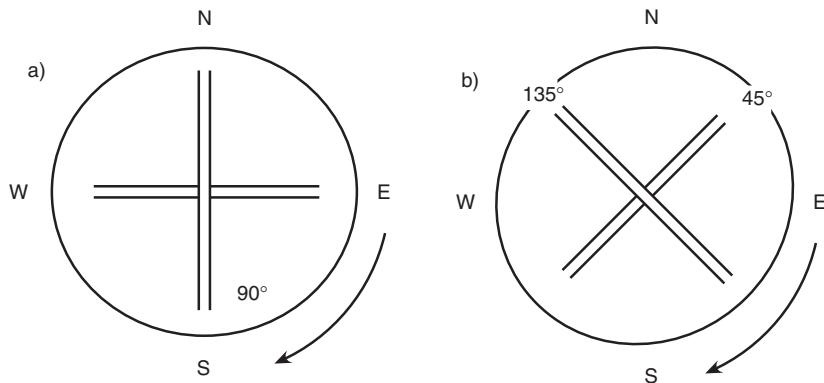


Figure 3A-2 A fiber is a typical anisotropic material. In this figure, a fiber is shown at different orientations on the rotating stage. a) Locations of extinction occur every 90°. In the E–W and N–S positions the fiber is in orientations that allow light to pass that is parallel to the polarizer but perpendicular to the analyzer. The analyzer then blocks the light resulting in extinction. b) Locations of maximum birefringence occur at the 45° and 135° positions. Here the fiber interacts with the polarized light producing two rays with two refractive indices. Some of the light is vibrating parallel to the analyzer and allowed to pass.

Another common microscopic examination of materials is to test for pleochroism. This is the phenomenon observed when a sample displays different inherent colors depending on its orientation with the polarizer's vibration direction. The sample is rotated with the analyzer removed from the light path. A pleochloric sample will show a change in inherent color upon rotation of the stage. Since pleochroism is a unique property, it can be used to compare and distinguish otherwise similar samples. The range of pleochroism for samples may be anywhere from slight differences in shade to a completely different color. Some materials show two colors or shades (called dichroic) and some show three colors (trichroic).

The optical properties of materials are used to characterize and distinguish different samples. Whether a sample is isotropic or anisotropic, its degree of birefringence and pleochroism are characteristics that are commonly determined for forensic samples.

EQUIPMENT AND SUPPLIES

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

McCrone™ Particle Reference Set

Micro kit

Tetraphenylcyclopentadienone

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your laboratory. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your laboratory. Dispose of glass in an appropriate container.

PART I: PARTS OF A POLARIZED LIGHT MICROSCOPE

Label the parts of the Leica DM EP™ polarized light microscope (see Figure 3A-3) by writing the name next to the appropriate number. A copy of this worksheet can be obtained from <http://www.wileyeurope.com/college/wheeler>.

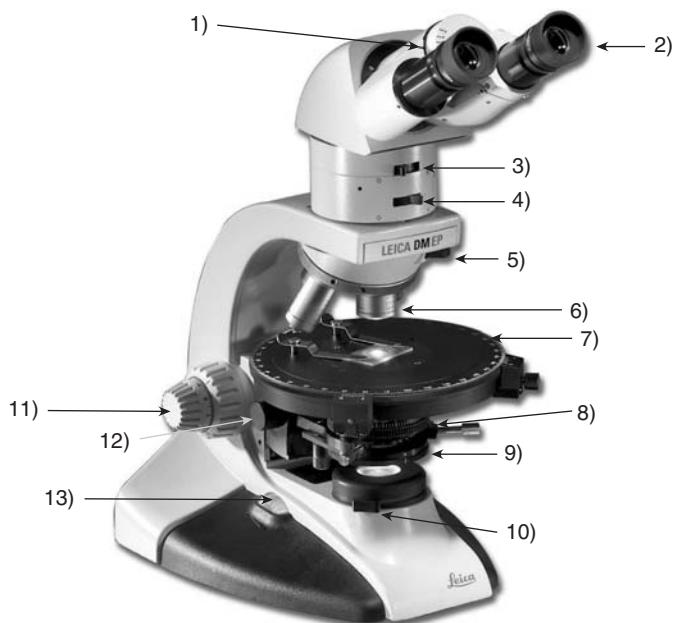


Figure 3A-3 Photograph of a DM EP™ polarizing light microscope. (Reproduced with permission of Leica Microsystems, Inc.)

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

PART II: OPERATION OF THE POLARIZED LIGHT MICROSCOPE

Procedure

1. Familiarize yourself with the polarized light 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. Set up Köhler illumination and have your instructor sign the Köhler Check Sheet.
5. Determine the orientation of the polarizer on your microscope.
6. Determine the orientation of your analyzer.

PART III: ISOTROPIC VERSUS ANISOTROPIC

Procedure

1. Using the polarized light microscope, set the polarizer and analyzer so that you have obtained ‘crossed polars.’ If the polars are properly crossed, the field of view should go completely black when the analyzer is introduced into the light path of the microscope. If the field of view does not go perfectly black, the polars are not perfectly perpendicular and either the polarizer or the analyzer must be adjusted to correct this. The polarizer may be adjusted by simply holding the polarizer and rotating it. It should be left in the position that results in the darkest field of view possible.
2. Examine the following prepared microscope slides, documenting what you see. Make sure you note the sample, the magnification, orientation of polarizing filters (crossed/uncrossed), and sample orientation for each drawing:

quartz	olivine
calcite	ground glass
mineral wool	diatoms

Drawings should be made in both polarized settings. Record whether the particles in each of the prepared slides are isotropic (I) or anisotropic (A).

PART IV: EXTINCTION POINTS AND BIREFRINGENCE

Procedure

1. Place a prepared slide of nylon on the microscope stage.
2. Align the fiber with the orientation of the polarizer.
3. Place the analyzer in position. The field of view should be black, including the fiber.
4. Rotate the fiber 90°. Does the fiber go to extinction?
5. Place the fiber at a 45° angle. Describe and draw what you see.
6. Repeat steps 2–5 with acetate fibers.

PART V: COLOR AND PLEOCHROISM

Procedure

1. Mount a few crystals of tetraphenylcyclopentodienone (TPCPN).
2. Pick a bright grain under cross polars. Rotate to extinction.
3. Take analyzer out. Note the inherent color.
4. Rotate the sample 90°. Note the color. If the crystal did not exhibit pleochroism the color would be the same regardless of the orientation.
5. Draw the colors for both orientations, labeling your notes.

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 basic components of a PLM? What function does each component perform in PLM?
2. Explain the optics used in a polarized light microscope.
3. Name three types of evidence that could be examined with a PLM. Of what would the examination consist?
4. What types of evidence would not be examined with the PLM? Why?
5. Explain plane polarized light.
6. What is isotropic? How can you determine if something is isotropic?
7. What is anisotropic? How can you determine if something is anisotropic?
8. What is birefringence?
9. What is pleochroism?
10. During a given rotation of the stage, how many times does a sample that exhibits typical birefringence exhibit extinction?
11. What are the locations of maximum birefringence during a complete stage rotation? How many times does this occur during one stage rotation?
12. Do a web search of the items in Part III. State their composition. Where are they likely to be found in casework? Why are they isotropic or anisotropic?

Experiment 3B: Determining Refractive Index of Anisotropic Materials

Recommended pre-lab reading assignments:

Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006; 580–6.

De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 305–9.

OBJECTIVE

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

1. difference in isotropic and anisotropic materials
2. use of polarized light to orient anisotropic materials
3. measurements of two refractive indices for fibers (n-parallel and n-perpendicular)
4. calculation of birefringence of fibers

INTRODUCTION

When samples are placed on the stage of the polarizing light microscope in the optical light path, they react in a variety of manners. Some samples, which have no effect on a polarized light beam regardless of their orientation, are said to be ‘isotropic.’ Isotropic materials have no repeating crystal structure and therefore have only one refractive index. The best example of isotropic materials is glass. Determination of refractive index using the Becke Line immersion method is used to determine the refractive index of isotropic materials as in Experiment 2D (Chapter 2).

As demonstrated in Experiment 3A, anisotropic materials glow under crossed polarizers producing interference colors. Anisotropic materials have more than one refractive index. All crystalline materials except for cubic crystals produce two polarized light components corresponding to the extraordinary (e-ray) and ordinary ray (o-ray). When the material has more than one refractive index the refractive index is found to vary with the orientation of the material with respect to the vibration direction of the light. This is because anisotropic materials contain a repeating structure, which differs by direction creating two or more paths for light to travel. The Becke Line immersion method can also be used to measure multiple refractive indices within a single sample, however the procedure now requires control of the orientation of the sample. It is the use of polarized light that allows the orientation of the sample to be controlled always with respect to the vibration direction of light.

We will focus on fibers for this experiment because it is relatively easy to control the orientation of the sample. Fibers are composed of repeating monomer units so that they vary in density in two directions. This creates two axes causing light to be separated into two rays inside the fiber. One

direction is parallel to the length of the fiber, n_{\parallel} -parallel, while the other is perpendicular to the length of the fiber, n_{\perp} -perpendicular. For fibers it will always be true that in one direction light will encounter a higher density of atoms. This causes the light to be retarded in that direction. Light moving in this direction is called the slow ray. Perpendicular to this direction light encounters a lower density of atoms and moves at a faster velocity. Light moving in this direction is called the fast ray. Refractive index has been defined in the previous chapter by Equation 2D-1 (page 30), and it can easily be shown that the slower ray has a higher refractive index and vice versa. In order for fibers to be identified both refractive indices must be measured.

The polarizing light microscope is used to determine both refractive indices. The polarizer allows light to pass, which vibrates in only one direction, typically, E–W polarized. Since plane polarized light is necessary for this procedure the analyzer is removed from the light path. To determine n_{\parallel} -parallel, the fiber is oriented on the stage so that the length is parallel to the polarizer (Figure 3B-1).

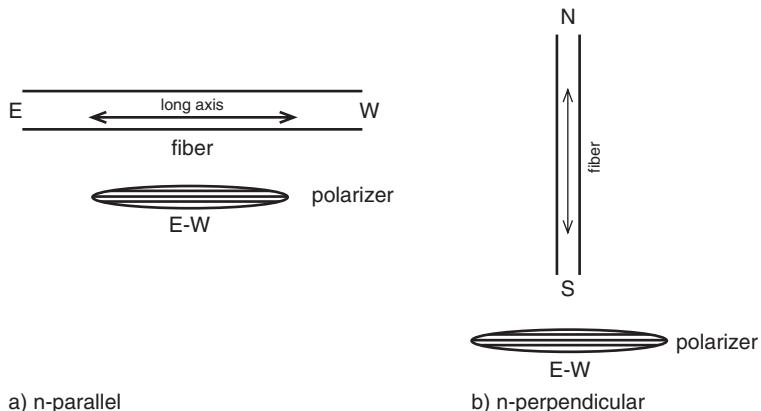


Figure 3B-1 a) A fiber oriented with its length parallel to the vibration direction of the polarizer allows n_{\parallel} -parallel to be measured. b) A fiber oriented with its long axis perpendicular to the vibration direction of the polarizer allows n_{\perp} -perpendicular to be measured. In order to properly orient the fiber the vibration direction of the polarizer must be known.

Once in this orientation, the Becke Line immersion method is used to determine if the immersion oil has a higher or lower refractive index than the fiber's n_{\parallel} -parallel value. As in the case of glass refractive index determination, the Becke Line will move to the medium of higher refractive indices when the focal distance is increased. To determine n_{\perp} -perpendicular, the fiber is rotated so that the length is perpendicular to the polarization direction of the polarizer. For an E–W polarizer, this results in a N–S orientation of the fiber. The Becke Line immersion method is repeated until minimum relief is observed in this direction. Once n_{\parallel} -parallel and n_{\perp} -perpendicular have been determined the birefringence can be determined by subtraction according to the following equation:

$$\text{Birefringence of fibers} = n_{\parallel} - n_{\perp} \quad (3B-1)$$

Although not as common, some tables report a single weighted average known as the isotropic refractive index for fibers where

$$\text{isotropic refractive index of fibers} = \frac{1}{3}(n_{\parallel} + 2n_{\perp}) \quad (3B-2)$$

For anisotropic minerals and crystals, the orientation of crystal must be known in order to determine the respective refractive indices. This represents a more complicated situation since it is possible for the mineral to have up to three refractive indices. Orientation of crystals with respect to vibration direction is determined using conoscopic illumination and is covered in Experiment 18A.

EQUIPMENT AND SUPPLIES

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

McCrone™ Particle Reference Set

Unknown fibers

Microscope slides and cover slips

Cargille™ refractive index oils

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by you laboratory. Dispose of glass in an appropriate container.

PART I: OBSERVING n-PARALLEL AND n-PERPENDICULAR

Procedure

1. Set up the polarizing light microscope for Köhler illumination.
2. Next you will determine the vibration direction of the polarizer on your microscope. Using a 10X objective, focus on a prepared slide of Dacron from the McCrone™ Particle Reference Set. Select a straight portion of the fiber. With the polarizer in and no analyzer, rotate the fiber so that you observe its relief in both the E-W and N-S orientation. Since the mounting medium of the slide is near the n-parallel refractive index, it will exhibit minimum relief when it is parallel to the vibration direction of the polarizer. If this occurs when the fiber is oriented in the E-W direction then your polarizer allows light vibrating in the E-W direction to pass.
3. OPTIONAL: Adjust the polarizer to match the cross hairs of your microscope. Rotate the Dacron fiber to align directly with the orientation of the polarizer. For example, rotate the fiber until it is perfectly E-W if your polarizer is E-W polarized. Now rotate your polarizing filter until you obtain minimum birefringence. This requires that the polarizer not be in a fixed position.
4. Remove the analyzer. Determination of n-parallel and n-perpendicular is performed with plane polarized light.
5. Close down the condenser aperture to increase contrast and to prevent the formation of a false Becke Line. A false Becke Line may be observed as a second bright line, which moves in the opposite direction of the Becke Line. This is visible when the difference of the refractive index of a sample and the immersion liquid is low and the true Becke Line is faint.
6. Place a single polyester fiber on a microscope and add a cover slip.

7. Choose Cargille™ oil close to one of the known refractive indices for polyester (n -parallel: 1.720; n -perpendicular: 1.540). Allow a drop of it to be drawn under the cover slip.
8. Place the microscope slide on the stage with the length of the fiber parallel and perpendicular to the plane of polarized light. Examine the fiber under high power. Explain what you see in these two orientations. At what orientation does it disappear? Why?
9. Rotate the fiber to the direction that is not a match. Using the focus, adjust the position while observing the edges of the fiber. When the fiber is slightly out of focus, the Becke Line will appear either inside or outside the fiber edge. The Becke Line will move towards the higher refractive index when the focal distance is increased. Does the Becke Line move into or out of the fiber? Why?

PART II: DETERMINING n -PARALLEL AND n -PERPENDICULAR OF AN UNKNOWN FIBER

Procedure

1. Obtain an unknown fiber.
2. Using the polarizing light microscope, check the lighting to ensure that samples will be viewed using Köhler illumination.
3. Place the unknown fiber on a microscope slide and add a cover slip. Place a drop of Cargille™ oil on the edges of the cover slip and allow it to be drawn up under the cover slip.
4. Place the microscope slide on the stage and focus using high magnification and plane-polarized light.
5. Rotate the fiber so that the length of the fiber is parallel to the plane of polarized light. For most microscopes this would be E-W.
6. Close down the condenser to prevent the formation of a false Becke Line. A false Becke Line may be observed as a second bright line, which moves in the opposite direction of the Becke Line. This is visible when the difference of the refractive index of a sample and the immersion liquid is low and the true Becke Line is faint.
7. Using the focus knob, adjust the focus while observing the edges of the fiber. When the fiber is slightly out of focus, the Becke Line will appear either inside or outside the fiber edge. The Becke Line will move towards the higher refractive index when the focal distance is increased. Document which medium has the higher refractive index.
8. Rotate the fiber so it is oriented perpendicular to the plane of polarized light. For most microscopes this would be N-S.
9. Once again focus the edges to determine which medium has the higher refractive index and document the answer.
10. Repeat with different Cargille™ oils until you are able to determine the refractive indices for your fiber. Determine which liquid to use next, depending on your previous results.
11. Continue until you have determined the minimum relief for the two directions of your fiber. Report n -parallel and n -perpendicular for the room temperature.
12. Using the temperature coefficient on the bottle of Cargille™ oils that were a match, report the refractive index at 25°C.
13. Calculate the birefringence of the fiber at room temperature using Equation 3B-1. Also, calculate the refractive index at 25°C using Equation 2D-4. Using the data found in Appendix A and B identify your unknown fiber.

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. Define isotropic and anisotropic.
2. Why do fibers have more than one refractive index?
3. Explain the difference between plane polarized light and crossed polars.
4. If a fiber is oriented in the N–S direction, which refractive index is being measured using E–W polarized light? Why?
5. Why was the analyzer removed from the light path?
6. Can this procedure be used on a compound light microscope?

Experiment 3C: Determining Birefringence and Sign of Elongation

Recommended pre-lab reading assignments:

De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 284–5.

Recommended website:

Griffin JD, Johnson ID, Davidson MW. Specialized Microscopy Techniques – Polarized Light Microscopy – Compensators and Retardation Plates. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited 2007 October 25]; Available from: <http://www.olympusmicro.com>.

OBJECTIVE

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

1. common compensators
2. sign of elongation of fibers
3. birefringence of fibers
4. use of the polarizing light microscope to determine sign of elongation
5. use of compensators to determine birefringence of fibers

INTRODUCTION

Many optical properties can be determined for samples with the aid of a polarizing light microscope and a compensator. As learned in the previous experiments, birefringence is the numerical difference in the refractive indices for a given sample. This can be determined by Equation 3A-1 or by several other methods with the aid of a compensator. The sign of elongation of anisotropic samples is a characteristic that can be determined with a polarized light microscope. It is a term that refers to how sample is elongated in relation to its refractive indices. If the refractive index for light parallel to the long axis of the sample, n_{parallel} , exceeds that of the refractive index of light perpendicular to the long axis of the sample, $n_{\text{perpendicular}}$, the sign of elongation is said to be positive. This means that the slow component vibrates in the longest direction of the fiber. If the reverse is true, the sign of elongation is negative.

Sign of elongation can be determined by two methods:

- by inserting a compensator into the optical path

- by measurement of both refractive indices followed by mathematical subtraction to determine the birefringence and sign of elongation according to Equation 3B-1 (p. 49)

In the previous experiment you used the Becke Line immersion method to determine the refractive index of n-parallel and n-perpendicular. These values were subtracted to give the birefringence. If the value obtained was a positive value, the sign of elongation was positive. Likewise, if the value obtained was a negative value, the sign of elongation was negative. In this experiment you will use a compensator to determine the sign of elongation and birefringence.

Compensators are made of various anisotropic crystalline materials. The amount of retardation is clearly marked on the compensator. They are usually marked to identify their slow direction, which is the higher refractive index direction. First-order compensators are made of selenite or quartz and produce a retardation of approximately 550 nm. Quarter-wave compensators are usually made of mica or quartz and produce a retardation of approximately 137 nm. Quartz wedges are composed of a ‘wedge’ of quartz of variable thickness and are used to compensate the retardation produced by a sample. First-order compensators and quartz wedges are most commonly used for forensic applications. When inserted into the light path under crossed polars a compensator will either add or subtract retardation according to their thickness and refractive indices. A change in the interference color observed is caused by either ‘addition’ or ‘subtraction’ to the light path. If the slow components of both the sample and the compensator are parallel, addition occurs. If the slow components of both the sample and the compensator are perpendicular to each other, then subtraction will occur. The Michel-Lévy chart located in Appendix C can be used to determine if the change in color is due to addition or subtraction. If the insertion of the compensator causes a new color that corresponds to a higher retardation color, addition has occurred and the color is said to have ‘moved up the chart.’ If the color change results in a lower retardation color, subtraction has occurred and the color is said to have ‘moved down the chart.’

We will focus on fibers for this experiment since the sign of elongation and birefringence is a characteristic that is commonly determined to identify and compare fiber evidence. A fiber has two optical axes in that light is separated into two rays inside the fiber. One ray is parallel to the long axis of the fiber while the other is perpendicular to the long axis. Because of how the fiber is organized internally, light will travel at different speeds in the two directions. Light that is retarded in one direction is said to be the slow ray and that direction will have a higher refractive index. Fibers are first observed in the NE–SE or 45° position under crossed polars. If interference colors other than gray are observed the fiber has a positive sign of elongation. That is, the slow ray is parallel to the length of the fiber and the refractive index in the elongated direction is greater than the refractive index in the perpendicular direction. If the fiber has a gray color in this position it can have a negative sign of elongation or a positive sign of elongation coupled with very low birefringence. Addition of a compensator adds a thickness to the optical path, which has the effect of changing the retardation of light. It is possible to add enough thickness so that the fast ray is completely retarded to match the speed of the slow ray. This causes the fiber to appear dark and mimics an isotropic sample. A quartz wedge has a variable thickness and allows the analyst to estimate the retardation needed to equalize the speed in the two directions. This retardation is used to calculate the birefringence provided the thickness can be determined using Equation 3A-3 (p. 43).

EQUIPMENT AND SUPPLIES

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

McCrone™ Particle Reference Set

Prepared slides of unknown fibers

First-order compensator

Quartz wedge

Michel-Lévy chart

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: SIGN OF ELONGATION USING THE FIRST-ORDER COMPENSATOR

Procedure

1. Obtain a prepared slide of acrylic.
2. After turning on the light source, adjust the microscope to obtain Köhler illumination.
3. Using the lowest magnification, place the prepared sample on the microscope stage and bring it into focus. Observe the acrylic fiber under crossed polars. Rotate this fiber into a NW–SE or 45° orientation. Draw what you see. Remember, if interference colors other than gray are observed the fiber has a + sign of elongation.
4. Insert the first-order compensator and redraw the sample.
5. Rotate the stage 90° to the 135° position (see Figure 3A-2) and redraw the colors.
6. Consult the Michel-Lévy chart and determine if addition or subtraction of interference colors is occurring in both positions. Is this a positive or negative fiber? Explain.
7. Next, obtain a prepared slide of acetate fibers.
8. Observe the fiber under crossed polars. Rotate the fiber into a NW–SE or 45° orientation. Draw what you see.
9. Insert the first-order compensator and redraw the colors.
10. Rotate the stage 90° to the 135° position and redraw the colors.
11. Consult the Michel-Lévy chart and determine if addition or subtraction of interference colors is occurring in both positions. Is this a positive or negative fiber? Explain.

PART II: SIGN OF ELONGATION USING THE QUARTZ WEDGE

Procedure

1. Obtain a prepared slide of polyester.
2. Observe the fiber under crossed polars. Rotate the fiber into a NW–SE or 45° orientation. Draw what you see.
3. Insert the quartz wedge and observe the color change.
4. Continue inserting the wedge until the center of the fiber becomes black. As you insert the quartz wedge you are increasing the retardation of the light. When the fiber goes black you

- have slowed down the fast ray until it equals the speed of the slow ray. The sample and quartz wedge are now isotropic (equal in all directions).
5. Rotate the stage 90° and observe what happens to the black color. Draw what you see. Because the slow direction of the quartz wedge is known, you can determine the slow and fast direction of the sample. Likewise, the elongation can be determined since you will know whether n-parallel or if n-perpendicular is greater. Is this a positive or negative fiber?
 6. Repeat steps 1–5 with a prepared slide of rayon and a prepared slide of nylon.

PART III: BIREFRINGENCE MEASUREMENT USING A QUARTZ WEDGE

Procedure

1. Obtain a prepared slide of your unknown fiber.
2. Observe the fiber under crossed polars. Rotate the fiber into a NW–SE or 45° orientation.
3. Determine if the unknown fiber is positive or negative using the procedure in Part I or Part II.
4. Measure the diameter of the unknown fiber using your calibrated ocular eyepiece.
5. Using crossed polars rotate the fiber into a NW–SE or 45° orientation.
6. Insert the quartz wedge while observing the color change at the center of the fiber. This is the location that corresponds to colors on the Michel-Lévy chart. Continue inserting the wedge until the center of the fiber becomes black. As you insert the quartz wedge you are increasing the retardation of the light. When the fiber goes black you have slowed down the fast ray until it equals the speed of the slow ray. The sample and quartz wedge are now isotropic and the center color of the fiber is said to be distinguished.
7. Read the amount of retardation from the quartz wedge. If your wedge is not calibrated (as is more often the case), you can determine the retardation by counting the number of red bands that pass on the outside of the fiber on its way to extinction. Each red band corresponds to 550 nm.
8. From the retardation calculate the birefringence of the fiber. For example, a nylon fiber is found to be 49 μm in diameter. Upon insertion of the quartz wedge, the color in the center of the fiber is extinguished between 5 and 6 orders of a quartz wedge. Its retardation is therefore 5.5 orders × 550 nm or 2,750 nm. The birefringence is determined using Equation 3A-3:

$$2,750 \text{ nm} = B \times 49 \text{ } \mu\text{m} \times 1000 \text{ nm}/\mu\text{m}$$

$$B = 0.056$$

From Appendix B this fiber can be identified as nylon 6,6.

9. Compare the sign of elongation and birefringence to the table found in Appendix B to identify your unknown. Justify your answer with both drawings and a paragraph.

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. Define the sign of elongation. How can it be determined?
2. What is the difference between sign of elongation and birefringence?
3. How is the sign of elongation used in forensic examinations?
4. What are the advantages and disadvantages of this optical property for forensic samples?
5. Two compensators were used in this experiment:
 - a) Draw a quartz wedge and explain what happens when it continues to be inserted.
 - b) How many wavelengths of retardation are added for a 550 nm compensator?
 - c) How many wavelengths of retardation are added for a 137 nm compensator?
6. A fiber is viewed in the NE–SW position under crossed polars. The background is black. The fiber has a positive sign of elongation.
 - a) Explain why the background changes from black to red upon insertion of the first order red compensator.
 - b) With the first order red compensator in position the fiber is rotated 90° to the NW–SE position. Would the color change be i) no change, ii) higher order, or iii) lower order? Fully explain your answer.
7. Explain why the center of the fiber went dark upon insertion of the quartz wedge in Parts II and III.

RECOMMENDED AND FURTHER READING

- Abramowitz M, Davidson MW. Polarization of Light. [Java Interactive Tutorial]: Olympus American Inc.; 2007 [updated 2007; cited 2007 October 23]; Available from: <http://www.olympusmicro.com/>.
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- Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006.
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- Griffin JD, Johnson ID, Davidson MW. Specialized Microscopy Techniques – Polarized Light Microscopy – Compensators and Retardation Plates. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited 2007 October 25]; Available from: <http://www.olympusmicro.com/>.
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- McCrone WC. The Case for Polarized Light Microscopy. *American Laboratory*. 1996; 28(9): 12.
- McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978.
- Robinson P, Bradbury S. *Qualitative Polarized Light Microscopy*. Oxford, United Kingdom: Oxford Science Publications (RMS), Oxford University Press, 1992.
- Weaver R. Rediscovering Polarized Light Microscopy. *American Laboratory*. 2003; 35(20): 55.

The Fluorescence Microscope

In the previous chapters we discussed microscopes used in many types of forensic examinations as well as many different sections of a forensic laboratory. Similar to the polarized light microscope, the fluorescence microscope is generally used for specialized examinations in the trace section. Using high magnification and the additional components found in a fluorescence microscope, an analyst can identify many important characteristics for a sample, which can be used for identification and comparison purposes.

Like other microscopes, the fluorescence microscope uses a combination of lenses to produce a magnified image. In addition to the basic components, several additional parts are added to enhance the analytical ability of the microscope. A fluorescence microscope has the following basic parts:

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

In addition, some or all of the following components are added:

- ultra-violet light source
- excitation filters
- barrier filters

Basically, the short wavelength light in a fluorescence microscope originates from a specialized illuminator. The light from the illuminator then passes through an excitation filter. The excitation light is redirected through the objective by a mirror so that it reaches the sample. Part of this light is absorbed by the sample and re-emitted as fluorescence. The re-emitted light then passes through a barrier filter. This filter allows light to pass that is in the visible range (fluorescence from the sample), permitting it to reach the oculars. Since the barrier filter blocks light emitted by the excitation filters, the background will appear black, allowing only fluorescence from a sample to be viewed.

Fluorescence microscopes generally use oculars and objectives that provide total magnification within the 40X to 400X range. Because of the higher total magnification, a smaller field of view and less depth of field are obtained. Samples may initially be viewed with transmitted light; however, reflected filtered light is sometimes used to obtain certain microscopic characteristics.

The fluorescence 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.

Experiment 4A: Familiarization with the Fluorescence Microscope

Recommended pre-lab reading assignments:

Siegel JI. Fluorescence Microscopy. *American Laboratory*. 1982; April: 65–9.

Recommended website:

Abramowitz M, Herman B, Murphy DB, Davidson MW. Anatomy of a Fluorescence Microscope. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited 2007 December 19]; Available from: <http://www.olympusmicro.com>.

OBJECTIVE

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

1. components of the fluorescence microscope
2. fluorescence
3. use of the fluorescence microscope to observe fluorescence

INTRODUCTION

The theory behind a fluorescence microscope is fairly simple. Some materials possess the property that when specific wavelengths of light fall upon them, they absorb this light and in turn re-emit light of a longer wavelength. This property is called fluorescence. The fluorescence microscope allows the forensic scientist to observe this property by selectively irradiating the sample and viewing the resultant fluorescence. Although many samples fluoresce, we will study the fluorescence properties of fibers in this exercise. Fiber evidence is class evidence (i.e., not unique) because many fibers from different sources could be indistinguishable. The discovery of a fiber and its identification as a particular fiber type (e.g., acrylic, cotton, nylon, polyester) may not, of itself, provide much support for a forensic investigation. The probative value of particular fibers found at a crime scene depends on their uniqueness relative to the background of fibers normally encountered. What is often required is information that makes the trace evidence more specific and discriminating. The fluorescence property of a questioned fiber may be unique, allowing it to be easily distinguished from other fibers.

In earlier experiments we learned that a microscope is an optical instrument that uses a combination of lenses to produce a magnified image of small objects. Just like a compound or polarized microscope, the fluorescence microscope uses several basic optical 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. These basic optical components are: light source, condenser, sample stage, objective, support and alignment portions, and oculars. In addition, the fluorescence microscope uses additional parts that enhance the microscope's analytical ability. These additional components are

excitation and barrier filters and a light source, which extends into the UV region of the spectrum for excitation. Excitation filters are placed between the illuminator and the sample while the barrier filters are placed between the sample and the oculars.

The operation of a fluorescent microscope is shown in Figure 4A-1. In a fluorescence microscope, the short wavelength light originates from a specialized illuminator. Since fluorescence is easily examined with reflected light, the illuminator is usually mounted at the top of the microscope. Usually a high-pressure xenon or mercury lamp is used. The light from the lamp is passed through an excitation filter, which allows only a narrow band of specific wavelengths to pass through. Excitation filters are normally in the UV range of light. The excitation light is redirected

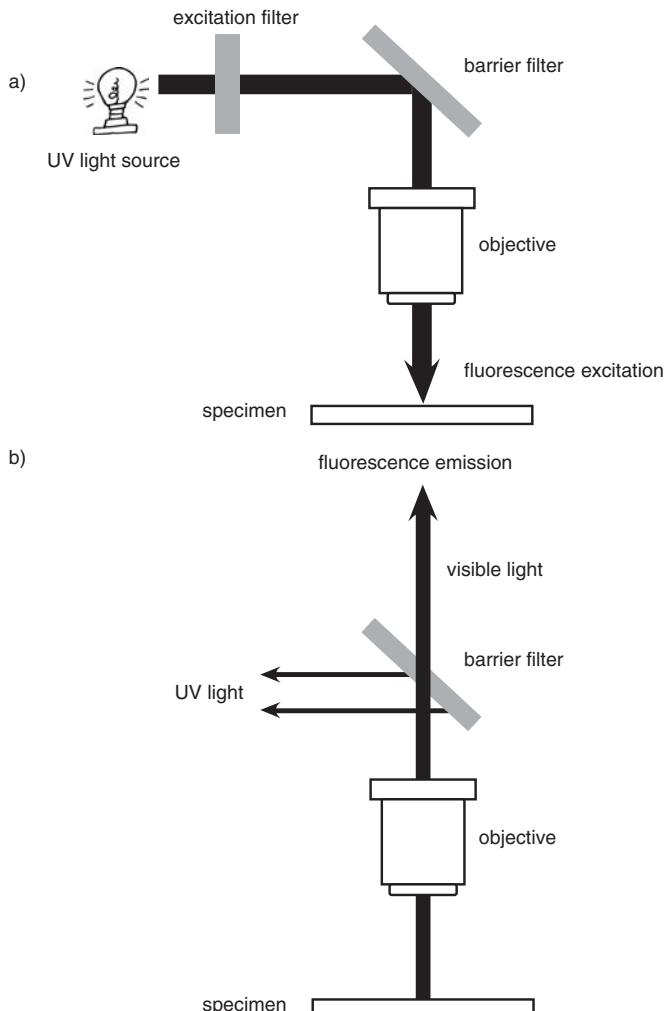


Figure 4A-1 a) Fluorescence excitation occurs when UV light travels from the light source to the specimen. The wavelengths used to cause excitation vary with each sample so a variety of excitation filters are used. b) Fluorescence excitation is detected by measuring the light coming off the specimen. UV light from the light source is blocked by a barrier filter.

through the objective by a mirror so that it reaches the sample. Part of this light is absorbed by the sample and re-emitted as fluorescence. The re-emitted light then passes through a barrier filter, which is used to separate the light used to excite the sample from the fluorescence. It does this by only allowing light to pass that is in the visible range (fluorescence from the sample), permitting it to reach the oculars. Since the barrier filter blocks light emitted by the excitation filters, the background will appear black, allowing any fluorescence from a sample to be easily viewed.

Samples may initially be viewed with transmitted light; however, reflected filtered light is used to obtain their fluorescent microscopic characteristics. A series of four excitation filters – ultraviolet, violet, blue, and green – are usually recommended for forensic use. It is important to note that glass slides and most mounting mediums can absorb UV radiation or fluoresce in and of themselves. A strongly fluorescing sample may still be visible with a glass cover slip, but weak fluorescence might be missed. In order to minimize interfering fluorescence, it is best to use quartz slides and cover slips. Since most of the common mounting media also fluoresce, it is important to use mounting mediums such as methanol, xylene, Norland 65TM, and XAMTM, which do not fluoresce.

EQUIPMENT AND SUPPLIES

Fluorescence microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and various excitation and barrier filters

Micro kit

Fibers with known fluorescence

Quartz microscope slides

Quartz cover slips

Mounting medium such as methanol, xylene, Norland 65TM, or XAMTM

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Mercury or xenon lamps generate extreme heat and may explode. Do not look at the lamp directly to avoid eye damage. Use the safety shield if it is provided. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: PARTS OF A FLUORESCENCE MICROSCOPE

Label the parts of the fluorescence microscope in Figure 4A-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>.

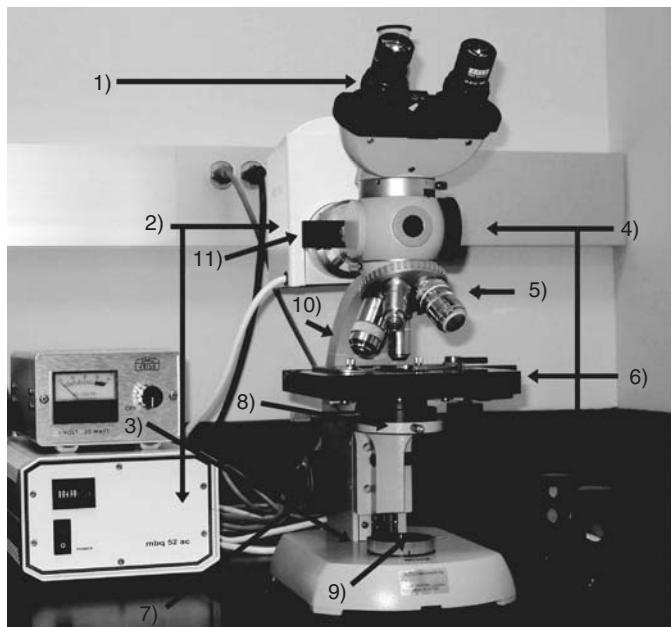


Figure 4A-2 A typical fluorescence microscope with additional filter cubes.

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

PART II: OPERATION OF THE FLUORESCENCE MICROSCOPE

Procedure

1. Familiarize yourself with the fluorescence microscope. Locate each part of the microscope. Place a prepared microscope slide on the stage. After turning on the transmitted light source, manipulate the oculars of the microscope to adjust the interpupillary distance so that when viewing an object, the right and left images merge 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. Set up Köhler illumination and have your instructor sign the Köhler Check Sheet.
5. Determine the location of the specialized illuminator.
6. Determine the location of the excitation and barrier filters.

PART III: OBSERVING SAMPLE FLUORESCENCE

Procedure

1. Turn on the power supply for the specialized illuminator. Slide lamp diaphragm into the closed position while the lamp warms up.
2. Prepare the sample using non-fluorescent slides, cover slips, and mounting medium.
3. Turn on the power supply for the base light illuminator. Adjust the microscope to obtain Köhler illumination.
4. Using the lowest magnification, place the prepared sample on the microscope stage and bring it into focus. Lower the intensity of the base light and room lighting. (Steps 3 and 4 are only necessary if the base light will be used to aid in the visualization of samples.)
5. Open the lamp diaphragm on the specialized illuminator. Refocusing of the sample may be necessary.
6. Observe the sample and note the color of any light being emitted. Change excitation and barrier filters and repeat your observations. In your notes fully describe the fluorescence characteristics of your sample.
7. Repeat this examination with three more samples.

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. Explain how a fluorescence microscope is used in forensic science. Give the purpose and value of the examination.
2. What is fluorescence? How is fluorescence initiated?

3. What are the advantages and disadvantages of this optical property for forensic samples?
4. Explain the optics used in a fluorescence microscope.
5. If a forensic scientist has a polarizing microscope such as a Leica DMEP, which they want to convert to a fluorescence microscope, could they use the same light bulb? Why or why not?
6. What is the purpose of the excitation and barrier filters?
7. How are excitation and barrier filters paired?
8. Does the color of light emitted from a fluorescent sample have any significance in distinguishing and identifying the sample?
9. Why were quartz slides and cover slips used?
10. Name two types of evidence that could be examined with a fluorescence microscope. Of what would the examination consist?
11. What types of evidence would not be examined with the fluorescence microscope? Why?

RECOMMENDED AND FURTHER READING

- Abramowitz M, Herman B, Murphy DB, Davidson MW. Anatomy of a Fluorescence Microscope. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited 2007 December 19]; Available from: <http://www.olympusmicro.com>.
- Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006.
- Fong W. Analytical Methods for Developing Fibers as Forensic-Science Proof – a Review with Comments. *Journal of Forensic Sciences*. 1989; 34(2): 295–311.
- Herman B. *Fluorescence Microscopy*. 2nd ed. Oxford, UK: Bios Scientific Publishers; Springer in Association with the Royal Microscopical Society, 1998.
- Siegel JI. Fluorescence Microscopy. *American Laboratory*. 1982; April: 65–9.
- Wang XF, Herman B. *Fluorescence Imaging Spectroscopy and Microscopy*. New York: John Wiley & Sons, Inc., 1996.
- Wiggins K, Holness JA. A Further Study of Dye Batch Variation in Textile and Carpet Fibres. *Science & Justice*. 2005; 45(2): 93–6.

The Phase Contrast Microscope

The stereomicroscope and compound light microscope are used for many applications in different sections of a forensic laboratory. Similar to the polarized light microscope, the phase contrast microscope is used for specialized examinations in the trace section. Using higher magnification and additional components a phase contrast microscope allows the analyst to identify important characteristics of a forensic sample.

Like other optical microscopes, the phase contrast microscope uses a combination of lenses to produce a magnified image. In addition to the basic components, several additional parts are added to enhance the analytical ability of the microscope. A phase contrast microscope has the following basic parts:

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

The following components are added:

- phase annulus
- phase plate

Basically, the light from the illuminator is passed to the condenser. In the phase contrast microscope the condenser is modified by replacing the substage iris with a phase annulus. The phase annulus is a centerable mechanical device that allows only a ring of light to pass into the condenser and to be focused upon the specimen. The purpose of the phase annulus is to separate the light into direct rays and diffracted rays. Once the light has exited the specimen, it is once again modified by a phase plate. A phase plate is a standard objective modified with a ring of material that retards the light coming through the objective. This directly corresponds to the light being allowed through by the phase annulus. This phase retardation interferes destructively with the direct light and reduces its amplitude, usually by $\lambda/2$. The light being diffracted by the sample does not go through this ring, and its amplitude is therefore not affected. The net effect of this light diffraction is that there is a bigger difference in the amplitude of the direct and diffracted rays, which in turn makes the image more visible.

Phase contrast microscopes generally use oculars and objectives that provide total magnification within the 40X to 400X range. 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 phase contrast 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.

Experiment 5A: Familiarization with the Phase Contrast Microscope

Recommended pre-lab reading assignments:

McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978; 61–4.

Recommended website:

Abramowitz M, Davidson MW. Specialized Microscopy Techniques – Phase Contrast. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited November 24, 2007]; Available from: <http://www.olympusmicro.com>.

OBJECTIVE

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

1. components of the phase contrast microscope
2. use of the phase contrast microscope to determine refractive index

INTRODUCTION

A microscope is an optical instrument that uses a combination of lenses to produce a magnified image of small objects. The phase contrast microscope accomplishes this using 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. In a phase contrast microscope, there are additional parts that enhance the microscope's analytical ability. It contains the basic components: light source, condenser, sample stage, support and alignment portions, and oculars, with the addition of a phase annulus or ring that modifies the condenser, and an objective with a phase plate.

The microscopist uses the difference in the refractive index of the mounting medium and the sample to enhance the contrast in the images seen. If the microscopist cannot improve the contrast of a sample by changing the mounting medium, as is the case with biological samples or when working with very tiny particles, he or she may need to work with a phase contrast microscope. The theory behind a phase contrast microscope is fairly simple. Since most transparent samples diffract light, a phase shift occurs in the rays of light that pass through them. Phase contrast microscopes take advantage of this shift by transforming the differences in light waves, to differences in the image. Because the sample being observed is either very thin or has a refractive index close to the mounting medium, the phase difference is undetectable by a routine compound light microscope. The phase contrast microscope renders undetectable phase differences into easily visible light and dark. This makes the image appear as if it has been ‘stained,’ which in turn enhances the ability to view the sample. Essentially, the

phase contrast microscope allows the forensic scientist to observe samples with greater contrast, which can be defined as the difference in brightness between the light and dark areas of an image.

This phenomenon takes place in the phase contrast microscope by the special placement of a phase annulus and phase plate in the light path as illustrated in Figure 5A-1. The phase annulus in the condenser separates the direct and diffracted light. This occurs because the phase annulus is a mechanical device that allows only a ring of light to pass into the condenser and to be focused upon the specimen. After passing through the sample, it is directed to the objective and phase plate. A phase plate is a standard objective with a ring of material that retards the light coming through, and corresponds to the light being allowed through by the phase annulus (see Figure 5A-2).

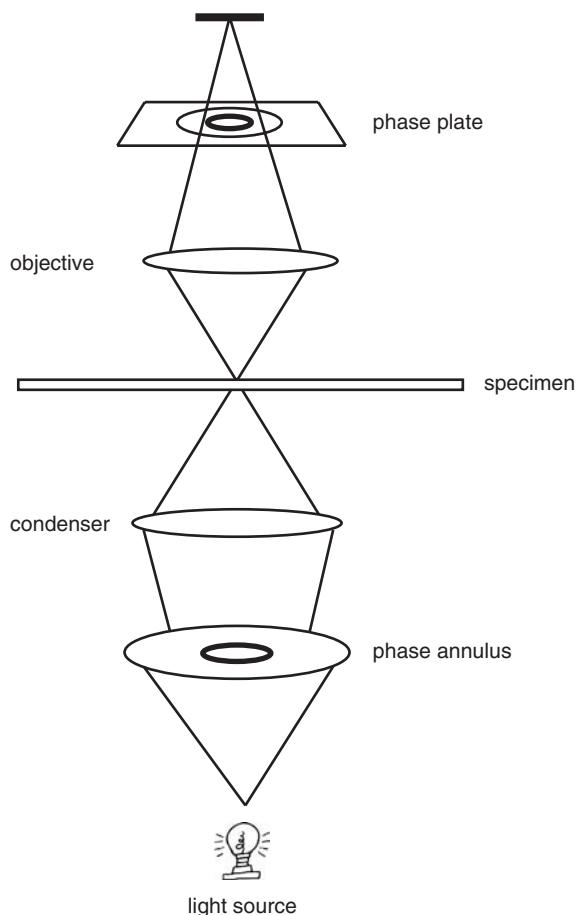


Figure 5A-1 Optical path of a phase contrast microscope.

At this point, the light that passes through the central part of the light path is recombined with the light that travels around the periphery of the sample. This phase retardation interferes destructively with the direct light and reduces its amplitude. The light being diffracted by the sample does not go through this ring, and its amplitude is therefore not affected. The interference produced by

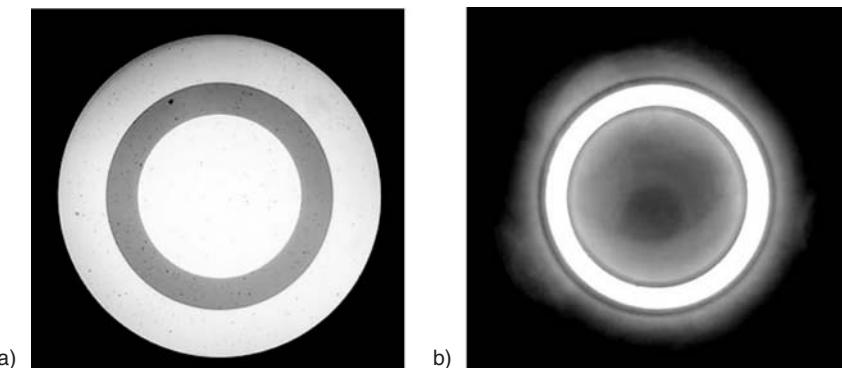


Figure 5A-2 a) Image of the back focal plane of the objective with a typical phase objective. This shows the phase plate. b) An image of the phase plate and phase annulus of condenser superimposed and correctly centered. (Reproduced with permission of David Walker, <http://www.micscape.org>)

these two paths produces images in which the samples appear darker than the background, making samples more visible.

EQUIPMENT AND SUPPLIES

Phase contrast microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X)
Micro kit
Glass samples
Microscope slides
Cover slips
Cargille™ refractive index liquid mounting set

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: PARTS OF A PHASE CONTRAST MICROSCOPE

The phase contrast microscope is a compound microscope that has two additional components, the phase annulus and phase plate. If necessary, re-familiarize yourself with the components of the compound light microscope (see Experiment 2A on page 14). The phase annulus is located in the condenser and is adjusted with each sample. Adjustments are made to the phase annulus by using setscrews attached to the condenser (see Figure 5A-3a). By adjusting these screws, the phase annulus is centered, so that the objective can form an image of the annular ring of light in the back focal plane. The phase plate is pre-set and located in the objective.



Figure 5A-3 A specialized condenser used on a phase contrast microscope. a) Adjustment screws are attached to the phase annulus, so that optimal contrast may be obtained for each sample. b) The phase annulus is a specialized condenser that separates the direct and diffracted light by allowing only a ring of light to pass and be focused upon the specimen. (Photo (b) reproduced with permission of David Walker, <http://www.micscape.org>)

PART II: OPERATION OF THE PHASE CONTRAST MICROSCOPE

Procedure

1. Familiarize yourself with the phase contrast microscope. Various brands of phase contrast microscopes are available. Familiarize yourself with the operations manual of the microscope so that you are able to center the phase rings prior to use.
2. 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 images merge as one.
3. Adjust the focus up and down. Using the non-adjustable ocular, focus on an item to obtain a clear image of an item.
4. Focus the second ocular if necessary.
5. Set up Köhler illumination and have your instructor sign the Köhler check sheet.
6. Determine the location of the phase annulus.
7. Determine the location of the phase plate.

PART III: USING THE PHASE CONTRAST MICROSCOPE

Procedure

1. Obtain a glass sample. Mount a small portion of the sample on a microscope slide and cover with a cover slip. Choose a Cargille™ oil close to the estimated refractive index of the glass sample. Place a drop of the oil next to the cover slip so that it is drawn under the cover slip. Turn on the power supply for the base light illuminator and adjust the light to provide an even, bright illumination over the entire field of view.
2. With the microscope set on the lowest magnification, adjust the interpupillary distance for the oculars, so that the right and left images merge into one.
3. Place the mounted glass samples on the stage and focus; if necessary focus (using the non-adjustable ocular) on the sample by using the coarse and fine focus knobs. Next, adjust the second ocular so that a crisp, clear image is viewed.

4. Check alignment of the phase annulus and phase plate.
5. Observe the sample, looking at the Becke Line. Using the focus knob, adjust the focus while observing the edges of the glass sample. When the glass is slightly out of focus, the Becke Line will appear either inside or outside the fiber edge. The Becke Line will move towards the higher refractive index when the focal distance is increased. Document which medium has the higher refractive index.
6. Repeat with an oil of different refractive index until you are able to determine the refractive index of the glass sample. Determine which liquid to use next, by depending on your previous results.
7. Repeat steps 1–6 for all assigned samples.

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 specialized components of a phase contrast microscope? What function does each component perform in phase contrast microscope?
2. Name two types of specimens that would be best viewed with a phase contrast microscope. Of what would the examination consist?
3. Why is this microscope commonly used by biologists?
4. Give an example of how this microscope is used by forensic scientists?
5. Explain the optics used in a phase contrast microscope.
6. Name two types of evidence that could be examined with a phase contrast microscope. Of what would the examination consist?
7. What are the advantages and disadvantages of using this microscope for forensic samples?

RECOMMENDED AND FURTHER READING

- Abramowitz M, Davidson MW. Specialized Microscopy Techniques – Phase Contrast. [Java Interactive Tutorial]: Olympus American, Inc.; 2007 [updated 2007; cited November 24, 2007]; Available from: <http://www.olympusmicro.com>.
- Allen R, Brault J, Zeh R. Image Contrast and Phase Modulation Light Methods in Polarization and Interference Microscopy. *Advances in Optical and Electron Microscopy*. 1966; 1: 77–114.
- De Forest PR. Foundations of Forensic Microscopy. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 301–5.
- Gerlovin BY. Calculation of the Optimum Dimensions of the Phase Rings of Phase-Contrast Microscope Objectives. *Soviet Journal of Optical Technology*. 1987; 54(10): 596–9.
- Hostounsky Z, Pelc R. Phase-Contrast Versus Off-Axis Illumination: Is a More Complex Microscope Always More Powerful? *Advances in Physiology Education*. 2007; 31(2): 232–5.
- Kong XG, Feng TS, Jin GF. Reflection Zernike Phase-Contrast Microscope. *Applied Optics*. 1990; 29(10): 1408–9.

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

Ojena SM, De Forest PR. Precise Refractive Index Determination by the Immersion Method Using Phase Contrast Microscopy and the Mettler Hot Stage. *Journal of the Forensic Science Society*. 1972; 12: 315–29.

Ross KFA. *Phase Contrast and Interference Microscopy for Cell Biologists*. London: Edward Arnold, 1967.

Application Experiments

Experiment 6: Physical Match Examinations

Recommended pre-lab reading assignments:

Thornton JI. Fractural Surfaces as Models of Physical Matches. *Journal of Forensic Sciences*. 1986; 31(4); 1435–38.

Adolf FP. Physical Fits Between Textiles. *Proceedings of the 3rd Meeting of the European Group*. Linkoping, Sweden. 1995.

OBJECTIVE

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

1. physical match examinations

INTRODUCTION

A comparative examination is the process of ascertaining whether two or more objects have a common origin. One way that this is accomplished is by a physical match examination. Once a comparative examination has been completed, the forensic scientist must be prepared to render a conclusion with respect to the origins of the specimens. Do they or do they not originate from the same source? Evidence that can be associated with a common source with an extremely high degree of probability is said to possess individual characteristics. Evidence is said to possess class characteristics when it can be associated only with a group and never with a single source.

Physical matching of broken, cut, or torn objects can be encountered in many types of forensic investigations. These types of examinations are performed to associate an item with the material from which it is thought to have originated. Physical match determinations may be performed in situations in which an item has been cut, torn, broken, or otherwise undergone a separation into two or more pieces. If a separation is the result of a random process, the particular separation

would not be expected to be repeated and should be considered unique. It is therefore possible that broken pieces could be fit together in a unique fashion to demonstrate a common origin.

The process of determining a physical match depends on the actual items themselves. For most items that have been cut or torn, a forensic scientist is able to determine whether or not the items were one piece at an earlier time by using edge shapes and surface features. These items are examined as a whole with the aid of a stereomicroscope (see Figure 6-1). Edges are examined for a physical ‘fit,’ similar to what is done when working a jigsaw puzzle. Surface features such as color, patterns, or unique markings, may also be used to help determine the actual placement of a section. In some cases, it may be necessary to use surface features only, because items that have been separated by ‘sawing’ will have had a small portion of the newly formed edge removed.



Figure 6-1 A Zeiss™ stereomicroscope. Placing a stereomicroscope on a boom stand allows more flexibility for various sized samples.

Items that have been broken may also be examined using edge shapes and surface features. However, at times additional examinations are also necessary. Prior to breaking, some items tend to bend or stretch. This can cause unique markings along the break edge. These markings, which appear as mirror images, can also be examined to determine whether or not two items were one piece at an earlier time.

Several conclusions can be reached for physical match examinations. First, if a physical match is obtained, it can be determined that the items were one at an earlier time. Sometimes a physical match is not possible. This may be a result of differences or the lack of characteristics to make a determination. In these situations, no indication should be given as to whether or not the items were one at an earlier time. However, when appropriate, further testing may be suggested to prove or disprove similarities between the items.

EQUIPMENT AND SUPPLIES

- Stereomicroscope
- Previously prepared sets of plastic pieces, cut up
- Previously prepared sets of playing cards, cut up
- Previously prepared sets of matchbooks and matches
- Previously prepared sets of fabric, cut up
- Previously prepared sets of broken microscope slides

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor.

PART I: PHYSICAL MATCH EXAMINATIONS USING EDGE SHAPE

Procedure

1. Obtain a set of plastic pieces.
2. Using the stereomicroscope, examine the edges of the pieces.
3. Describe the pieces. Any physical characteristics such as color and edge shape should be noted.
4. Using the circle template located in Appendix F, draw what you see for each piece.
5. Determine whether the pieces were ‘one’ at an earlier time. If you obtain a physical match, draw and state what you see. If you do not obtain a physical match, state the differences you found.
6. As a forensic scientist you must learn to form a conclusion and be able to communicate your conclusion verbally and in writing. Clearly state if you found a physical match between the plastic pieces. Explain how you reached this conclusion. If you do not obtain a physical match, state the differences you found.

PART II: PHYSICAL MATCH EXAMINATIONS USING EDGE SHAPE AND SURFACE FEATURES

Procedure

Playing cards

1. Obtain a set of playing card pieces.
2. Using the stereomicroscope, examine the edge shapes and surface features of the pieces.
3. Describe the playing card pieces. Any physical characteristics such as color, patterns, and edge shapes should be noted.
4. Draw what you see for each piece.

5. Determine whether the pieces were ‘one’ at an earlier time. If you obtain a physical match, draw and state what you see. If you do not obtain a physical match, state the differences you found.
6. Clearly state if you found a physical match between the playing card pieces. Explain how you reached this conclusion. If you do not obtain a physical match, state the differences you found.

Matches and matchbook

1. Obtain a match and matchbook set.
2. Using the stereomicroscope, examine the edge shapes and surface features of the match and matchbook.
3. Describe the match and matchbook in your notes. Any physical characteristics such as color, width, length, and thickness should be noted. Most matchbooks are made from reprocessed paper. Sheets of paper are cut and dipped. Examination of the sides of the match and the edge where it was separated from the matchbook may be performed. Examination of these edges may reveal fibers, which can be traced from one piece to another. Inclusions consisting of a large variety of colored fibrous material, aluminum foil, and other contaminants that were involved in the production of the paper may also be visible.
4. Draw what you see for the match.
5. Draw what you see for the matchbook.
6. Determine if the match and the matchbook were ‘one’ at an earlier time. If you obtain a physical match, draw and state what you see. If you do not obtain a physical match, state the differences you found.
7. Clearly state if you found a physical match between the match and matchbook. Explain how you reached this conclusion. If you do not obtain a physical match, state the differences you found.

Fabric

1. Obtain a set of fabric pieces.
2. Using the stereomicroscope, examine the edge shapes and surface features of the pieces.
3. Describe the fabric pieces. Any physical characteristics such as color, patterns, and edge shapes should be noted.
4. Draw what you see for each piece.
5. Determine whether the pieces were ‘one’ at an earlier time. If you obtain a physical match, draw and state what you see. If you do not obtain a physical match, state the differences you found.
6. Clearly state if you found a physical match between the fabric pieces. Explain how you reached this conclusion. If you do not obtain a physical match, state the differences you found.

PART III: PHYSICAL MATCH EXAMINATIONS AND ‘MIRROR IMAGES’

Procedure

1. Choose a broken glass microscope slide set.
2. Using the stereomicroscope, examine the edge shapes and actual edge of the glass pieces. Glass can be thought of as a super-cooled liquid. It will try to bend and stretch before it breaks. This creates ‘rib’ markings on the edge upon breaking.

3. Draw the ‘rib’ markings on one piece of glass.
4. Determine if the pieces ‘fit’ together. Make a drawing of the fit. (Some maneuvering is necessary.) Write a statement about whether or not the pieces fit together next to your drawing.
5. Clearly state whether you found a physical match between the glass pieces. Explain how you reached this conclusion. If you do not obtain a physical match, state the differences you found.

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 is a physical match?
2. Explain why physical matches are possible.
3. Explain the three general types of physical examinations possible and how examinations are performed for each. Give an example of another item in addition to what we examined in lab for each type of comparison analysis.
4. A glass examiner examines a small piece of glass and is able to make a physical fit to a windowpane found at the crime scene. The glass examiner makes an opinion as to whether or not the piece of questioned glass came from the windowpane? Which one of the following opinions can be made? Fully explain your choice.
 - a) Positive opinion of identity
 - b) Opinion of probable common source
 - c) Positive opinion of nonidentity
 - d) Opinion that no conclusion could be reached
5. A broom handle has been *sawed* in half. One piece is used to break a window at a crime scene (and left behind) and the other piece is still at the suspect’s garage. Can a physical match be made between the two pieces? Explain your answer.

RECOMMENDED AND FURTHER READING

- Adolf FP. Physical Fits between Textiles. *Proceedings of the 3rd Meeting of the European Group*. Linkoping, Sweden. 1995.
- Bisbing RE. Fractured Patterns: Microscopical Investigation of Real Physical Evidence. *The Locard Exchange*. 2004; January 29: 1–5.
- Farmer NL, Ruffell A, Meier-Augenstein W, Meneely J, Kalin RM. Forensic Analysis of Wooden Safety Matches – a Case Study. *Science & Justice*. 2007; 47: 88–98.
- Inman K, Rudin N. The Origin of Evidence. *Forensic Science International*. 2002; 126(1): 11–6.
- Lee HC, Harris HA. *Physical Evidence in Forensic Science*. Tucson: Lawyers & Judges Pub. Co., 2000.
- McJunkins SP, Thornton JI. Glass Fracture Analysis: A Review. *Forensic Science*. 1973; 2(1): 1–27.
- Thornton JI. Fractural Surfaces as Models of Physical Matches. *Journal of Forensic Sciences*. 1986; 31: 4.
- Tsach T, Wiesner S, Shor Y. Empirical Proof of Physical Match: Systematic Research with Tensile Machine. *Forensic Science International*. 2007; 166(1): 77–83.

Experiment 7: Construction Examinations of Evidence

Recommended websites:

Wellington Leisure Products. [Web Page] Macungie, PA 18062: The Lehigh Group; [cited 2007 October 30]; Available from: <http://www.wellingtoninc.com>.

The Carpet and Rug Institute. [Web Page] Dalton, GA: The Carpet and Rug Institute; [cited 2007 October 30]; Available from: <http://www.carpet-rug.org>.

OBJECTIVE

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

1. examinations to determine construction features of evidence
2. common construction features for ropes, carpets, and duct tape

INTRODUCTION

A construction examination is the process of ascertaining how an item is made, and in some situations, of what it is composed. Many times, this examination provides indications as to whether or not two items originated from the same source. Evidence that can be associated with a common source with an extremely high degree of probability is said to possess individual characteristics. Evidence is said to possess class characteristics when it can be associated only with a group and never with a single source. Once a construction examination is completed, the forensic scientist is able to determine whether additional testing is required.

Various compositions and methods of construction can be identified when performing a simple construction examination. These types of examinations are performed to associate an item with a similar item from which it is thought to have originated. For instance, a section of duct tape retrieved from a victim may be compared back to a roll of duct tape. The process of determining

construction depends on the actual item itself. For most items, this involves examining the item to such a degree that details can be obtained to determine identifying characteristics about the construction and composition. For instance, ropes are classified into two categories: twisted or braided. Twisted ropes usually consist of three strands. Strands are composed of twisted yarns, which are sections of fibers that have been spun together. Strands may be either right-laid (Z twist) or left-laid (S twist) (see Figure 7-1).

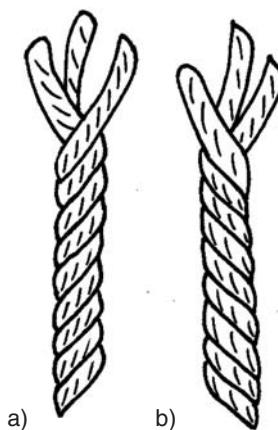


Figure 7-1 Two different twists for yarns. a) Z-twist is right-laid and b) S-twist is left-laid.

Braided ropes are made by the interweaving of three or more strands, which occurs in a diagonal overlapping pattern. Braided ropes are found in a variety of patterns: solid braid, diamond braid with or without a core, and twisted braid. Solid braid is the simplest braid, formed by interweaving numerous strands. Diamond braid without a core (also called hollow braid) is a looser braid with no core. Diamond braid with a core is a doubly braided rope. It is composed of a braid over a braid. Twisted braid ropes are composed of twisted strands that have been braided. Common rope configurations are found in Figure 7-2.

When examining a section of carpet, details can be learned about the backing and method of construction. Carpets are usually woven, knitted, or tufted. Woven carpets are produced on a loom, similar to cloth production. The carpet is created by interweaving the surface pile and the backing. This may be produced by a variety of different methods. Figure 7-3 shows side views of Wilton™, Axminster™, velvet, and chenille woven carpets.

These carpets are produced on different style looms, or in the case of chenille carpets, on several looms. Knitted carpets are created by a process that loops the backing yarn, the stitching yarn, and the pile yarn. Tufted carpets are produced on a tufting machine. After the backing has been woven, yarns are introduced that may be cut or left as loops. Most carpets manufactured today are either woven or tufted. Figure 7-4 shows yarns that have been tufted into two different backings.

In addition to the construction of the carpet, backing and adhesives holding the actual loops or tufts of the carpet yarns themselves may also be examined. Each loop or tuft can be examined for more detail as to yarn construction and fiber count. Differences in the yarn construction, fiber count, and whether or not the yarns are sheared or unsheared provide various textures. Woven and tufted carpets are constructed to provide a variety of textures. Figure 7-5 shows various types of tufted carpets that provide a wide assortment of textures.

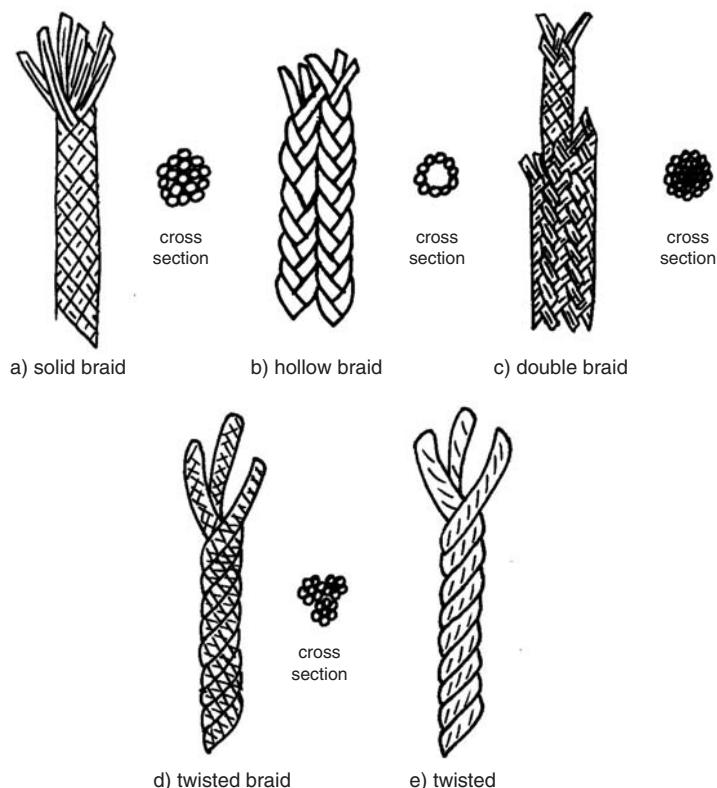


Figure 7-2 Common rope configurations shown in longitudinal and cross-sectional view.

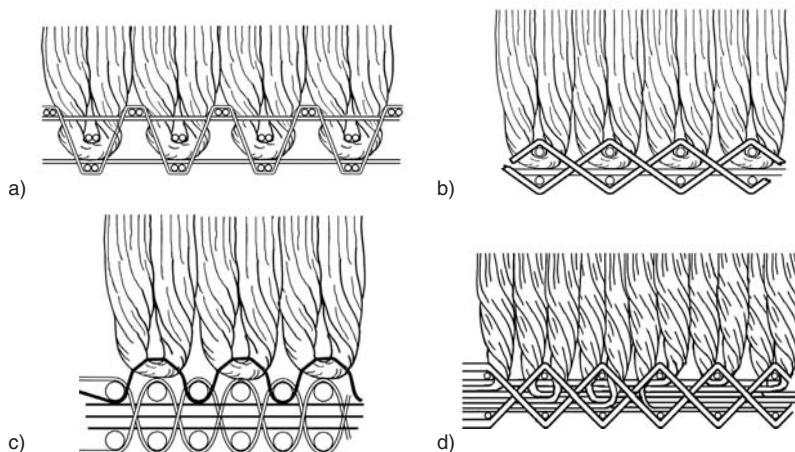


Figure 7-3 Common woven carpets: a) Axminster™; b) velvet; c) chenille; and d) Wilton™.

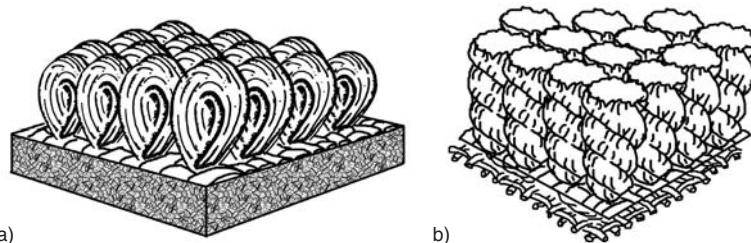


Figure 7-4 Tufted carpets with a) foam backing or b) double woven backing.

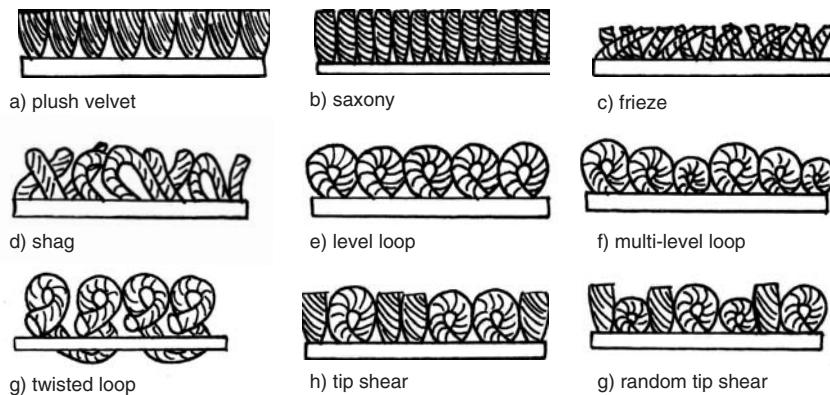


Figure 7-5 Common tufted carpet styles.

Duct tape may also be composed of various constructions. The simplest duct tape is composed of a vinyl backing with an adhesive. Many duct tapes are now reinforced with fibers. Fiber reinforcements may also be constructed in a variety of ways. Yarn count and whether or not the fiber reinforcement is woven and non-woven should be considered.

Since most construction examinations involve comparison to a known sample, conclusions are generally simple. If the construction and composition is similar, a conclusion may be reached which would indicate that the items may have originated from the same source. Further testing would be recommended to prove or disprove these similarities. At times, differences are also found that indicate more than one source.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Micro kit

Previously prepared sections of rope

Previously prepared sections of carpet

Previously prepared sections of duct tape

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor.

PART I: CONSTRUCTION EXAMINATIONS USING THE STEREOMICROSCOPE

Procedure

The stereomicroscope will be used to examine various items for their construction. Worksheets for each exam are located at the end of this experiment and are available at <http://www.wileyeurope.com/college/wheeler>.

Rope

1. Obtain a sample of rope.

Using the stereomicroscope, determine the construction of the sample. A rope worksheet is located at the end of this experiment. Draw the construction with further details about each portion. Any physical characteristics such as color, diameter, type of construction (twisted or braided), number of strands, direction of twist (Z or S see Figure 7-1.), type of braid, (see Figure 7-2) core, and/or sheaths present should be noted.

2. Repeat steps 2 and 3 using a second rope sample.

Carpet

1. Obtain a carpet sample.
2. Using the stereomicroscope, determine the construction of the sample.
3. A carpet worksheet is located at the end of this experiment. Draw the construction with further details about each portion. Any physical characteristics such as color, type of construction, texture, style, and carpet components to include details of the backings and tufts should be noted (see Figures 7-3, 7-4, and 7-5).
4. Repeat steps 2 and 3 using a second carpet sample.

Duct tape

1. Obtain a duct tape sample.
2. Using the stereomicroscope, determine the construction of the sample.
3. A duct tape worksheet is located at the end of this experiment. Draw the construction with further details about each portion. Any physical characteristics such as color, type of construction, backing, adhesive, width, thickness, yarn count, and weave pattern of fiber reinforcement should be noted.
4. Repeat steps 2 and 3 using a second tape 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. Explain why construction examinations are important.
2. Describe one type of rope construction in addition to the type you determined your samples to be.
3. What fibers are commonly used for rope production?
4. Describe one method of carpet construction in addition to the type you determined your samples to be.
5. What components are commonly used for backings, bonding agents, and fiber in carpet production?
6. Describe one method of tape construction in addition to the type you determined your samples to be.
7. What components are commonly used for backings, adhesives, and fiber reinforcements in duct tape production?

RECOMMENDED AND FURTHER READING

The Carpet and Rug Institute. [Web Page] Dalton, GA: The Carpet and Rug Institute; [cited 2007 October 30]; Available from: <http://www.carpet-rug.org>.

David SK, Pailthorpe MT. *Classification of Textile Fibres: Production, Structure, and Properties*. 2nd ed. London: Francis & Taylor, 1999.

Joseph ML. *Joseph's Introductory Textile Science*. 6th ed. New York: International Thomson Publishing, 1992.

Laux DL. Identification of a Rope by Means of a Physical Match between the Cut Ends. *Journal of Forensic Sciences*. 1984; 29(4): 1246–48.

Wellington Leisure Products. [Web Page] Macungie, PA 18062: The Lehigh Group; [cited 2007 October 30]; Available from: <http://www.wellingtoninc.com>.

Wiggins K. *Ropes and Cordage*. 2nd ed. London: Francis & Taylor, 1999.

Wiggins KG. Recognition, Identification and Comparison of Rope and Twine. *Science & Justice*. 1995; 35(1): 53–8.

ROPE WORKSHEET**Physical Characteristics:**

Color_____ Diameter_____

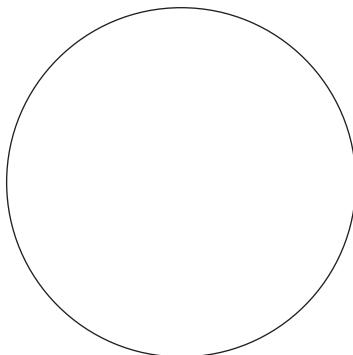
Construction type: twist_____ : (Z or S)

strands_____ threads/strand

braid_____ : solid_____ hollow_____ double braid_____

diamond braid_____ twisted braid_____

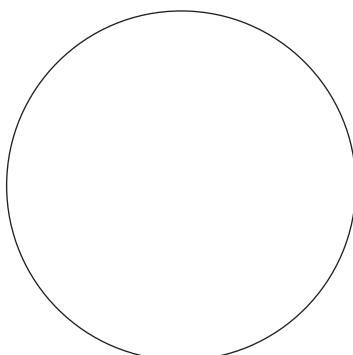
strands_____ threads/strand



core_____

strands_____ threads/strand

sheath_____



CARPET WORKSHEET**Physical Characteristics:**

Color_____

Construction type: texture and style_____

tufts/inch: warp_____ fill_____

adhesive_____

primary backing_____

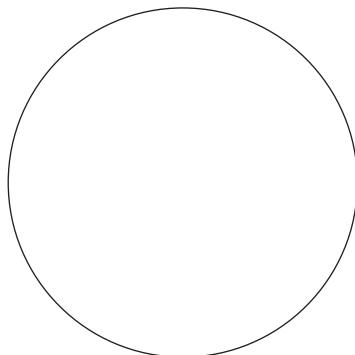
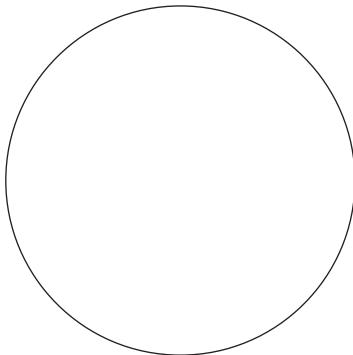
Warp: count_____ twist_____

Fill: count_____ twist_____

secondary backing_____

Warp: count_____ twist_____

Fill: count_____ twist_____



DUCT TAPE WORKSHEET**Physical Characteristics:**

Backing color_____ Adhesive color_____

Width_____

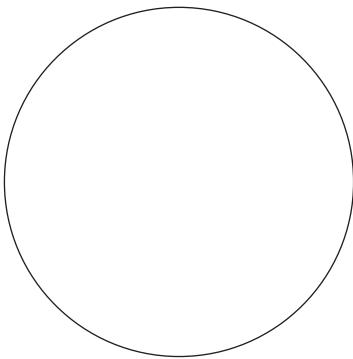
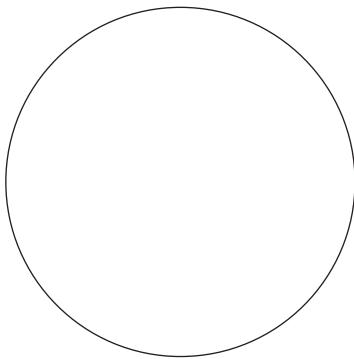
Fiber reinforcement/weave_____

Warp: count_____ fiber type_____ twist_____

Fill: count_____ fiber type_____ twist_____

Microscopic Test:Warp: _____

_____Fill: _____

_____

Experiment 8: Lamp Filament Examinations

Recommended pre-lab reading assignments:

- Baker JM, Fricke LB, Baker KS, Ayock TL. Lamp Examination for ‘On’ or ‘Off’ in Vehicle Collisions. *Traffic Collision Investigation*. 9 ed. Evanston, IL: Northwestern University Center for Public Safety; 2001; 301–66.
- Becker T. *Lamp Examination for Traffic Collision Investigators*. Jacksonville, FL: Institute of Police Technology and Management, 1995.

OBJECTIVE

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

1. lamp filaments
2. lamp filament examinations

INTRODUCTION

At times, it may be necessary for a forensic scientist to examine a lamp filament from a vehicle to determine whether or not a filament was illuminated when a collision occurred. These examinations of filaments can be performed using the stereomicroscope. However, prior to discussing filament examinations, it is important to understand how a lamp is constructed and how it works.

Nearly all lamps are incandescent electric lamps. When a filament produces light, it is at approximately 4000°C. At this temperature, the electric current produced by the lamp raises the filament’s temperature until it reaches incandescence or produces ‘white light.’ Tungsten is generally used for the filament because it does not melt at incandescent temperatures. Wires are used to support the filament and carry the electrical current through the lamp. Since tungsten oxidizes rapidly in air at incandescent temperatures, the filaments and supports must be enclosed in a glass bulb that

has had the air removed and replaced with nitrogen or another inert gas. Many newer vehicles use halogen gas technology for headlamps. This makes the tungsten filaments more efficacious producers of light – more lumens out per watt in – and gives drivers more light than was available from non-halogen filaments at the same power consumption. However, even in normal use, the tungsten will gradually ‘boil off.’ This produces weak spots in the filament. Over time the heat continues to weaken the filament until a gap develops, leading to lamp failure.

Another type of failure can be caused by fracture. This failure can be produced by accidental breakage, or by vibrations or force, which is applied while the filament is cold. Filament examinations identify the microscopic characteristics that can be associated with each type of lamp failure, allowing the forensic scientist to determine whether or not a lamp was incandescent at the time of impact.

The majority of lamp filament examinations involve vehicle lamps, however, these examinations can be performed on any type of lamp filament. Lamps come in a variety of sizes and shapes and may be used for more than one purpose. Vehicle lamps may also have one or more filaments in many different shapes and sizes and can serve many purposes. Headlamps, which are the most commonly examined, come in two varieties: Sealed beam headlamps can have one or two filaments contained within the glass enclosure; Semi-sealed beam headlamps contain a halogen bulb that is placed in a plastic lens assembly. Many other lamps are also used in vehicles and may at times need to be examined. Some have single filaments and others may be dual-purpose filaments.

A lamp filament examination involves a careful examination of the entire lamp. Knowledge of the lamp’s actual placement in the vehicle is extremely important with respect to the final conclusions that can be reached. To begin the microscopic examination, it is necessary to be able to view the filaments. Special care should be taken if it is necessary to open a sealed beam headlamp or halogen bulb because these are highly pressurized. Characteristics will vary depending on the actual circumstances of the lamp. Characteristics indicating a normal lamp are included in Table 8-1.

It is important to recognize aged and normal burn out filament characteristics, because they can be misinterpreted at times. The easiest way to distinguish these filament characteristics from impact characteristics is the even coil spacing. With aged filaments, coils will remain evenly spaced even though there may be a downward sag in the filament arch. With normal burn out, the filament ends are separated with a tapered or balled shape (see Figure 8-1). This shape is caused by the ‘boiling off’ of the tungsten wire, which causes the short or burnout.

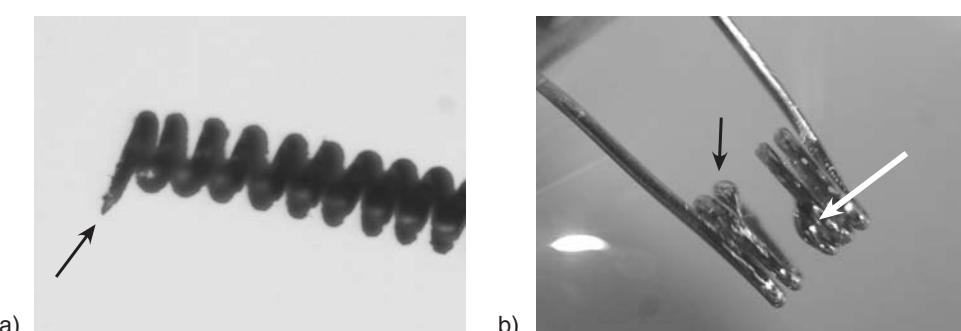


Figure 8-1 Filament ends from a normal burn out bulb. Coils are evenly spaced with distinct shaped ends. a) slight bulbous tapered end and b) teardrop shaped ends.

Table 8-1 Characteristics of normal lamps.

	Bright luster to the filament. Bright luster to the filament supports. Evenly spaced coils to the filament.
NEW	Longitudinal draw lines in the filament. Normal arch to the filament. No darkening of the glass enclosure. Working circuit.
	Bright luster to the filament. Bright luster to the filament supports. Evenly spaced coils of the filament.
AGED	Rough or pitted filament. Downward sag in the filament arch. Possible darkening of the glass enclosure. Working circuit.
	Bright luster to the filament. Bright luster to the filament supports. Evenly spaced coils of the filament.
BURN OUT	Separated filament. Separated ends are usually rounded or 'balled' but may also have a slight taper. Possible darkening of the glass enclosure. Open circuit.

There are additional characteristics often associated with an impact. These may occur when a lamp is 'hot' (incandescent) or 'cold' (off) during impact. If the lamp was on, the characteristics that may be present are referred to as 'hot shock.' Table 8-2 summarizes the characteristics associated with 'hot shock.' The most common characteristic for 'hot shock' is the greatly stretched or distorted coils of the filament (see Figure 8-2).

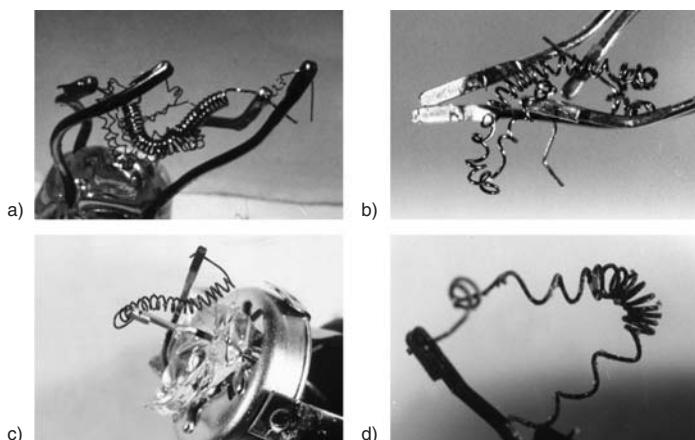
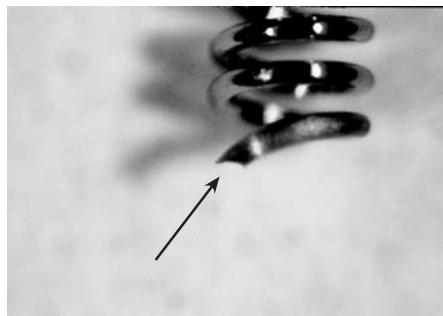
**Figure 8-2** Examples of 'hot shock' with the glass broken and the filament present.

Table 8-2 Characteristics of incandescence at impact (hot).

GLASS UNBROKEN, filament intact	Bright luster to the filament. Bright luster to the filament supports. Greatly stretched or distorted coils of the filament. If filament is separated, ends would appear tapered. Possible darkening of the glass enclosure (depending on age). Working circuit is possible.
GLASS BROKEN, filament intact	Coloring or blackening to the filament. Coloring to the filament supports. Greatly stretched or distorted coils of the filament. If filament is separated, tapered ends. Possible darkening of the glass enclosure (depending on age). Possible fusing of glass on the filament and supports. Oxidation on the filament and supports. Open circuit.
GLASS BROKEN, filament missing	Coloring or blackening to filament fragment. Coloring to the filament supports. Possible darkening of the glass enclosure (depending on age). Possible fusing of glass on the supports. Oxidation on the supports. Open circuit.

If the lamp was off during impact the characteristics are referred to as ‘cold shock.’ Table 8-3 summarizes the characteristics associated with ‘cold shock.’ The most common characteristic for ‘cold shock’ is the fractured brittle ends to an evenly spaced filament (see Figure 8-3).

**Figure 8-3** Blunt end of a ‘cold shock’ filament.

Keep in mind that in some situations only a portion of these characteristics may be present. This is especially true when the filament is at a distance from the impact area. In these circumstances, an inconclusive conclusion may be reached.

Three conclusions are generally reached for filament examinations. First, a filament may be identified as incandescent at impact when indications are found of ‘hot shock’ distortion, fused glass particles, or oxidation. Second, a filament may show no indications of incandescence. In

Table 8-3 Characteristics of non-incandescence at impact (cold).

GLASS UNBROKEN, filament intact	Bright luster to the filament. Bright luster to the filament supports. No distortion of the coils of the filament. If filament is separated, fractured brittle ends. Possible darkening of the glass enclosure (depending on age). Working circuit is possible.
GLASS BROKEN, filament intact	Bright luster to the filament. Bright luster to the filament supports. No distortion of the coils of the filament. If filament is separated, fractured brittle ends. Possible darkening of the glass enclosure (depending on age). Open circuit.
GLASS BROKEN, filament missing	Bright luster to filament fragment. Bright luster to the filament supports. Possible darkening of the glass enclosure (depending on age). Open circuit.

this situation, a filament may exhibit ‘cold shock’ or it may have been too far from the impact to introduce characteristics. Third, a filament may lack enough characteristics for a determination to be made.

EQUIPMENT AND SUPPLIES

- Stereomicroscope
- Micro kit
- Propane torch to open pressurized headlamps
- Previously prepared lamp filaments

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious when opening lamps, because they are highly pressurized. Dispose of glass in an appropriate container.

PART I: LAMP FILAMENT EXAMINATIONS

Procedure

A single filament worksheet and double filament worksheet are located at the end of this experiment and at <http://www.wileyeurope.com/college/wheeler>.

1. Obtain a lamp.
2. Using the stereomicroscope, examine the lamp.

3. Describe the overall condition of the bulb, noting any markings on the bulb.
4. If possible, check the electrical circuit to determine if the lamp is operational.
5. If necessary, clean the glass enclosure so that the filament can be visualized.

If the filament cannot be viewed, it may be necessary to open the enclosure. *Proceed with caution, because lamps can be highly pressurized.* Lamps that are contained in a plastic lens assembly may be cut open. To open glass enclosures, place the lamp face down (onto a towel) and use the scribe to make a circular scratch around the base. Next, use a propane torch in a continuous motion to apply heat to the lamp and the area around the scratch. Immediately place a towel that has been ‘iced’ onto the hot headlamp in the area of the scratch. The glass should pop open where the scribe has created the stress.

6. Determine whether the lamp is a single or double filament lamp.
7. Examine the color of the filament.
8. Examine the shape of the filament.
9. If the filament is broken, examine the ends of the filament.
10. Determine if there are any particles *fused* to the filament.
11. Examine the color and shape of the filament supports.
12. Determine if there are any particles *fused* to the supports.
13. Repeat steps 7–12 if the lamp is a double filament lamp.
14. Draw what you see.
15. Determine whether or not the lamp was incandescent at impact.
16. Repeat steps 2–15 using two more lamps.
17. For each lamp filament examination, you were asked to evaluate evidence. As a forensic scientist you must learn to form a conclusion and be able to communicate your conclusion verbally and in writing. Clearly state your results. Explain how you reached the conclusions.

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. Explain how a lamp creates light.
2. Explain how a double filament lamp works. Give an example of a double filament lamp and state the use of each filament.
3. Explain ‘hot shock’ and differentiate it from ‘cold shock.’
4. Give an example of when a lamp filament examination would be valuable information in an investigation.
5. Is it possible for a driver’s side lamp filaments to show characteristics of incandescence and the passenger’s side lamp filaments (of the same vehicle) to show no characteristics? Explain your answer.
6. If the vehicle being examined has extensive damage to the front so that no headlamps or side lights can be examined, how could you determine if the lights were on or off at impact?

RECOMMENDED AND FURTHER READING

- Baker JM, Fricke LB, Baker KS, Ayock TL. *Lamp Examination for ‘on’ or ‘Off’ in Vehicle Collisions*. Traffic Collision Investigation. 9 ed. Evanston, IL: Northwestern University Center for Public Safety; 2001; 301–66.
- Baudoin P, Lavabre R, Vayne F. An Unusual Oxidation Type on Bulb Filaments after a Car Crash Dive. *Journal of Forensic Sciences*. 2002; 47(2): 377–80.
- Becker T. *Lamp Examination for Traffic Collision Investigators*. Jacksonville, FL: Institute of Police Technology and Management, 1995.
- Fu L, Leutz R, Ries H. Physical Modeling of Filament Light Sources. *Journal of Applied Physics*. 2006; 100(10): 103528.
- Lavabre R, Baudoin P. Examination of Light bulb Filaments after a Car Crash: Difficulties in Interpreting the Results. *Journal of Forensic Sciences*. 2001; 46(1): 147–55.
- Menon VJ, Agrawal DC. A Theory of Filament Lamp’s Failure Statistics. *European Physical Journal – Applied Physics*. 2006; 34(2): 117–21.

SINGLE FILAMENT WORKSHEET

Bulb location: _____

Bulb type/markings: _____

Bulb condition:

Glass intact_____

Darkened_____

White deposit_____

Glass broken_____

Darkened_____

White deposit_____

Electrical circuit: _____

Single filament condition

Shiny_____ Colored_____

Evenly stretched_____ Distortion_____

Broken_____

ends_____

Deposits_____ Fused glass_____

Other_____

Missing_____

Filament supports:

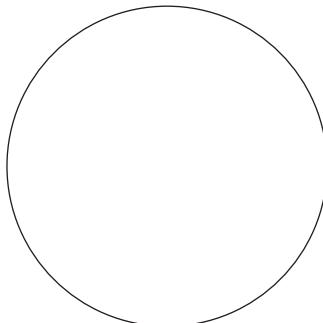
Bent_____

Broken_____

Colored_____

Deposits_____

Other_____



DOUBLE FILAMENT WORKSHEET

Bulb location: _____

Bulb type/markings: _____

Bulb condition:

Glass intact _____

Darkened _____

White deposit _____

Glass broken _____

Darkened _____

White deposit _____

Electrical Circuit: _____

Double filament condition – thick filament

Shiny _____ Colored _____

Evenly stretched _____ Distortion _____

Broken _____
ends _____

Deposits _____ Fused glass _____

Other _____

Missing _____

Filament supports:

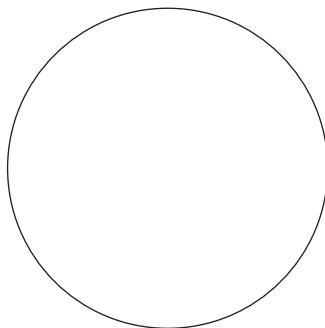
Bent _____

Broken _____

Colored _____

Deposits _____

Other _____



Double filament condition – thin filament

Shiny _____ Colored _____

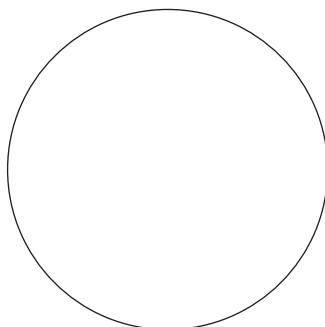
Evenly stretched _____ Distortion _____

Broken _____
ends _____

Deposits _____ Fused glass _____

Other _____

Missing _____



Filament supports:

Bent _____

Broken _____

Colored _____

Deposits _____

Other _____

Experiment 9: Fingerprint Examinations and Comparison¹

Recommended pre-lab reading assignments:

US Department of Justice; *The Science of Fingerprinting*; US Government Printing Office; 1990.
US Department of Justice; *Fingerprint Training Manual*; US Government Printing Office; 1993.

OBJECTIVE

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

1. general characteristics of fingerprints
2. use of the stereomicroscope to visualize the characteristics of fingerprints
3. fingerprint comparison

INTRODUCTION

Fingerprints are an impression of friction ridges found on the tips of the palm side of fingers and thumbs. Fingerprints may be found in two forms: patent or latent. Patent prints are those that are easily viewed with the naked eye. Examples of patent prints are ridge patterns in blood, grease, or on a soft substrate such as the adhesive on duct tape. Latent prints are those that are viewed with the aid of a visualizing agent. Common visualizing agents are dusting powders (black, colored, fluorescent, or magnetic), ninhydrin, and cyanoacrylate ester.

Friction ridges are formed during the third to fourth month of fetal development. They are formed as the result of the fusion of the main two layers of the epidermis, the stratum corneum and the stratum mucosum. The stratum corneum covers the surface, and the stratum mucosum is just beneath the covering surface. It is the combination of these two layers that form friction

¹ This experiment adapted courtesy of Joe Wallace, Department of Criminal Justice Training, Richmond, KY 40475.

ridges. Sections of the stratum mucosum are folded to form ridges. The ridges run lengthwise and correspond to the surface ridges. These ridges are twice as numerous, because the deeper ridges corresponding to the middle of the surface ridges alternate with smaller ones corresponding to the furrows. Sweat pores run in single rows along the ridges. Through these pores, perspiration releases and deposits on the skin. Fingerprint impressions are created through direct contact with wet or soft surfaces (patent prints) or when the perspiration deposits and any oil that is also on the skin are transferred to items (latent prints).

When an individual bruises or slightly cuts the outer layer of the skin of the finger, the friction ridges will not be permanently changed. However, if a more serious injury is incurred, which damages the stratum mucosum, the skin will heal, but not in its original formation. A scar will appear in the area of injury, changing the friction ridge pattern.

Fingerprints offer a dependable means of personal identification. Other personal characteristics may change, but fingerprints are formed prior to birth and remain constant until death and decomposition. Even identical twins, who have the same DNA, do not have the same fingerprints! Individuals may have the same pattern types; however, the ridge detail of every fingerprint of every person is different. This is also true of the palms of the hands, toes, and soles of the feet.

Fingerprints are classified into three main categories: arch, loop, and whorl. These patterns may be further divided into sub-groups by means of the smaller differences existing between the patterns in the same general group. Initially, a pattern area must be determined. The pattern area is the part of the print in which the cores, deltas, and ridges appear. The pattern areas of arches are open, and type lines enclose the pattern area of loops and whorls. Type lines may be defined as the two innermost ridges that start parallel, diverge, and surround or tend to surround the pattern area. Within the pattern areas, focal points are used to classify them. These points are called deltas and/or cores (see Figure 9-1). A delta is the point on a ridge at, or in front of and nearest, the center of the divergence of the type lines. A delta may be a bifurcation, ending ridge, dot, short ridge, meeting of two ridges, or a point on the first recurring ridge located nearest to the center and in front of the divergence of the type lines. The core is the approximate center of the fingerprint impression, and is placed on the innermost sufficient recurve. The core is situated upon the end of the center rod whether it touches the looping ridge or not when the innermost sufficient recurve contains an uneven number of rods rising as high as the shoulders. The core will be placed upon the end of the farther one of the two center rods when the innermost sufficient recurve contains an even number of rods.

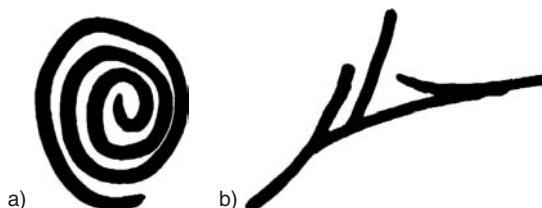


Figure 9-1 The core and the delta are two characteristics used to classify fingerprints. a) The core is the approximate center of a fingerprint. b) A delta is the point on a ridge where a divergence occurs.

An arch is a ridge pattern that is characterized by lines that enter the print from one side and exit from the other. As it crosses the pattern area, there is a rise in the ridge pattern. The difference in this rise determines whether it is classified as a plain arch or a tented arch (see Figure 9-2).

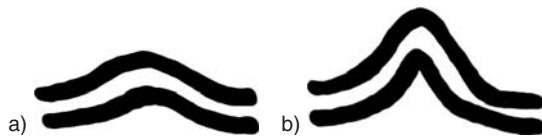


Figure 9-2 The arch classification has ridges that enter the print from one side and exit on the other. Arches may be either a) plain or b) tented.

A plain arch is the most simple of all fingerprint patterns. There are no recurring ridges or deltas. The ridge pattern will follow a general contour, however, various ridge formations such as ending ridges, bifurcations, dots, and islands may also be involved in this type of pattern. For a tented arch, most of the ridges enter one side of the print and exit out the other side; however, the ridge or ridges at the center do not. One or more ridges at the center form an up thrust. An up thrust is an ending ridge of any length rising at a sufficient degree from the horizontal plane (at 45° or more). Tented arches are sometimes confused with loops.

A loop is a ridge pattern that is characterized by lines that enter on one side of the print and recurve, touch, or pass an imaginary line drawn from the delta to the core, and terminate or tend to terminate on or toward the same side of the print from which such ridge or ridges entered (see Figure 9-3). A loop is distinguished as being ‘radial’ or ‘ulnar.’ The terms ‘radial’ and ‘ulnar’ are derived from the radius and ulna bones of the forearm. Loops that flow in the direction of the ulna bone (toward the little finger) are called ulna loops and those that flow in the direction of the radius bone are called radial loops. A loop pattern will always have a delta.

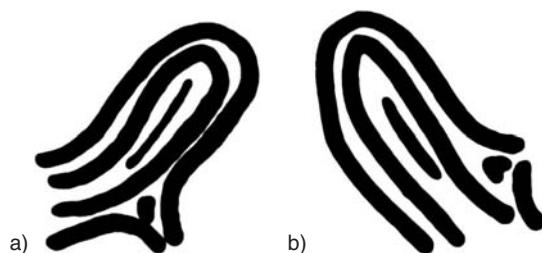


Figure 9-3 Loops are classified as either radial or ulnar. a) Loops that form toward the right are radial loops and b) loops that form toward the left are ulnar loops.

A whorl is a ridge pattern that is generally rounded in shape and has at least two deltas (see Figure 9-4). A whorl may be further sub-divided into four categories: plain, central pocket loop, double loop, or accidental. The plain whorl has two deltas and at least one ridge making a complete circle. This circle may be spiral, oval, circular, or any variant of a circle. An imaginary line drawn between the two deltas must touch or cross at least one of the recurring ridges within the inner pattern area. The central pocket loop type of whorl has two deltas and at least one ridge, which makes or tends to make a complete circle. The circle may be spiral, oval, circular, or any variant of a circle. An imaginary line drawn between the two deltas must not touch or cross any recurring ridges within the inner pattern area. The double loop consists of two separate loop formations, with two separate and distinct sets of shoulders, and two deltas. The accidental whorl is a pattern consisting of a combination of two different types of patterns, with the exception of the plain

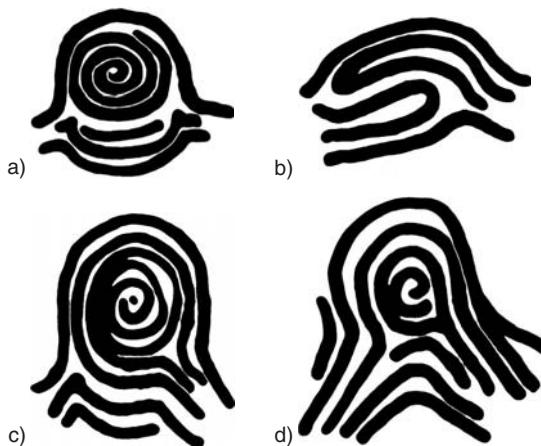


Figure 9-4 These ridge patterns are generally rounded and have at least two deltas. They are classified as whorls: a) plain whorl; b) double loop whorl; c) central pocket loop whorl; d) accidental whorl.

arch, with two or more deltas; or a pattern possessing some of the requirements for two or more different types; or a pattern that conforms to none of the definitions.

Once ridge patterns have been determined, comparisons may next be considered. Fingerprints are compared by noting the ridge characteristics in two fingerprint impressions to determine whether or not they match. An identification is established when a number of these characteristics occupy the same relative position in the two fingerprint impressions. When making a comparison the first observation would be using a magnifying glass or stereomicroscope to determine whether the fingerprints are of the same type (arch, loop, whorl).

If the pattern types are the same, the next step is to examine the fingerprint for specific characteristics: bifurcations, short ridges, ridge endings, and/or enclosures. These characteristics are called minutia features (see Figure 9-5). A ridge ending is the point at which a friction ridge stops. Enclosures are a ridge that completely encloses another. Short ridges are ridges that are significantly smaller than the average ridge length of the print. Bifurcations are points at which a

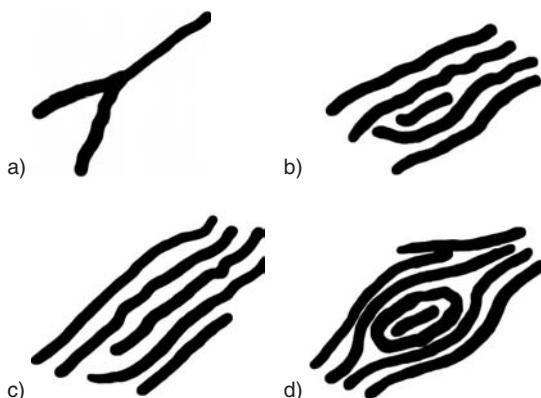


Figure 9-5 Minutia features used in fingerprint classifications: a) bifurcation; b) short ridge; c) ridge ending; and d) enclosure.

single ridge splits into two ridges. The examiner begins by looking for the most obvious points of identification: the characteristics that capture one's attention when first looking at the fingerprint. This characteristic or point is compared to the same area of the suspect print to make a potential match. If the same characteristic or point is present in both prints, the number of ridges is counted from the first characteristic or point to the second characteristic. Continue this process until you have determined that the two fingerprint impressions are the same (see Figure 9-6) or different.

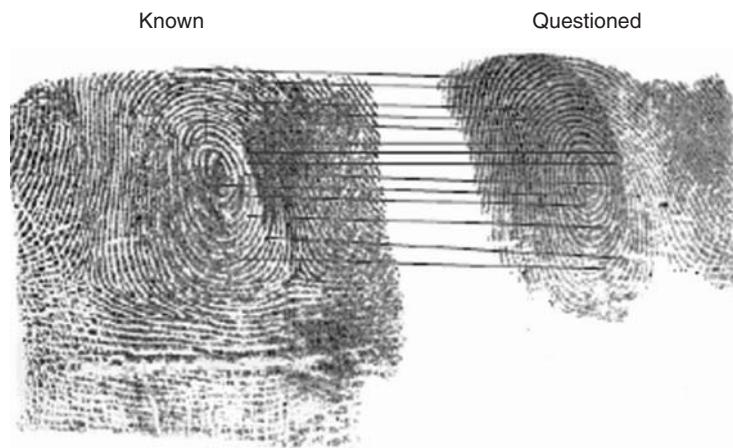


Figure 9-6 Fingerprint comparisons for a latent lift to a known fingerprint sample.

There is no standard number of characteristics required to establish an identification. The fingerprint examiner must determine when enough points have been located to make an identification. This number may vary due to clarity, uniqueness of formations, experience, and ability.

EQUIPMENT AND SUPPLIES

- Fingerprint ink
- Print cards
- Stereomicroscope
- A set of known prints
- Unknown latent prints

SAFETY

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

PART I: FINGERPRINT EXAMINATION

Procedure

1. Obtain a fingerprint card.
2. Using the fingerprint ink, ink and roll each of your fingers on the card in the designated areas.
3. Using the stereomicroscope or a magnifying glass, determine the pattern type and sub-division for each finger.
4. Locate any delta(s) of the prints if applicable.
5. Locate any cores of the prints if applicable.
6. Using the circle template located in Appendix F, draw the main characteristics that you see to determine the pattern type and sub-division. Include any cores or deltas found.

PART II: FINGERPRINT COMPARISON

Procedure

1. Obtain a set of known prints.
2. Obtain a set of unknown prints.
3. Determine if any of the unknown prints match the known prints using the above procedure.
4. Draw the comparison of any prints that you consider to be a match.

REPORT REQUIREMENTS

Include all drawings, comparisons, 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 is a patent print? How would you compare this to known samples?
2. What is a latent print? How would you compare this to known samples?
3. What are the main groups of fingerprints? Describe each.
4. What are the two subgroups of arches?
5. What are the two subgroups of loops?
6. What are the four subgroups of whorls?
7. What is a delta?
8. What is the core?
9. What are the four types of minutia used to compare fingerprints? Describe each.
10. Can partial prints be compared?
11. How many characteristics must match in order to make a positive identification? Explain.
12. Would identical twins be able to be distinguished by their fingerprint? By their DNA?

RECOMMENDED AND FURTHER READING

- Cole SA. Witnessing Identification: Latent Fingerprinting Evidence and Expert Knowledge. *Social Studies of Science*. 1998; 28(5–6): 687–712.
- Cole SA. What Counts for Identity? The Historical Origins of the Methodology of Latent Fingerprint Identification. *Science in Context*. 1999; 12(1): 139–72.
- Cole SA. Grandfathering Evidence: Fingerprint Admissibility Rulings from Jennings to Llera Plaza and Back Again. *American Criminal Law Review*. 2004; 41(3): 1189–276.
- Cole SA. More Than Zero: Accounting for Error in Latent Fingerprint Identification. *Journal of Criminal Law & Criminology*. 2005; 95(3): 985–1078.
- Egli NM, Champod C, Margot P. Evidence Evaluation in Fingerprint Comparison and Automated Fingerprint Identification Systems – Modelling within Finger Variability. *Forensic Science International*. 2007; 167(2–3): 189–95.
- Grant A, Wilkinson TJ, Holman DR, Martin MC. Identification of Recently Handled Materials by Analysis of Latent Human Fingerprints Using Infrared Spectromicroscopy. *Applied Spectroscopy*. 2005; 59(9): 1182–7.
- Kahn HS. Enhanced Collection of Fingerprints and Ridge Counting. *American Journal of Human Biology*. 2005; 17(3): 383.
- Kempton JB, Sirignano A, Degaetano DH, Yates PJ, Rowe WF. Comparison of Fingernail Striation Patterns in Identical-Twins. *Journal of Forensic Sciences*. 1992; 37(6): 1534–40.
- Kulkami JV, Patil BD, Holambe RS. Orientation Feature for Fingerprint Matching. *Pattern Recognition*. 2006; 39(8): 1551–4.
- US Department of Justice. *The Science of Fingerprinting*. Washington, DC: Government Printing Office; 1990.
- US Department of Justice. *Fingerprint Training Manual*. Washington, DC: Government Printing Office; 1993.
- Wilson JD, Cantu AA, Antonopoulos G, Surrency MJ. Examination of the Steps Leading up to the Physical Developer Process for Developing Fingerprints. *Journal of Forensic Sciences*. 2007; 52(2): 320–9.
- Zhu E, Yin JP, Hu CF, Zhang GM. A Systematic Method for Fingerprint Ridge Orientation Estimation and Image Segmentation. *Pattern Recognition*. 2006; 39(8): 1452–72.
- Zhu Y, Dass SC, Jain AK. Statistical Models for Assessing the Individuality of Fingerprints. *IEEE Transactions on Information Forensics and Security*. 2007; 2(3): 391–401.

Experiment 10: Tool Mark Examinations

Recommended pre-lab reading assignments:

Burd D, Kirk PL. Tool Marks: Factors Involved in Their Comparison and Use as Evidence. *Journal of Police Science*. 1942; 32(6): 465.
Miller J. An Introduction to the Forensic Examination of Tool Marks. *AFTE Journal*. 2001; 33(3): 233–48.

OBJECTIVE

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

1. tool mark examinations
2. use of the comparison stereomicroscope for tool mark examinations

INTRODUCTION

Tool mark identifications involve the basic principle of uniqueness, where all objects are unique to themselves and thus can be differentiated from each other. The mechanism for differentiating tool marks depends on class and individual characteristics. Class characteristics are intentional or design characteristics that would be common to a particular group of items. The size and shape of a tools working surface is an example of class characteristics. For instance, a significantly larger impression may be left by one flat-head screwdriver than another. The shape can also leave varying impressions as shown by flat-head and Phillips-head screwdrivers. The distance between a toothed instrument's teeth are also considered class characteristics. Individual characteristics are marks produced by random imperfections or irregularities of a tool surface. These characteristics may be produced through the machining process or by random imperfections or irregularities, which are produced incidental to manufacture and/or caused by use, corrosion, or damage. For instance, small irregularities left in the edge of the tool as a result of the machining process used to cut

and finish edges provide individual characteristics. The shape and pattern of such irregularities are also modified by wear and damage to the tool. This can create a unique pattern for every tool, allowing for the possibility of performing tool mark examinations.

A tool mark is any impression, cut, gouge, or abrasion caused by a tool coming into contact with another object. Because the tool is usually the harder surface, when it comes into contact with a softer object, it will impart its unique marks on that object.

A microscopic examination of the markings reveals irregularities that can be compared to the working surface of the suspected tool. Usually a reverse image of the tool or tool mark must be made so that comparisons can be performed. Silicon casting materials are used to obtain the reverse images.

In some situations, the investigator may request identification of the possible tool. For this, the examiner determines the class characteristics of the impression. From this information, a class of tools can be determined.

In other situations a complete examination may be requested. In these cases, if the class characteristics are consistent with a suspected tool, test marks are then made using lead sheets. The test marks are then compared to each other to ensure that the tool produces unique, consistent individual markings. This examination is done on a comparison stereomicroscope with a special stage. The stage used for tool mark identifications will allow the sample to be moved and rotated so that markings can be easily compared on various surfaces (see Figure 10-1).



Figure 10-1 This specialized stage holds tool mark samples for viewing on a comparison stereomicroscope. Since the sample area of the stage is positioned on a ball and socket joint, samples can be easily maneuvered for comparison examinations.

A comparison stereomicroscope is composed of two stereomicroscopes that are connected by an optical bridge. The bridge contains prisms and mirrors that are used to direct the light to a common set of oculars. A set of knobs is used to adjust the field of view, so that items can be viewed from either microscope independently or combined. In the combined field of view the image is split showing portions of the field of view from each microscope. This allows the examiner to view two items side-by-side on a microscopic scale. If unique, consistent markings are found, comparisons can then be performed with the evidence samples. When comparisons are performed, the forensic examiners usually reach one of three results:

- an identification; the exhibit was produced by contact with the tool and a sufficient correlation between individual characteristics is found;

- a non-identification or elimination; the exhibit was not produced by the tool because class or individual characteristics disagree;
- inconclusive; the class characteristics agree but there is an insufficient correlation between individual characteristics.

EQUIPMENT AND SUPPLIES

Comparison stereomicroscope
Unknown tool mark impressions
Casting material (Mikrosil™, Duplicast™, or Epse Impregum™)
Tools
Lead sheets

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor.

PART I: CASTING A TOOL MARK

Procedure

1. Mix the casting material according to manufacturer's instructions.
2. Apply the casting material over the tool or tool mark to be cast.
3. When the casting material has sufficiently set (or cooled), gently tap the area to loosen the cast.
Some casting materials may require more force to separate the cast from the area.

PART II: TOOL MARK COMPARISON

1. Examine the tool mark that you have been assigned. Make a cast, if necessary.
2. Identify the class characteristics to determine a possible tool.
3. Obtain a possible tool and using the tool, make several test tool marks using lead sheets.
4. Attach the test tool marks to the stages of the comparison microscope. Examine the test tool marks to determine if the tool produces unique, consistent markings.
5. If consistent, compare these markings to those of your cast. Not only must the size and shape match up, but the irregularities or longitudinal striations on each must also coincide. Comment on the striations and draw regions that appear similar using the circle template located in Appendix F. Is there anything unusual with any of the knowns or unknowns?

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. For tool mark evidence, what properties are class characteristics? Using a tool, describe its class characteristics.
2. What are the individual characteristics for tool mark evidence? Using a different tool, describe individual characteristics.
3. Write a statement about your comparison with the ‘known tool.’ Could the tool have produced the tool mark impression? Back up your written statement with why you think they match/or don’t match referring to your drawings if necessary.

RECOMMENDED AND FURTHER READING

- Burd D, Kirk PL. Tool Marks: Factors Involved in Their Comparison and Use as Evidence. *Journal of Police Science*. 1942; 32(6): 465.
- Liukkonen M, Majamaa H, Virtanen J. The Role and Duties of the Shoeprint/Toolmark Examiner in Forensic Laboratories. *Forensic Science International*. 1996; 82(1): 99–108.
- Miller J. An Introduction to the Forensic Examination of Tool Marks. *AFTE Journal*. 2001; 33(3): 233–48.
- Nichols RG. Firearm and Toolmark Identification Criteria: A Review of the Literature. *Journal of Forensic Sciences*. 1997; 42(3): 466–74.
- Nichols RG. Firearm and Toolmark Identification Criteria: A Review of the Literature, Part II. *Journal of Forensic Sciences*. 2003; 48(2): 318–27.
- Nichols RG. Defending the Scientific Foundations of the Firearms and Tool Mark Identification Discipline: Responding to Recent Challenges. *Journal of Forensic Sciences*. 2007; 52(3): 586–94.
- Novoselsky Y, Tsach T, Klein A, Volkov N, Shor Y, Vinokurov A. Unusual Contact Marks: Connecting the Hubcap to the Wheel of the Car. *Journal of Forensic Sciences*. 2002; 47(3): 630–2.
- Petraco N, Petraco ND, Pizzola PA. An Ideal Material for the Preparation of Known Toolmark Test Impressions. *Journal of Forensic Sciences*. 2005; 50(6): 1407–10.

Firearms Examinations

Experiment 11: Firearms Examinations

Recommended pre-lab readings:

Hamby J. Identification of Projectiles. *AFTE Journal*. 1974; 6(5/6): 22.

Nicols R. Firearms and Tool mark Identification Criteria: A Review of the Literature. *Journal of Forensic Sciences*. 1997; 42(3): 466–74.

Nicols R. Firearms and Tool Mark Identification Criteria: A Review of the Literature – Part 2. *Journal of Forensic Sciences*. 2003; 48(2): 18–327.

Recommended website:

FirearmsID [Web Page] Louisville, KY; [cited 2007 October 30]; Available from: <http://www.firearmsid.com>.

OBJECTIVE

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

1. firearms examinations
2. use of the comparison stereomicroscope for firearms examinations

INTRODUCTION

As a firearm is shot, several actions occur that make firearms examinations possible. First, the cartridge enters the chamber. The firing pin then strikes the primer of the cartridge case, igniting it. The spark from the primer ignites the propellant. The burning gases from the propellant expand the cartridge case to seal it against the chamber wall. This causes the projectile to be pushed in the direction that releases the pressure, down the barrel. Once the projectile leaves the barrel the

pressure is released, allowing the cartridge case to be removed from the chamber. These actions cause unique impressions or markings on the bullet and cartridge case, which firearms examiners use for identification.

Firearms identification is actually a form of tool mark identification. A tool mark is any impression, cut, gouge, or abrasion caused by a tool coming into contact with another object. For firearms examinations, the firearm itself acts as the tool. Since the components of the firearm are harder than the ammunition, it leaves impressed or striated marks on the ammunition components with which it comes into contact. Studies have shown that no two firearms, even those with the same make and model, will produce the same unique markings on fired bullets and cartridge cases.

To perform an examination, the firearms examiner deals with class and individual characteristics. Class characteristics are intentional or design characteristics that would be common to a particular group of items. The class characteristics, which the examiner must take into consideration, are number of lands and grooves, width of lands and grooves, direction of twist, degree of twist, and caliber (or gauge). During the manufacturing process of a rifled firearm, the barrel goes through a rifling process. Rifling of a barrel can occur through several methods. The most common methods used are broaching, button rifling, hammer forging, and electrochemical etching. These processes create the unique spiral lands and grooves that the firearms examiner uses for identification of bullets. The raised portion of the rifling impression is called lands and the recessed portions are called grooves. Firearms can be manufactured with any number of lands and grooves in their barrels. They vary in widths and also in the direction of twist, spiraling either left or right. The final class characteristic is the size of the firearm. Caliber is the size of a rifled firearm, and is the distance measured between opposing lands and grooves. Gauge is the size of a smooth barrel firearm. It is the number of solid spheres of a diameter equal to the inside diameter of the barrel that could be made from a pound of lead.

Individual characteristics are marks produced by random imperfections or irregularities of a tool surface. These characteristics may be produced through the machining process or by random imperfections or irregularities produced incidental to manufacture and/or caused by use, corrosion, or damage. The individual characteristics that the examiner must take into consideration are: rifling impressions, striated action marks, and impressed action marks. For the projectile, a closer examination is done of the rifling impressions. Imperfections on the interior of the barrel leave microscopic striations or scratches on the projectile as it passes through the barrel. For cartridges, a closer examination is done of the striated action marks and impressed action marks. Striated action marks are scratches produced as the cartridge case moves laterally against the chamber. These may be in the form of chamber marks, shear marks, extractor marks, or ejector marks. Chamber marks are scratches produced by the walls of the chamber as the cartridge is loaded and removed from the chamber. Scratches resulting from the movement of the primer area of the cartridge case against the firing pin aperture are called shear marks. Firing pin drag marks are scratches produced as the firing pin drags across the primer during extraction. Extractor marks are scratches resulting from the extractor 'hook' removing a cartridge from the chamber and ejector marks are striated marks created as the cartridge contacts the ejector during ejection. Impressed action marks are the result of sufficient impact by a portion of the firearm causing impressed or indented marks. Firing pin marks are indentations produced when the firing pin strikes the primer. Impressions resulting from the head of the cartridge striking the breech face of the firearm are called breech marks. Ejector marks are indentations created as the cartridge is ejected from the chamber.

In some situations, the investigator may request identification of the possible weapon. For this, the examiner determines the class characteristics of the projectile or cartridge case. From this information, possible weapons can be determined. In other situations a complete examination may

be requested. In these cases, if the class characteristics are consistent with a suspected firearm, test fires are then made using similar ammunition. The test fires are then compared to each other to ensure that the firearm produces unique, individual markings. This examination is done on a comparison stereomicroscope. The universal bullet stage is designed to allow the sample to be moved and rotated so that markings can be easily compared on various surfaces (see Figure 11-1). This accommodates the numerous sizes and shapes of bullets, cartridge casings, and shot shell casings. A comparison stereomicroscope is composed of two stereomicroscopes that are connected by an optical bridge. The bridge contains prisms and mirrors, which are used to direct the light to a common set of oculars. A set of knobs is used to adjust the field of view, so that items can be viewed from either microscope independently or combined. The combined field of view shows portions from each microscope. This allows the examiner to view two items side-by-side on a microscopic scale (see Figure 11-2). If unique, consistent markings are found, comparisons can then be performed with the evidence samples.



Figure 11-1 The specialized stage on a firearms comparison microscope. This stage allows complete movement in all directions of the sample.

When comparisons are performed, the firearms examiners usually reach one of four results:

- the exhibit was identified as having been fired from or in the suspected weapon;
- the exhibit could neither be identified nor eliminated as having been fired from the weapon;
- the exhibit was eliminated as having not been fired from or in the suspected weapon;
- the exhibit was identified as having passed through the action of the suspected weapon.



Figure 11-2 A comparison of cartridge case samples.

EQUIPMENT AND SUPPLIES

Comparison stereomicroscope

Previously prepared known and unknown bullet sets

Previously prepared known and unknown cartridge case sets

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Students should know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. Refer to the Material Safety Data Sheet (MSDS) as needed. Students should wear personal protective equipment such as goggles and nitrile gloves for all parts of this lab. All work done with chloroform and organic solvents should be completed in a hood or with adequate ventilation. Several of the reagents contain concentrated acid solutions and should be handled with caution.

PART I: PARTS OF THE COMPARISON STEREOMICROSCOPE

Label the parts of the Leitz™ comparison stereomicroscope (see Figure 11-3) by writing the name next to the appropriate number. A copy of this worksheet is available from <http://www.wileyeurope.com/college/wheeler>.

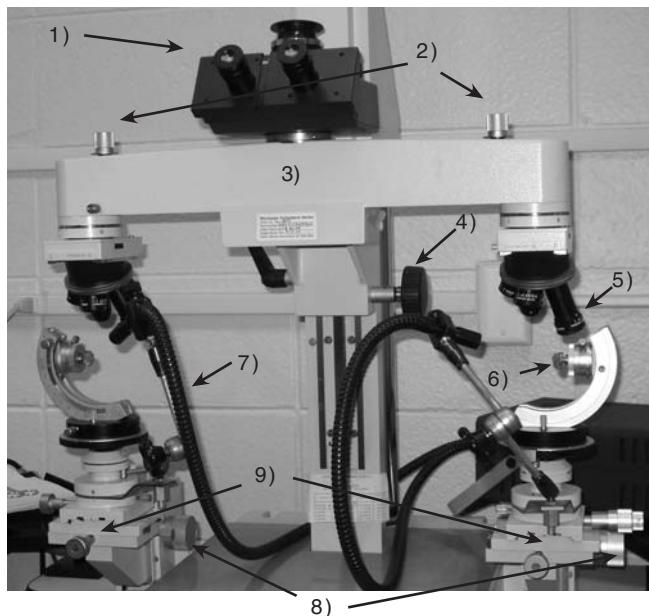


Figure 11-3 Photograph of a Leitz™ comparison stereomicroscope.

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

PART II: BULLET AND CARTRIDGE COMPARISONS

Procedure

You will use a comparison stereomicroscope to compare bullets and cartridge casings shot from several weapons. This procedure will be followed for both the knowns (A, B, and C) and your assigned unknown.

Bullets

1. Examine the unknown bullet for the number of lands and grooves. Hold the bullet with the tip end away from you and look at it under the stereomicroscope.
2. Mark one of the lands with a pen or marker. Use something that can be washed off after examination is complete.
3. Using wax, press the bullet onto the rotating stage mount. Rotate the bullet and count the number of land impressions until the bullet is rotated completely around to your mark. This is your number of lands and grooves.
4. Examine the bullet for the direction of twist. Holding the bullet with the tip end away from you, determine the direction of twist. If the land and groove impressions go toward the right, the bullet has a right twist; if they go toward the left, the bullet has a left twist.
5. Examine the known bullets for the number of lands and grooves and also direction of twist as performed in steps 1–3. Determine if your unknown bullet shares class characteristics with any of the known bullets.
6. Using the comparison stereomicroscope, examine the unknown bullet for the rifling striations.
7. Look at the striations for comparison to any possible knowns. Not only must the lands and grooves of the questioned and known bullets match but the longitudinal striations on each must also coincide. Comment on the striations and draw regions that appear similar using the circle template in Appendix F. Is there anything unusual with any of the knowns or unknowns?

Cartridges

1. A cartridge casing can be compared for both the firing pin impression, breech marks, ejector marks, and the extractor marks on the side of the casing.
2. Draw the firing pin impression for each cartridge examined (known and unknown).
3. Determine if any of the cartridge casings were fired from the same weapon.

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. For firearms evidence, what are the class characteristics? Discuss each.
2. How can the caliber of a firearm be determined from a fired bullet?

3. What are the individual characteristics for firearms evidence? Discuss each.
4. Write a statement about your comparison with the ‘known bullet samples.’ Could the weapon from which they (known samples) had been fired also be the weapon from which the unknown was fired? Back up your written statement with why you think they match, referring to your drawings if necessary.
5. Write a statement about your comparison with the ‘known cartridge case samples.’ Could the weapon from which they (known samples) had been fired also be the weapon from which the unknown was fired? Back up your written statement with why you think they match referring to your drawings if necessary.

Experiment 11A: Gunshot Residue Examinations

Recommended pre-lab readings:

Dillon J. The Sodium Rhodizonate Test: A Chemically Specific Test for Lead in Gunshot Residues. *AFTE Journal*. 1990; 22(3): 26.

Lekstrom JA, Koons RD. Copper and Nickel Detection on Gunshot Targets by Dithiooxamide Test. *Journal of Forensic Sciences*. 1986; 31(4): 1283.

Anon. Gunshot Residues and Shot Pattern Test. *FBI Law Enforcement Bulletin*. 1970; 39(9): 7.

Fiegel F, Anger V. *Spot Tests in Inorganic Analysis*. 6 ed. Amsterdam: Elsevier, 1972.

Recommended website:

FirearmsID [Web Page] Louisville, KY; [cited 2007 October 30];

Available from: <http://www.firearmsid.com>.

OBJECTIVE

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

1. gunshot residue
2. chemical tests used for gunshot residue examinations
3. chemical tests performed on gunshot residue for distance determinations
4. chemical tests performed on samples for copper determination

INTRODUCTION

At times, it may be important to examine an item for gunshot residue. When a gun is fired the firing pin strikes the back of the cartridge and sparks the primer that ignites the ‘gunpowder’ and causes it to burn. This results in the formation of a gaseous vapor, which forces the bullet down the barrel of the gun and out the muzzle. The majority of the vapor proceeds out the muzzle but the vapor can also escape from the cylinder gap in a revolver or the ejection port in a pistol. The vapor that escapes from these areas is called gunshot residue (GSR). It includes primer and gunpowder residues, plus metallic residues from projectiles.

To understand GSR, we must first discuss some of the components of ammunition, because this is where the components for gunshot residue originate. A modern cartridge consists of a bullet (projectile), the casing (which holds all the parts together), the propellant (powder), the rim, the portion of the casing used for loading, and the primer (which ignites the propellant). A modern shotgun shell consists of: shot (projectile), the casing (which holds all the parts together), the wad (which separates the shot from the propellant), the propellant (powder), a brass head, and the primer (which ignites the propellant). Three elements are associated with GSR: barium, lead, and antimony. Examinations are also performed to identify the presence of

nitrates from nitrocellulose and/or nitroglycerin, which are the propellants and primers used in ammunition.

A propellant is a chemical that is used to move an object by applying a force. Black powder and smokeless powder are used in ammunition as propellants or gunpowder. Black powder contains charcoal, potassium nitrate, and sulfur. Smokeless powder may be single-based or double-based. Single based smokeless powder contains cellulose nitrate with a stabilizer and modifier. Double based smokeless powder contains cellulose nitrate and nitroglycerin, with stabilizers and modifiers. Stabilizers react with the nitrogen oxides. If these are not removed, the propellants are extremely unstable and prone to auto ignition. Common stabilizers are diphenylamine and ethyl centralite. Modifiers are either plasticizers or flash suppressants. Plasticizers soften the powder, which prevents the powder from fracturing. Flash suppressants interrupt the reaction of muzzle gases. Common modifiers are nitroglycerin, ethyl centralite, dibutyl phthalate, 2,4-dinitrotoluene, and triacetin. Common flash suppressants are potassium nitrate and potassium sulfate. The ignition component of a cartridge is the primer. Originally mercury fulminate and potassium chlorate were used as primers. These compounds deteriorated the weapons. Currently lead azide, lead styphnate, antimony sulfide, barium nitrate, and tetracene are used.

Although most laboratories will perform instrumental techniques to identify barium, lead, and antimony in GSR (Experiment 25), chemical spot testing can also be performed. The sodium rhodizonate test is specific for lead and barium. The Harrison-Gilroy test is used to identify antimony. These tests are generally used to determine the physical characteristics of bullet holes.

The gunpowder used in most ammunition is smokeless powder made of nitrocellulose or a nitroglycerine-nitrocellulose combination. Unburned nitrocellulose and nitroglycerine can be identified with the Griess test. This testing is also performed to determine the physical characteristics of bullet holes.

Chemical spot tests may also be performed for copper. Since many bullets are copper coated, this in combination with the sodium rhodizonate, and Harrison-Gilroy tests may give an analyst important information about the entrance or exit holes produced by bullets. It can also provide information about the type of bullet used to produce the holes.

Materials may be examined to determine if any of the GSR components are present. Bullets that are fired through clothing or items often leave traces of lead and copper. The lead may come from the surface of the bullet, the barrel of the weapon, or from primer components. Copper may come from the bullet itself. Examinations may also be performed for the presence of barium, antimony, and nitrates, other components of the ammunition that are released upon firing. When these examinations are done in combination, distance determinations can sometimes be made. It is important to perform the tests in a particular sequence, so that reagents do not interfere with results. The modified Griess test is performed first, with the dithiooxamide test second, followed by the sodium rhodizonate test. A distance determination is done when it is important to determine the possible distance from a weapon to the target. Known test fire samples are made from various distances; for instance, from 12 inches (30 cm) away from the target, from 18 inches (45 cm) away from the target, and from 24 inches (60 cm) away from the target. These samples are then processed using the color spot tests to give visual patterns. The closer the weapon is to the target, the more pattern there will be. Likewise, when distances greater than three feet (90 cm) are tested, patterns become less visible. The unknown sample is chemically treated and compared to the known test fire samples to determine a possible weapon to target distance.

Chemical spot tests are used because they are both inexpensive and easy to perform. They can be very effective and their ease of use allows them to be performed by personnel with very

minimal training. As with all presumptive tests, these spot tests must be confirmed with additional testing (see Experiment 25).

EQUIPMENT AND SUPPLIES

Reagents and samples as listed with each test.

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Students should know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. Refer to MSDS as needed. Students should wear personal protective equipment such as goggles and nitrile gloves for all parts of this lab. All work done with organic solvents should be completed in a hood or with adequate ventilation. Several of the reagents are hazardous and should be handled with caution.

PART I: COLOR TESTS FOR BARIUM, LEAD, AND ANTIMONY

Spot tests to detect the presence of barium (Ba), lead (Pb), and antimony (Sb) from the bullet primer were developed in the 1960s. These tests are considered more conclusive than the test for nitrates, because in combination the elements are rarely found in the natural environment. When all three of these elements are found in significant levels on a person, recent exposure to GSR is probable.

Sodium rhodizonate test for Ba and Pb

You will need the following reagents:

- Sodium rhodizonate: Prepare the sodium rhodizonate solution by adding 0.2 g of sodium rhodizonate to 100 mL of distilled water and stir. This reagent has a short shelf life and should be made fresh; discard any portions not used. Place in a spray bottle and label.
- Buffer solution, pH 2.8: Dissolve 1.9 g of sodium bitartrate and 1.5 g of tartaric acid in 100 mL of distilled water. This solution may be stored. Place in a spray bottle and label.
- Hydrochloric acid (HCl): Combine 5 mL of concentrated hydrochloric acid in 95 mL of distilled water. This solution may be stored. Remember to gently pour the acid into the water. Calculate the molarity of the HCl and place in a labeled spray bottle.

Known samples: 1 mg/mL Pb standard solution, 1 mg/mL Ba standard solution.

Unknown sample: obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. First, perform a positive control. Obtain a large circle of filter paper pre-treated with acetic acid. Divide it in half. Label one half as Ba and the other as Pb. Add 1–2 drops of the appropriate

- standard solution. Use a hot air gun to dry the filter paper. Note any color changes. Saturate, by spraying, the test papers with the sodium rhodizonate solution.
2. Allow the papers to sit for approximately one minute. The test area should turn a yellowish-orange color.
 3. Next, spray the area with the buffer solution. The test area for lead should turn pink.
 4. To confirm the presence of lead, spray the area with the hydrochloric acid solution. Test areas that turn deep blue-violet indicate the presence of lead.
 5. Next, perform a negative control. Using a blank section of paper, saturate the paper with the sodium rhodizonate solution.
 6. Continue with steps 2–4.
 7. Obtain an unknown. Saturate the sample with the sodium rhodizonate solution.
 8. Continue with steps 2–4.

Harrison-Gilroy (Triphenylmethylarsonium iodide) test for Sb

You will need the following reagents:

- Triphenylmethylarsonium iodide: Dissolve 10 g of triphenylarsine, 10 g of methyl iodide, and 20 mL of absolute ethanol. Reflux the solution for three hours. To reflux, place the solution in a round bottom flask and attach a fractionating column and condenser, so that the refluxed liquid can be trapped. Add ether to the trapped liquid to precipitate the triphenylmethylarsonium salt. The salt can be purified by re-dissolving in ethanol and precipitating with ether.

Known samples: 1 mg/mL Sb standard solution.

Unknown sample: obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. Perform a positive control. Obtain a large circle of filter paper that has not been pre-treated with acetic acid. Divide it in half. Label one half as Sb and the other as negative control. Add 1–2 drops of the appropriate standard solution. Use a hot air gun to dry the filter paper. Note any color changes. Add 1–2 drops of triphenylmethylarsonium iodide solution where the Sb standard was placed. If antimony ion is present, an orange precipitate will appear in about 30 seconds. No further color development will occur after two minutes. Record your observations.
2. Perform a negative control. Place a few drops of distilled water to the blank filter paper. Add 1–2 drops of triphenylmethylarsonium iodide at the location you want to test. No color change should result. Record your observations.
3. To test your unknown, add 1–2 drops of the triphenylmethylarsonium iodide solution. Record your observations.

PART II: COLOR TEST FOR NITRATES

These spot tests will test for nitrates and nitrite ions commonly found in GSR. Since nitrates are ubiquitous compounds commonly found in many consumer products positive results must be interpreted with caution. These tests are commonly used to identify gunpowder patterns so

that distance determinations can be made. You will perform the modified Greiss test and the diphenylamine (DPA) test.

Modified Griess test

You will need the following reagents:

- Greiss reagent A: Prepare 10 mL of 1 % (w/w) solution of sulfanilic acid (MW = 173.84 g/mol). Place in a dropper bottle and label.
- Greiss reagent B: Prepare 10 mL of 0.1 % (w/w) 1-naphthylamine (MW = 143.19 g/mol) in methanol. Place in a dropper bottle and label.

Known samples: Sodium nitrite solution: Dissolve 0.6 g of sodium nitrite in 100 mL of distilled water. Place in a dropper bottle and label.

Unknown sample: obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. First, perform a positive control. Add a few drops of the sodium nitrite solution to a cotton swab. Use a hot air gun to dry the swab.
2. Next add a few drops of Greiss reagent A to the swab. Follow this with a few drops of Greiss reagent B. Note the reaction. An orange-red color indicates the presence of nitrites. Record your observations.
3. Perform a negative control. Place a few drops of dilute HCl (about 1.5 M) to a blank swab. Next add a few drops of Greiss reagent A followed by a few drops of Greiss reagent B to the swab. No color change should result. Record your observations.
4. To test your unknown, add a few drops of Greiss reagent A followed by a few drops of Greiss reagent B to the swab. A positive result indicating the presence of nitrate is an orange-red color. Record your observations.

DPA test

You will need the following reagents:

1. DPA reagent: Add 10 mL concentrated sulfuric acid to 2 mL distilled water under constant stirring. Add to this solution 0.05 g diphenylamine and stir until the solid is completely dissolved. Store in a dark glass bottle. Place into a dropper bottle and label.

Known samples: $\text{NaNO}_3(\text{s})$.

Unknown sample: obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. To test the reagent, first add a couple of crystals of NaNO_3 to a test tube. Add a few drops of water and mix until the NaNO_3 dissolves. Dip a clean dry cotton swab into the solution and

let it absorb. Remove the swab and add a drop of DPA reagent. Note the reaction. A positive result indicating the presence of nitrate is a blue-purple color.

2. Record your observations.
3. Perform a negative control. Place a few drops of DPA on a blank swab. No color change should result. Record your observations.
4. To test your unknown, add a few drops of the reagent to the swab. Record your observations.

PART III: COLOR TESTS FOR COPPER

This spot test will test for copper. Simple cast lead slugs are the simplest form of bullets. At times, the lead may be blended with another metal to give the bullet additional hardness. It is not uncommon to have leaded bullets with a thin coating of copper plating. These bullets are referred to as ‘copper-washed.’ This test provides important information about the entrance or exit holes produced by bullets. It can also provide information about the type of bullet used to produce the holes.

Dithiooxamide (DTO)

You will need the following reagents:

- Dithiooxamide solution: Dissolve 0.2 g of dithiooxamide in 100 mL of ethanol. This solution should be stored in a tightly sealed container to prevent evaporation. Transfer to a spray bottle just before use.
- Ammonia solution: Dilute 50 mL of concentrated ammonium hydroxide with 50 mL of distilled water. Always use a ventilated hood when mixing ammonium hydroxide solutions. Place in a spray bottle and label.

Known samples: 1 mg/mL Cu standard solution or a cooper-washed bullet.

Unknown sample: obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. Perform a positive control. Obtain a large circle of filter paper. Divide it in half. Label one half as Cu and the other as negative control. Add 1–2 drops of the Cu standard solution or swipe the copper-washed bullet over the paper several times. If the solution is used, dry the area with a hot air gun. Note any color changes.
2. Spray the test area with the ammonia solution.
3. After one minute, spray the area with DTO. A green color is a positive reaction for copper. (The presence of a yellow color may indicate the presence of lead.)
4. Perform a negative control. Place a few drops of ammonia followed by the DTO solution as performed in steps 2 and 3 above to a blank filter paper. No color change should result. Record your observations.
5. To test your unknown, add a few drops of the reagents to the swab. Record your observations.

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. Write a paragraph describing how a color test is performed including the value and purpose of the test.
2. Define ‘false positive.’ Give an example of a false positive color test.
3. What can be said if antimony and lead are confirmed on a test sample, but no barium is present? Can this still be attributed to GSR?
4. What are ‘significant levels’?
5. How is the Griess test used for distance determinations?
6. What effect would a bloody shirt have on the testing of a bullet hole? What can be done to compensate for this?
7. Is it important to test both sides of a test sample?

RECOMMENDED AND FURTHER READING

- Anon. Gunshot Residues and Shot Pattern Test. *FBI Law Enforcement Bulletin*. 1970; 39(9): 7.
- Banno A, Masuda T, Ikeuchi K. Three Dimensional Visualization and Comparison of Impressions on Fired Bullets. *Forensic Science International*. 2004; 140(2–3): 233–40.
- Basu S, Boone CE, Denio DJ, Miazga RA. Fundamental Studies of Gunshot Residue Deposition by Glue-Lift. *Journal of Forensic Sciences*. 1997; 42(4): 571–81.
- Bell S. *Forensic Chemistry Laboratory Manual*. Upper Saddle River, NJ: Pearson Education, 2006.
- Coumbaros J, Kirkbride KP, Kobus H, Sarvas I. Distribution of Lead and Barium in Gunshot Residue Particles Derived from 0.22 Caliber Rimfire Ammunition. *Journal of Forensic Sciences*. 2001; 46(6): 1352–7.
- D’Uffizi M, Falso G, Ingo GM, Padeletti G. Microchemical and Micromorphological Features of Gunshot Residue Observed by Combined Use of Afm, Sa-Xps and Sem Plus Eds. *Surface and Interface Analysis*. 2002; 34(1): 502–6.
- Deagetano D, Siegel JA. Survey of Gunshot Residue Analysis in Forensic-Science Laboratories. *Journal of Forensic Sciences*. 1990; 35(5): 1087–95.
- Dillon J. The Modified Griess Test: A Chemically Specific Chromophoric Test for Nitrate Compounds in Gunshot Residues. *AFTE Journal*. 1990; 22(3): 248.
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- Emmons RC. *The Universal Stage (with Five Axes of Rotation)*. Washington, Geological Society of America, Memoir 8, 1943.
- Hamby J. Identification of Projectiles. *AFTE Journal*. 1974; 6(5/6): 22.
- Houck MM, Siegel JA. *Fundamentals of Forensic Science*. Amsterdam; Boston: Elsevier Academic Press, 2006.
- Kage S, Kudo K, Kaizoji A, Ryumoto J, Ikeda H, Ikeda N. A Simple Method for Detection of Gunshot Residue Particles from Hands, Hair, Face, and Clothing Using Scanning Electron Microscopy/Wavelength Dispersive X-Ray (Sem/Wdx). *Journal of Forensic Sciences*. 2001; 46(4): 830–4.
- Klein A, Nedivi L, Silverwater H. Physical Match of Fragmented Bullets. *Journal of Forensic Sciences*. 2000; 45(3): 722–7.

- Lekstrom JA, Koons RD. Copper and Nickel Detection on Gunshot Targets by Dithiooxamide Test. *Journal of Forensic Sciences*. 1986; 31(4): 1283.
- Meng HH, Caddy B. Gunshot Residue Analysis – A Review. *Journal of Forensic Sciences*. 1997; 42(4): 553–70.
- Nichols RG. Defending the Scientific Foundations of the Firearms and Tool Mark Identification Discipline: Responding to Recent Challenges. *Journal of Forensic Sciences*. 2007; 52(3): 586–94.
- Nicols R. Firearms and Tool Mark Identification Criteria: A Review of the Literature. *Journal of Forensic Sciences*. 1997; 42(3): 466–74.
- Nicols R. Firearms and Tool Mark Identification Criteria: A Review of the Literature – Part 2. *Journal of Forensic Sciences*. 2003; 48(2): 18–27.
- Romolo FS, Margot P. Identification of Gunshot Residue: A Critical Review. *Forensic Science International*. 2001; 119(2): 195–211.
- Saferstein R. *Criminalistics: An Introduction to Forensic Science*. 7 ed. Upper Saddle River, NJ: Prentice Hall, 2001.
- Simpson BM, Grant RE. A Synopsis of Urban Firearm Ballistics: Washington, DC Model. *Clinical Orthopaedics and Related Research*. 2003(408): 12–6.
- Tillman WL. Automated Gunshot Residue Particle Search and Characterization. *Journal of Forensic Sciences*. 1987; 32(1): 62–71.
- Tugcu H, Yorulmaz C, Bayraktaroglu G, Ulner HB, Karslioglu Y, Koc S, et al. Determination of Gunshot Residues with Image Analysis: An Experimental Study. *Military Medicine*. 2005; 170(9): 802–5.
- Tugcu H, Yorulmaz C, Karslioglu Y, Uner HB, Koc S, Ozdemir C, et al. Image Analysis as an Adjunct to Sodium Rhodizonate Test in the Evaluation of Gunshot Residues – An Experimental Study. *American Journal of Forensic Medicine and Pathology*. 2006; 27(4): 296–9.
- Wilber CG, Lantz RK, Sulik PL. Gunshot Residue, 10 Years Later. *American Journal of Forensic Medicine and Pathology*. 1991; 12(3): 204–6.
- Zeichner A. Is There a Real Danger of Concealing Gunshot Residue (GSR) Particles by Skin Debris Using the Tape-Lift Method for Sampling GSR from Hands? *Journal of Forensic Sciences*. 2001; 46(6): 1447–55.
- Zeichner A, Levin N. More on the Uniqueness of Gunshot Residue (GSR) Particles. *Journal of Forensic Sciences*. 1997; 42(6): 1027–8.

Experiment 12: Shoe and Tire Print/Impression Examinations

Recommended pre-lab reading assignments:

Bodziak WJ. *Footwear Impression Evidence, Detection, Recovery and Examination*, 2 ed. Boca Raton, FL: CRC Press, 2000; 59–134.
McDonald P. *Tire Imprint Evidence*. Boca Raton, FL: CRC Press, 1993.

OBJECTIVE

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

1. shoe and tire print/impression examinations
2. use of the stereomicroscope for shoe and tire print/impression examinations

INTRODUCTION

Similar to tool mark impressions, shoe and tire print/impression examinations involve the basic principle of uniqueness; where all objects are unique to themselves and thus can be differentiated from each other. The mechanism for differentiating shoe or tire print/impressions depends on class and individual characteristics. Class characteristics are intentional or design characteristics that would be common to a particular group of items. The size and shape of a shoe/tire surface is an example of class characteristics. For instance, a significantly larger print may be left by a size 13 tennis shoe than a size 9. The pattern design and manufacturing characteristics of the tread can also leave varying impressions as shown by different tire brands. General wear patterns to both shoes and tires are also considered class characteristics. Individual characteristics are marks produced by random imperfections or irregularities of the tread surface. These characteristics may be produced through the machining process or by random imperfections or irregularities, which are produced incidental to manufacture and caused by use or damage. For instance, small irregularities left in the

edge of the tire as a result of the machining process used to cut and finish edges provide individual characteristics. More commonly, the shape and pattern of treads is modified by wear and damage to the tire or shoe. Foreign material such as stones or sticks may become attached or wedged into a shoe/tire, which may also provide individual characteristics in a print or impression. This can create a unique pattern for every shoe or tire, allowing for the possibility of individualizing the evidence.

Impression evidence such as shoe or tire prints or impressions can be divided into two general categories: 1) two dimensional impressions and 2) three dimensional surfaces. Prints made by depositing or removing material from a hard surface are two-dimensional; characteristics concerning length and width are considered. These prints are usually photographed and/or lifted. Impressions made in a pliable material are three-dimensional. They involve length, width, and depth. They should be photographed and casted (see Figure 12-1).

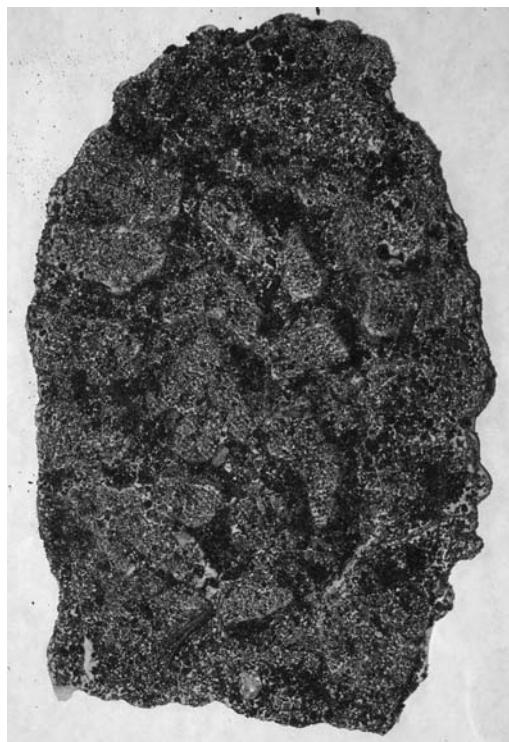


Figure 12-1 A cast made from a partial footprint in soil. The soil can be cleaned from this cast, so that comparisons to a suspect shoe can then be performed.

Many prints and impressions can be enhanced to improve the quality of the visible impression. This may involve various photographic techniques such as the use of filters, oblique lighting, ultra-violet lighting, infrared lighting, and high contrast lighting. An Electrostatic Dust Print Lifter may also be used to enhance outline prints and impressions on paper or dust prints. Fingerprint powders may enhance some prints and impressions, which have been left by wet surfaces. Gelatin and adhesive lifters can also be utilized to lift visible prints and impressions. Chemical enhancements can be done to visualize trace blood prints and impressions.

When several objects are pressed or stamped against one another, allowing the objects to transfer and retain characteristics from one to another, impression evidence is created. Although soil is the most common example, any material that can be manipulated with some degree of force may contain impression evidence. Shoes or tires that come into contact with a liquid (for example, blood, oil) may leave a stain or pattern when further movement occurs. Wet cement and snow may provide a surface for three-dimensional impressions.

In some situations, the investigator may request identification of the possible shoe or tire. For this, the examiner determines the class characteristics of the impression. From this information, a class of shoes or tires can sometimes be determined.

In other situations a complete examination may be requested. In these cases, if the class characteristics are consistent with a suspected shoe or tire, test marks are then made. The test marks are then compared to each other to ensure that the shoe or tire produces unique, consistent individual markings. This examination is done visually and with a stereomicroscope. If unique, consistent markings are found, comparisons can then be performed with the evidence samples. When comparisons are performed, the forensic examiners usually reach one of three results:

- an identification where the exhibit was found to be produced by contact with the shoe or tire and a sufficient correlation between individual characteristics was found;
- a non-identification or elimination where the exhibit was found not to be produced by the shoe or tire and class characteristics did not match;
- inconclusive, where class characteristics agree but there is an insufficient correlation between individual characteristics.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Micro kit

Unknown shoe and tire prints

Unknown shoe/tire impressions

Casting material (Dental StoneTM or Die StoneTM)

Cardboard (or another firm material to frame the impression)

Hair spray

Inkless print kit (paper and ink may also be used)

Shoes

Tires

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor.

PART I: CASTING AN IMPRESSION

Procedure

1. Make a frame or dam around the impression area.

2. If the foundation of the impression is in a loose material (for example, dry soil), spray the area with hair spray to firm up the top layer.
3. Mix the casting material according to manufacturer's instructions. The ideal mixture should be the consistency of batter; not watery, but also not too thick.
4. Apply the casting material over the impression to be cast. To prevent the casting material from breaking up the impression, gently pour the mixture over a spatula. For large impressions, more than one cast may be necessary.
5. When the casting material has begun to set, mark an area with identifying marks.

PART II: PRINT COMPARISON

Procedure

1. Examine the unknown print that you have been assigned.
2. Identify the class characteristics to determine a possible shoe or tire.
3. Obtain a possible shoe or tire and make several test prints using the inkless print kit. After pressing the shoe or tire on the print coater, press it again onto the chemically treated paper. Black prints will become visible.
4. Examine the test prints with the naked eye or stereomicroscope to determine if the shoe or tire produces unique, consistent markings.
5. Compare these markings to those of your unknown print. Not only must the size and shape match up, but also the irregularities or longitudinal striations on each must coincide. Comment on the striations and draw regions that appear similar. Is there anything unusual with any of the knowns or unknowns?

PART III: IMPRESSION COMPARISON

Procedure

1. Examine the unknown impression that you have been assigned.
2. Make a cast of the impression.
3. Once it has thoroughly dried, clean off the cast with water and a soft brush. Take special care not to damage the cast.
4. Identify the class characteristics to determine a possible shoe/tire.
5. Obtain a possible shoe or tire, and make several test prints using the inkless print kit. After pressing the shoe or tire on the print coater, press it again onto the chemically treated paper. Black prints will become visible. If necessary, test impressions/casts can also be made of the known shoe/tire.
6. Examine the test prints with the naked eye or stereomicroscope to determine if the shoe or tire produces unique, consistent markings.
7. Compare these markings to those of your unknown print. Not only must the size and shape match up, but also the irregularities or longitudinal striations on each must coincide. Comment on the striations and draw regions that appear similar. Is there anything unusual with any of the knowns or unknowns?

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. For shoe or tire print/impression evidence what properties are class characteristics? Using a shoe, describe its class characteristics.
2. What are the individual characteristics for shoe or tire print/impression evidence? Using a tire, describe individual characteristics.
3. Write a statement about your print comparison with the ‘known.’ Could the shoe/tire have produced the print? Back up your written statement with why you think they match/or don’t match, referring to your drawings if necessary.
4. Write a statement about your impression comparison with the ‘known.’ Could the shoe/tire have produced the impression? Back up your written statement with why you think they match/or don’t match, referring to your drawings if necessary.

RECOMMENDED AND FURTHER READING

- Abbott Jr. *Footwear Evidence*. Springfield, IL: Charles C. Thomas, 1964.
- Bodziak WJ. Manufacturing Processes for Athletic Shoe Outsoles and Their Significance in the Examination of Footwear Impression Evidence. *Journal of Forensic Sciences*. 1986; 31(1): 153–76.
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- DuPasquier E, Hebrard J, Margot P, Ineichen M. Evaluation and Comparison of Casting Materials in Forensic Sciences – Applications to Tool Marks and Foot/Shoe Impressions. *Forensic Science International*. 1996; 82(1): 33–43.
- Kerstholt JH, Paashuis R, Sjerps M. Shoe Print Examinations: Effects of Expectation, Complexity and Experience. *Forensic Science International*. 2007; 165(1): 30–4.
- Liukkonen M, Majamaa H, Virtanen J. The Role and Duties of the Shoeprint/Toolmark Examiner in Forensic Laboratories. *Forensic Science International*. 1996; 82(1): 99–108.
- Majamaa H, Ytti A. Survey of the Conclusions Drawn of Similar Footwear Cases in Various Crime Laboratories. *Forensic Science International*. 1996; 82(1): 109–20.
- McDonald P. *Tire Imprint Evidence*. Boca Raton, FL: CRC Press, 1993.
- Novoselsky Y, Tsach T, Klein A, Volkov N, Shor Y, Vinokurov A. Unusual Contact Marks: Connecting the Hubcap to the Wheel of the Car. *Journal of Forensic Sciences*. 2002; 47(3): 630–2.
- Robbins LM. The Individuality of Human Footprints. *Journal of Forensic Sciences*. 1978; 23(4): 775–8.
- Shor Y, Kennedy RB, Tsach T, Volkov N, Novoselsky Y, Vinokurov A. Physical Match: Insole and Shoe. *Journal of Forensic Sciences*. 2003; 48(4): 808–10.
- Shor Y, Weisner S. A Survey on the Conclusions Drawn on the Same Footwear Marks Obtained in Actual Cases by Several Experts Throughout the World. *Journal of Forensic Sciences*. 1999; 44(2): 380–4.
- Wilshire B, Hurley N. Development to Two-Dimensional Footwear Impressions Using Magnetic Flake Powders. *Journal of Forensic Sciences*. 1996; 41(4): 678–80.

Experiment 13: Botanical Examinations

Recommended pre-lab reading assignments:

- Core HA, Cote WA, Day AC. *Wood Structure and Identification*. 2 ed. New York, NY: Syracuse University Press; 1979; 64–89, 98–122.
- Summitt R, Siliker A. *CRC Handbook of Materials Science*. Boca Raton, FL: CRC Press; 1980; 11–9.
- Hauber DJ. Marijuana Analysis with Recording Botanical Features Present and Without the Environmental Pollutants of the Duquenois-Levine Test. *Journal of Forensic Sciences*. 1992; 37(6): 1656–61.
- Small E. Morphological Variation of Achenes of Cannabis, *Canadian Journal of Botany*, 1974; 53: 978–87.
- Clarke RC. *Marijuana Botany: Propagation and Breeding of Distinctive Cannabis*. 2 ed. Berkeley, CA: Ronin Publishing, 1993.

OBJECTIVE

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

1. general characteristics of marijuana samples
2. use of the stereomicroscope to identify microscopic characteristics for marijuana
3. general characteristics for hard and soft woods
4. use of the compound microscope to identify microscopic characteristics for hard and soft wood

INTRODUCTION

At times, it may be necessary for a forensic scientist to examine plant material. The most common botanical examinations involve the examination of plant material to identify marijuana, a controlled substance containing delta 9-tetrahydrocannabinol (THC), or hard and soft woods, commonly incurred during various crimes. These examinations can be performed using the stereomicroscope and the compound microscope.

Marijuana

Marijuana is one of the most common illicit drugs. Therefore, examinations must be performed on plant material to identify marijuana and the chemical component contained within, THC. Marijuana examinations involve both microscopic and chemical tests. To understand the identifying morphological features of marijuana, we must first discuss the plant itself.

The species, *Cannabis Sativa L.*, has many varieties; however the identifying features are the same. The plant itself is a tall, weedy plant, sometimes growing to 18 feet (5.5 m) high. Leaves are arranged opposite one another on an erect, branching stem. Leaves are compound palmate, in the fact that they appear hand-like, radiating from a common origin with numerous leaflets. The number of leaflets is usually an odd number, and is most often found to be 7; however numbers between 3–9 have also been found. Leaflets are lanceolate (lance-like) shaped, with serrated (saw tooth) edges. There is a single central vein with further venation from the central vein to the serrated edges. Marijuana plants are considered dioecious, having male or female flowers. Male flowers grow in elongated clusters, whereas female flowers have more of a spike-like cluster and generally grow in pairs. Pistils may be present on female flowers growing from a bract (seed covering) near the base of the leaf stem. Seeds are oval in shape, with a distinct ridge along one side. They have a mottled tortoise shell appearance. Stems and roots may also be present.

The identifying microscopic characteristic for marijuana involves the hair found on each leaflet. Cystolithic hair is found on the top of the leaflet, while simple hairs are found on the bottom side. Cystolithic hairs are claw-shaped and point toward the end of the leaflet. Simple hairs are not tapered and are longer. They are more numerous and appear as a downy mass. Both cystolithic and simple hairs must be present on the same leaflet for a microscopic marijuana identification. Both of these are shown in Figure 13-1.

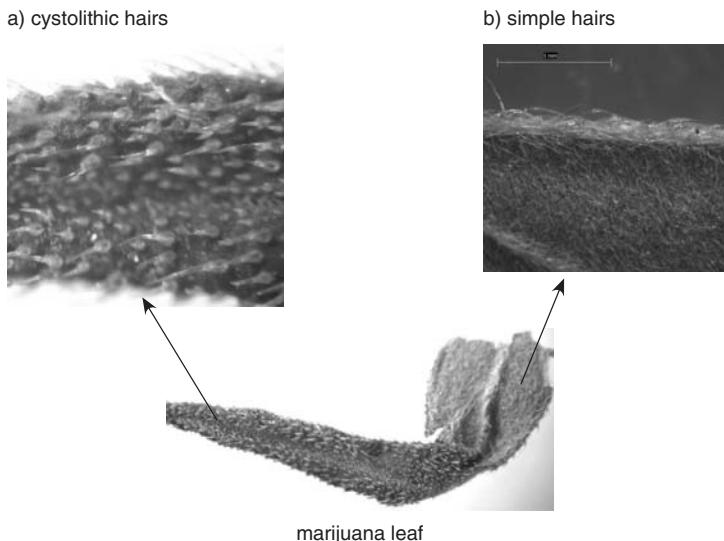


Figure 13-1 A marijuana leaflet showing the location of cystolithic and simple hairs. a) Cystolithic hairs are found on the top of marijuana leaves while b) simple hairs are found on the bottom of leaf. Both must be present in a sample for a positive identification. A full-color version of this figure can be found in the color plate section of this book.

In most situations, it is also necessary to perform further confirmatory tests. This is usually done by the modified Duquenois-Levine test described in Experiment 20, Thin Layer Chromatography, or instrumental analysis (GC-MS or LC-MS).

Two conclusions are generally reached for marijuana examinations. A sample may be identified as containing THC, a controlled substance, or no controlled substances may be identified in a sample.

Soft and Hard Wood

Wood is used for many purposes. Its use in furniture, tools, cutlery, and construction materials exposes the potential of it becoming evidence in almost every crime. A forensic scientist may also be asked to examine botanical samples to determine the species of various wood particles.

Wood is comprised of cellular structures called tracheids, which are elongated fibrous cells in the xylem. In soft woods (coniferous or non-porous), tracheids are long and usually have large prominent bordered pits on their radial walls. In hard woods (non-coniferous or porous), tracheids are shorter and associated with vessel elements that may possess small-bordered pits. The major difference between soft and hard woods is the presence of vessel elements or pores (see Figure 13-2). Vessel elements are wide openings that when present are used to transport water through the plant. Vessel elements are only found in hard woods.

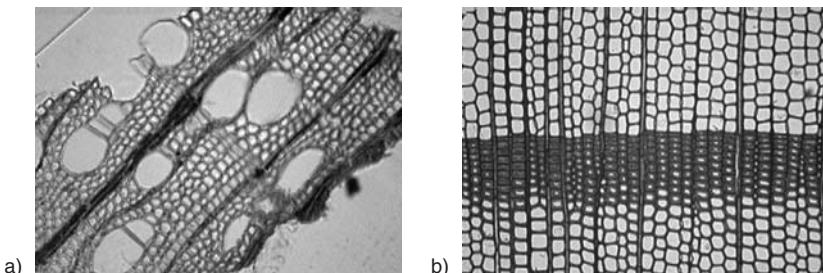


Figure 13-2 a) A microtome cutting of birch (hard wood) that shows vessel elements. To make the cellular structure more easily viewed, the sample was stained with food coloring. b) A microtome cutting of Douglas fir (soft wood). Note the lack of vessel elements. (Reproduced with permission of Lara Mosenthin)

To further distinguish soft and hard wood species, additional microscopic examinations must be performed. Radial and tangential sections should be examined with a polarized light microscope to identify the various microscopic features present within the sample.

Three conclusions are generally reached for wood examinations: 1) a sample may be identified as a particular hard wood; 2) a sample may be identified as a particular soft wood; 3) or the species may not be able to be determined for the sample.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and coverslips

Mounting medium (a colorless mounting medium in the refractive index range of 1.50–1.60)

Various plant samples, including marijuana

Hard and soft wood samples

SAFETY

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

PART I: MARIJUANA EXAMINATIONS

Procedure

1. Obtain a plant sample.
2. If possible, weigh the sample.
3. Using the stereomicroscope, examine the sample.
4. Describe the overall condition of the sample.
5. Observe whether or not the following features are present: palmate leaflets, lanceolate shaped leaflets, serrated edges, alternate venation or pinnate venation, seeds, stems, bracts, husks, female flowers, male flowers.
6. Continue to examine the leaflet under the stereomicroscope using slightly higher magnification.
7. Observe whether or not the following features are present: cystolithic hair, simple hair, resin glands. Using the circle template located in Appendix F, draw what you see.
8. Determine whether or not the sample is marijuana.
9. Repeat steps 1–8 using five more samples.

PART II: WOOD EXAMINATIONS

Procedure

1. Obtain a wood sample.
2. Prepare a cross-section for viewing by making a thin, clean cut perpendicular to the growing length of the sample.
3. Mount the sample so that it can be examined using the compound light microscope. Examine the surface for the presence or absence of vessel elements.
4. If necessary, enhance the image by soaking the cross-section in green food coloring. Allow to dry and re-examine the surface.
5. Draw what you see.
6. Identify the sample as hard or soft wood.
7. Repeat steps 1–6 with five additional samples.

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 microscopic characteristics used to identify marijuana?
2. Describe the difference between the hairs found on marijuana.
3. What is the difference between male and female marijuana plants?
4. What is the difference between soft and hard woods? Give examples of each.
5. What are the identifying characteristics for soft woods?
6. What are the identifying characteristics for hard woods?

RECOMMENDED AND FURTHER READING

- Clarke RC. *Marijuana Botany*. Berkeley, CA: And/Or Press, 1981.
- Clarke RC. *Marijuana Botany: Propagation and Breeding of Distinctive Cannabis*. Berkeley, CA: Ronin Publishing, 1993.
- Core HA, Cote WA, Day AC. *Wood Structure and Identification*. 2 ed. New York, NY: Syracuse University Press, 1979.
- Hauber DJ. Marijuana Analysis with Recording Botanical Features Present and Without the Environmental Pollutants of the Duquenois-Levine Test. *Journal of Forensic Sciences*. 1992; 37(6): 1656–61.
- Robards AW. *Botanical Microscopy*. Royal Microscopical Society (Great Britain). Oxford/New York: Oxford University Press, 1985.
- Schultes RE, Hoffman A. *The Botany and Chemistry of Hallucinogens*. Springfield, IL: Charles C. Thomas, 1980.
- Small E. Morphological Variation of Achenes of Cannabis. *Canadian Journal of Botany*. 1974; 53: 978–87.
- Summitt R, Siliker A. *CRC Handbook of Materials Science*. Boca Raton, FL: CRC Press, 1980.
- US Treasury Dept. *Marijuana and Its Identification*. Washington, DC: US Government Printing Office, 1948.
- Welch MJ, Ellerbe P, Tai SSC, Christensen RG, Sniegoski LT, Sander LC, et al. NIST Reference Materials to Support Accuracy in Drug-Testing. *Fresenius Journal of Analytical Chemistry*. 1995; 352(1–2): 61–5.
- Wheeler EA, Baas P. Wood Identification – a Review. *IAWA Journal*. 1998; 19(3): 241–64.

Experiment 14: Paint Examinations

Recommended pre-lab reading assignments:

Thornton J, Shemuel K, Lerner B, Kahane D. Solubility Characterization of Automotive Paints. *Journal of Forensic Science*. 1983; 28(3): 1004.

Thornton JI. Forensic Paint Examinations. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 458–73.

OBJECTIVE

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

1. general characteristics of paint samples
2. cross-sections
3. techniques that can be used to produce cross-sections of paint samples
4. solubility testing
5. paint solubility testing
6. use of the compound or comparison light microscope to visualize paint characteristics

INTRODUCTION

For many years, humans have used colored substances contained within some type of a substrate to adorn or protect surfaces. Decorative effects are produced by color, gloss, texture, lighting, or a combination of these properties. The protective function of paint includes resistance to air, chemicals, and water, in addition to properties such as hardness and abrasion resistance. Because of these decorative and protective features, the average person is surrounded by painted items. The main uses of paint include: automobiles, architectural or structural, and other miscellaneous uses (i.e., tools, non-automotive vehicles, appliances, artistic media, and cosmetic coatings). Since there is the possibility of contact with these items, microscopic examination of paint presents itself as indirect or circumstantial evidence in a court of law. This evidence is generally used in

conjunction with other forensic evidence. Paint examinations are performed to determine possible associations between people, places, and objects.

Paint is usually considered to be a pigment or extender that has been dispersed in a suitable vehicle or binder, and reduced to an application viscosity with the addition of solvents. Pigments, which may be organic or inorganic, provide color and concealing properties to paint. They furnish the dried paint film with color, opacity, gloss, and permeability. Extenders may be added to the formulation to disperse the pigment. These compounds are held together with the aid of a vehicle or binder. Paint vehicles are made of chemicals, generally resins, which form the thin film. The vehicle gives the adhesive, durability, flexibility, and reinforcement properties to the paint film. The thickness of the film may be adjusted with various solvents that are water- or organic-based. Solvents provide the properties that affect drying time and flow. Other additives may also be incorporated to modify the paint properties or application. At times components are added to increase gloss, hardness, and chemical resistance of the paint. UV and anti-mold components may also be added.

Paint examinations use of a variety of microscopes, techniques, and instruments. Examinations have two goals: 1) identify a paint type and 2) compare a paint sample back to a possible paint source when possible. Initially a stereomicroscope is used to examine the paint for layer structure and solubilities. FTIR (Experiment 22) and UV-VIS microspectrophotometry (Experiment 23), Scanning Electron Microscopy (Experiment 25), and Pyrolysis Gas Chromatography are additional techniques, which may be performed on paint.

The examination and comparison of cross-sections is an integral part of a paint examination. A cross-section of a solid is obtained by making a ‘slice’ of that solid. This shape can vary according to the orientation of the slice. A true cross-section is obtained with a 90° cut; however, at times, to view the cross-section of a paint sample, a 45° cut is preferred. With paint samples, a cross-section is used to identify the various layers of the paint sample and at times other characteristics within these layers. This layer structure is a characteristic of a paint sample and may be unique. The sequence of the layers also provides valuable information. Automotive paints are applied in layer sequences that may be related back to specific manufacturing companies. For instance, some manufacturers use a combination of three coats (clear coat, color coat, and primer), whereas others use four (clear coat, color coat, primer, primer). The individual layers may also provide additional manufacturer information. Thickness of paint layers may help indicate the application method utilized. Original automotive paints are very thin, however, most repaints are applied slightly thicker and at times uneven. The thickness of layers may also give indications as to whether the paint is from a vehicle or painted object, or a structural paint sample. Examination of each individual layer also provides the examiner with additional chemical information. Surface defects due to weathering and aging, and any contaminants, may also be noted during a cross-sectional examination.

A cross-sectional sample can be obtained using a scalpel to visualize the layers. By cutting the paint sample at an angle, layer structures can be examined. Placing the cut cross-section into a porcelain dish, along with a solvent such as methanol to cover the sample, assists in viewing thin or clear layers.

Solubility determinations can also be an important part of paint examinations. Although this method of testing is destructive, valuable information can be obtained from solubility testing. Determining the solubility of the different layers in paint samples has been used to discriminate between paint samples of differing pigment and binder composition that are otherwise similar in visual and macroscopic appearance. These tests are based on the solubility of the paint binders, in addition to the pigment and binder color interactions of oxidation, dehydration, and reduction. Solubility testing of paints involves a possible reaction of the paint with a variety of solvents. Since paints are made of different components and processes, they react differently in certain solvents.

Common reactions include: 1) the paint may be soluble or be partially soluble; 2) it may leach color; 3) it may swell, curl, discolor, soften, sink, and/or float. Occasionally the paint sample may react with the solvent, which provides additional limited information concerning the type of paint being tested. Table 14-1 lists some the solubility and reaction properties for common solvents and different paint types.

Table 14-1 Solubility and reactions for common solvents used in paint examinations.

Solvent	Solubility and reactions
Chloroform	Enamels remain insoluble. Acrylics will soften. Acrylic enamels will smoke and leave an oily residue. Alkyds show no reaction. Lacquers are soluble or soften. Nitrocellulose is soluble or softens.
Acetone	Most acrylics will soften. Acrylic lacquers are generally soluble. Lacquers are almost always soluble. Nitrocellulose is soluble or softens.
DPA: (reagent is a mixture of 3 g diphenylamine, 200 ml of concentrated sulfuric acid, and 100 ml of glacial acetic acid)	A color change to blue may indicate the presence of an oxidizing agent or nitrocellulose paint. Some paints will also effervesce.

EQUIPMENT AND SUPPLIES

- Stereomicroscope
- Paint samples
- Micro kit
- Spot plate
- Solvents (chloroform, methanol, acetone, DPA [prepare this reagent as described in Table 14-1])

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. The solvents should be handled with care. Proper ventilation, gloves, and eye protection should be used at all times. Refer to the MSDS if necessary.

PART I: CROSS-SECTIONS

Procedure

1. Obtain a paint sample.

2. Using the stereomicroscope, turn the paint chip up on edge.
3. Using the circle template located in Appendix F, draw the layer structure.
4. Cut a small chip from your sample. Using a spot plate, immerse the paint chip on edge in methanol.
5. Draw the layer structure (using methanol helps visualize thin and/or clear layers).
6. Lay the paint chip back down. Using a scalpel, slowly cut the edge of the paint chip at approximately a 45° angle to expose the underneath layers. Check the layer structure against your previous findings. Draw your findings.

PART II: SOLUBILITY

Procedure

1. Obtain two paint samples.
2. Cut three small sections from one of the paint samples.
3. Place each section in a separate spot well.
4. Add a drop (or two) of chloroform to the spot well.
5. Observe and document any reaction with the paint sample.
6. To the second spot well, add a drop (or two) of acetone.
7. Observe and document any reaction with the paint sample.
8. To the third spot well, add a drop (or two) of DPA.
9. Observe and document the reaction with the paint sample.
10. Repeat steps 2–9 with the second paint sample.
11. Create a chart with your findings.

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 three portions of a paint? What is the purpose for each portion?
2. What is a cross-section?
3. What information does a cross-section give an examiner for paint samples?
4. How can an examiner determine if an automotive paint sample is a repaint?
5. Describe the principle behind solubility testing.
6. Define solubility. Why is solubility testing performed during a paint examination?
7. What are the advantages of solubility testing?
8. What are the disadvantages of solubility testing?
9. What is the correct procedure for performing solubility testing on evidence?

RECOMMENDED AND FURTHER READING

- Allen TJ. Modifications to Sample Mounting Procedures and Microtome Equipment for Paint Sectioning. *Forensic Science International*. 1991; 52(1): 93–100.
- Allen TJ. The Examination of Thin-Sections of Colored Paints by Light-Microscopy. *Forensic Science International*. 1992; 57(1): 5–16.
- Allen TJ, Schnetz B. The Removal of Paint Smears from Tools and Clothing for Microscopic Examination and Analysis. *Forensic Science International*. 1991; 52(1): 101–5.
- Beam TL, Willis WV. Analysis Protocol for Discrimination of Automotive Paints by Sem-Edx Using Beam Alignment by Current Centering. *Journal of Forensic Sciences*. 1990; 35(5): 1055–63.
- Caddy B. *Forensic Examination of Glass and Paint: Analysis and Interpretation*. London: Ellis Horwood Ltd, 2001.
- Castle DA. The Forensic Examination of Paint. *Jocca-Surface Coatings International*. 1992; 75(7): 247.
- Crown DA. *The Forensic Examination of Paints and Pigments*. Springfield, IL: Charles C. Thomas, 1968.
- Giang YS, Wang SM, Cho LL, Yang CK, Lu CC. Identification of Tiny and Thin Smears of Automotive Paint Following a Traffic Accident. *Journal of Forensic Sciences*. 2002; 47(3): 625–9.
- Laing DK, Locke J, Richard RA, Wilkerson JM. The Examination of Paint Films and Fibers as Thin-Sections. *Microscope*. 1987; 35: 233–48.
- Stoecklein W. *The Role of Colour and Microscopic Techniques for the Characterization of Paint Fragments*. London, England: Taylor & Francis, 2001.
- Thornton J, Shemuel K, Lerner B, Kahane D. Solubility Characterization of Automotive Paints. *Journal of Forensic Sciences*. 1983; 28(3): 1004.
- Thornton JI. Forensic Paint Examinations. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 458–73.
- Zieba-Palus J. Selected Cases of Forensic Paint Analysis. *Science & Justice*. 1999; 39(2): 123–7.

Hair Examinations

Experiment 15: Hair Examinations

Recommended pre-lab reading assignments:

Ogle RR, Mitisinka GT. A rapid technique for preparing hair cuticle scale casts. *Journal of Forensic Sciences*. 1973; 18–82.

Recommended websites:

Deedrick DW, Koch SL. Microscopy of Hair Part I: A practical guide and manual for human hair. *Forensic Science Communications*. 2004; 6(1). Available from: <http://www.fbi.gov>.

Deedrick DW, Koch SL. Microscopy of Hair Part II: A Practical Guide and Manual for Animal Hairs. *Forensic Science Communications*. 2004; 6(3). Available from: <http://www.fbi.gov>.

OBJECTIVE

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

1. general characteristics of a hair
2. use of the stereomicroscope to visualize the characteristics of hair
3. use of the compound or comparison light microscope to visualize the characteristics of hair
4. preparing scale casts
5. identifying animal hair
6. identifying human hair

INTRODUCTION

Humans and most other mammals have hair located over their bodies. Although animal hair is limited in characteristics to determine specific species and somatic origin, human hair possesses characteristics that make these identifications and further comparisons sometimes possible. Therefore, microscopic examinations of hair presents itself as indirect or circumstantial evidence in a

court of law. This evidence is generally used in conjunction with other forensic evidence. Hair examinations are performed to determine possible associations between people, places, and objects.

The hair is divided into several regions for examination purposes (see Figure 15-1a). The root is the structure at the proximal end of a hair. This portion of the hair is the active growing site when attached to the hair follicle. The tip is the most distal end of a hair. The section in the middle is the hair shaft, composed of three main sections: cuticle, cortex, and medulla (see Figure 15-1b).

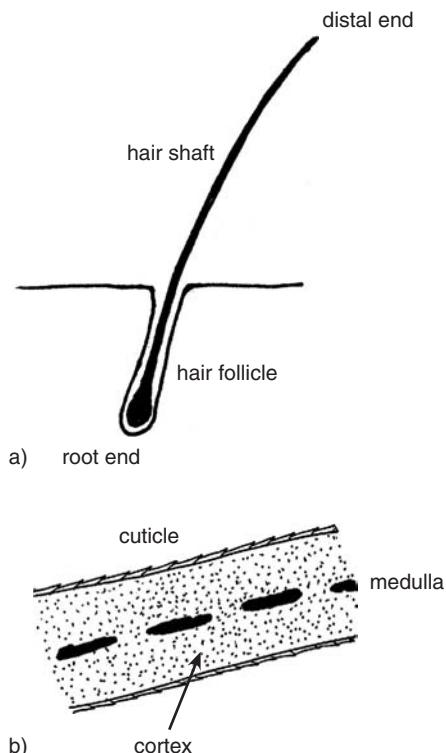


Figure 15-1 a) Hair grows in a structure called the hair follicle. The hair can be divided into three regions: root; shaft; and tip (distal end). Hair grows from the root end that is attached to the follicle during growth. b) The three sections of the hair are the cuticle, medulla, and cortex.

Microscopic analysis of hair includes using a stereomicroscope and a compound microscope. Examinations are performed to identify characteristics for the hair. These characteristics may include more obvious macroscopic features such as color, shaft form, diameter, and length of the hair, but also microscopic features such as cuticle scales, cortex characteristics including pigment granules, shape of medulla, and root and tip characteristics. Many times, the Medullary Index (MI) is also determined. The MI is simply the diameter of the medulla divided by the diameter of the hair shaft:

$$\text{Medullary Index} = \text{MI} = \frac{\text{diameter of medulla}}{\text{diameter of hair}} \quad (15-1)$$

Among other things, the MI is used to distinguish animal hair from human hair. Animals have a medullary index ranging from 0.5–0.9 whereas humans have a medullary index less than 0.3.

Macroscopic Characteristics

Characteristics such as color, form, diameter, and the length of the hair are considered macroscopic characteristics. Various colors, either natural or artificial, are found in hair (termed white, gray, blonde, red, brown, black, and other). These colors may range in various shades and also intensities. Visible coloring or banding patterns may also be present. Hair may display various shaft forms or shapes. Straight, waved, curled, kinked, and curved are examples of shaft form. The shaft diameter is another macroscopic characteristic of the hair structure. Diameter can either be recorded as a measurement or with terms such as: fine, medium, coarse, and varying. Finally, the shaft length is also measured for each hair sample. This is generally measured in inches or centimeters and described as a range for a sample.

Microscopic Characteristics

The reflected color of hair can be also be described while viewing the hair microscopically. Colorless, blonde, red, brown, black, and other are terms used to describe the microscopic color of hair. These colors may also be in various shades (i.e., gray-brown, yellow-brown, and red-brown), or varying intensities (light, medium, and dark). Description of the pigmentation further defines the coloring of the hair microscopically. Natural pigment granule color, size, shape, density, and distribution in addition to pigment aggregation, aggregate size, and density can provide additional information about the microscopic characteristics of a hair. Artificially colored hair can also be described with similar details.

The microscopic structure of hair can also be divided into several categories during a hair examination. Shaft forms such as buckling, convoluting, shouldering, undulating, twisting, and uniform can be determined from microscopic examinations. Buckling (see Figure 15-2d) is a disruption of the hair shaft resulting in an abrupt change in direction without a twist. Convoluting is an abrupt rotation of the hair shaft. Shouldering (see Figure 15-2e) is a partial variation of the diameter along the hair shaft, and undulating features changes in the diameter along the hair shaft.

The cross-sectional appearance can also provide additional information about a hair. Round, oval, flattened, and triangular cross-sections are commonly found for hair samples. The diameter of a hair is described in micrometers during a microscopic examination. This should be measured at several points along the hair shaft (from root to tip end) with an objective containing a calibrated micrometer.

A hair can be divided into three main regions: the root, the shaft, and the tip. The root (proximal end) of a hair can provide numerous microscopic characteristics. A hair lost from the scalp during the anagen stage (actively growing) will typically have an epithelial sheath adhering to the elongated pigmented root. A hair lost during the catagen stage (transition) will generally have a slightly enlarged root and may have bits of epithelial tissue adhering to the root. Hairs that are shed during the telogen (resting) stage typically have an enlarged root that lacks pigment and has no epithelial tissue attached. Shriveled, stretched, or damaged follicular tags may also provide information as to how or why the hair was shed. Post mortem changes in a hair root can also provide important information. The absence of a root may also provide information. The missing root end may be square cut, angular cut, angular cut with elongated tail, crushed, broken, and singed. Post mortem changes may also be described when the root end is missing.

Many microscopic characteristics are also found within the shaft (central portion) of a hair. The shaft can be divided into three sections: cuticle, cortex, and medulla.

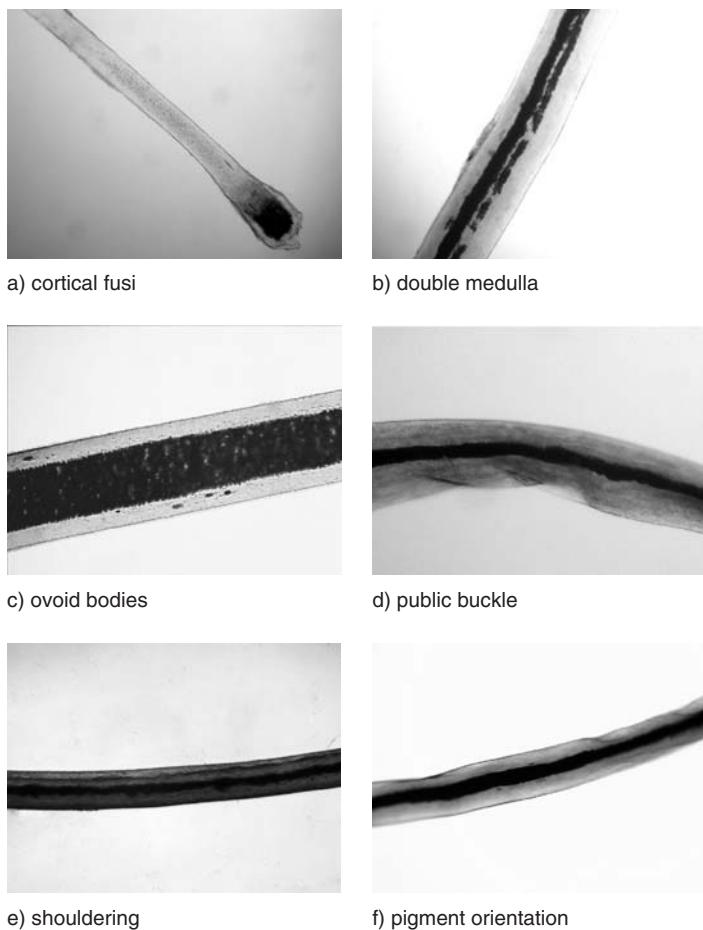


Figure 15-2 Several microscopic characteristics of hair that can be noted to ‘individualize’ a hair sample. A full-color version of this figure can be found in the color plate section of this book.

Cuticle

This portion of the hair consists of the multi-scale outer layer, which may be present or absent. The scales overlap to form a variety of patterns. There are three main patterns of scales: coronal, spinous, and imbricate (see Figure 15-3). The coronal scale pattern is created by the overlapping of scales that are shaped like small crowns or cups, stacked on top of each other. The spinous or petal-shaped scales are created by the overlapping of scales that are shaped like small triangular tiles, stacked on top of each other. Imbricate scales have irregular borders and margins. Once again, they are stacked on top of each other. Various scale patterns may be found in animal hair, however, human hair is always imbricate or irregular waved. Figure 15-4 contains several variations of spinous and imbricate scale patterns.

When present, the thickness and color of the cuticle should be noted: Thin is generally considered to be $2.5\text{ }\mu\text{m}$ or less; thick generally refers to $2.5\text{ }\mu\text{m}$ or greater. Colors are generally referred to as transparent, translucent, or yellow. The condition of the outer cuticle margin may also display a variety of microscopic differences, for example, smooth, flattened, serrated, looped, ragged, and

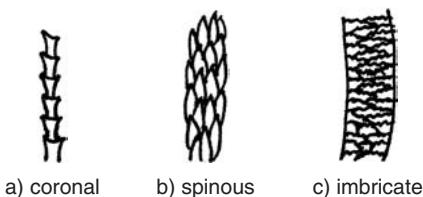


Figure 15-3 The three main patterns of hair scales.

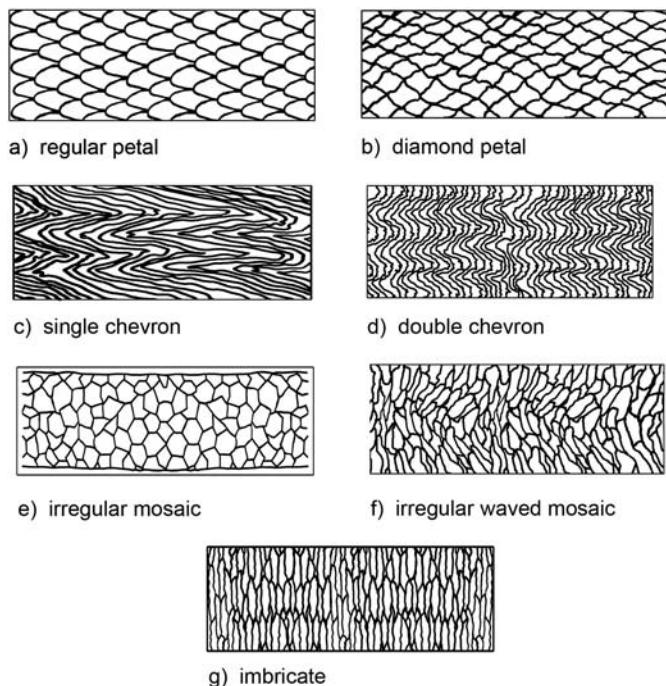


Figure 15-4 Variations of spinous and imbricate scale patterns. Scale patterns specific to the deer family and antelope are shown in Figure 15A-2.

cracked. The inner cuticle margin may be distinct or indistinct with pigment granules absent or present.

Cortex

This portion is the main body of the hair. It is composed of elongated and fusiform keratinized cells. Many of the microscopic characteristics are found within this portion of the hair. The cellular texture of the cortex may be apparent, extreme, or not visible. Color and pigmentation details are visualized in the cortex portion of the hair (see Figure 15-2f). Cortical fusi, which are small fusiform bodies that contain air, are sometimes found in the cortex. They are more commonly found near the root and the proximal portion of the hair (see Figure 15-2a), but may be seen at times toward the distal portion. Their size, shape, location, and abundance should be noted. Ovoid

bodies, which are large aggregates of pigment that have a spherical or oblong shape, may also be present (see Figure 15-2c). They range in diameter from 3 to 20 μm . When present, their size, shape location, and abundance should also be noted.

Medulla

This is the final portion of the hair. A medulla is located in the center of the hair, which may or may not be visible in each hair. Several cellular patterns or medulla forms are found in human and animal hair. Animal hair may have uniserial, multi-serial, lattice, vacuolated, and amorphous medullas, which are shown in Figure 15-5. Human hair is generally amorphous in appearance.

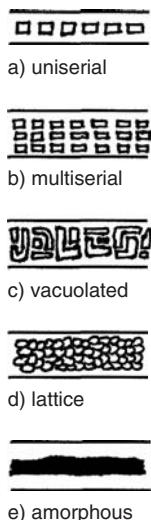


Figure 15-5 Medulla forms found in animal or human hair.

The distribution of the medulla is also an important microscopic characteristic. This describes the presence of the medulla along the hair shaft. Absent, continuous, or discontinuous are several terms used to describe the medulla distribution. The appearance of the medulla may be opaque, translucent, or absent. If the medulla is air filled, it will appear black or opaque with transmitted light (see Figure 15-2b). If the medulla is filled with a liquid, it will appear clear or translucent. The relative width of the medulla should also be noted using the Medullary Index, which is calculated by using Equation 15-1 (p. 150).

The tip of the hair also displays microscopic characteristics. Natural or uncut tips may be referred to as tapered. Cut tips may appear square, angular, or angular with a tail. Tips may also be broken, split, frayed, rounded, crushed, abraded, or singed.

Animal and human hair can easily be differentiated by macroscopic and microscopic characteristics. Animal hairs may contain various colors and color banding patterns, whereas human hair tends to be more consistent in color. Hair shaft diameter changes along the shaft are commonly found in animal hairs. Whereas human hair displays an imbricate or irregular-waved scale pattern, various scale patterns are found in animal hairs. Human hair generally has an amorphous medulla,

which may be absent, discontinuous, or continuous. Various medullary patterns are found in animal hairs. In addition to medullary patterns, the medullary index can also be a determining factor of whether hair is human or animal. Finally, various root shapes are found in some animal hairs.

Animal hair

The forensic scientist uses numerous characteristics to identify animal hair. These are summarized in Table 15-1. Pigmentation, shaft diameter, scale patterns, medullary patterns, and root shapes provide valuable information to the forensic hair examiner. Various pigmentation and color banding patterns are commonly found in animal hairs. These patterns provide information for specific identifications of animal hair. Shaft diameter and changes along the shaft are also important factors to take into consideration when identifying animal hair. Numerous scale patterns are found in animal hairs. Use of scale cast mounting preparation makes viewing scale patterns easier for the hair examiner. The size, shape, and pattern arrangement on the hair provides distinctive characteristics for certain animals. Several medullary patterns are also found in animal hairs. These patterns provide additional information for certain animal hair identifications. Various root shapes are found in some animal hairs. These root shapes provide information for specific identifications of animal hair. Animal hair is not routinely compared in forensic casework. However, in many cases it may be necessary to identify the species.

Table 15-1 Comparison of animal and human hair characteristics.

Characteristic	Animal	Human
Color	Often has color changes along shaft, may be in specific banding patterns	Color remains consistent
Pigmentation distribution	Pigment is denser towards medulla	Pigment even or denser towards cuticle
Cuticle	Various scale patterns, some changes along shaft	Imbricate pattern
Cortex	Width is thinner than the medulla	Width is the majority of the hair shaft
Medulla	Width is greater than 1/3 of hair shaft, usually continuous	Width when present is thin, mostly continuous
Medulla form	Various patterns	When present; amorphous
Root shape	Various shapes	Bulbous
Diameter of hair	Variation along shaft, 'shield'	Diameter moderate, changes gradual along shaft

Human hair

Numerous characteristics are used by the hair examiner to identify human hair (summarized in Table 15-1). Pigmentation, shaft diameter, scale pattern, medullary pattern, and root characteristics also give information to aid in the identification of human hair. Color is generally constant and shaft diameter is generally moderate with gradual changes. The scale pattern for human hair is imbricate or irregular-waved. The medulla is amorphous when present, with continuous or discontinuous distribution. Root shape may also give an indication for human hair. Once a hair has been identified as human, further characterization of the hair can be done.

Hair examinations involve the use of a variety of microscopes, techniques, and instruments. Examinations are usually performed to identify a hair and then, when necessary, to compare the hair back to a possible hair source. Initially a stereomicroscope is used to examine the hair for macroscopic characteristics such as color, length, and form. Further testing can be performed using a compound or comparison light microscope to determine the microscopic characteristics of the hair sample. To do this, scale cast, semi-permanent, or permanent mounts are used.

Since a compound microscope uses transmitted light, a scale cast can be used to view the external or surface features of a hair. A scale cast is an impression or semi-permanent mold made from the scale pattern of the hair. Scale patterns, along with other microscopic characteristics, are used primarily with animal hair for species identification. Semi-permanent and permanent mounts are used to visualize other microscopic characteristics of a hair. To observe these characteristics properly, a hair must be mounted in a medium of refractive index similar to that of the hair itself. The average refractive index for hair is 1.55. A synthetic, semi-permanent, or permanent mounting medium with a refractive index in the range of 1.5–1.6 is generally recommended.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound Light Microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and calibrated focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media

Nail polish and/or Polaroid film coating

Animal hair

Human hair

Unknown hair samples

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: MACROSCOPIC EXAMINATION OF HAIR

Procedure

Use the hair worksheet located at the end of this experiment or available from <http://www.wileyeurope.com/college/wheeler> for each hair examined.

1. Examine the hair with the stereomicroscope.
2. Measure the length of the hair. Record the value in centimeters.
3. Note the color and any banding patterns that may be present.
4. Note the shaft form of the hair.
5. Note the diameter of the hair.
6. Repeat steps 1–5 for four additional animal samples and one human hair sample.

PART II: SCALE CASTS

Procedure

1. Use the hair samples examined in Part I.
2. Label a microscope slide with identifying marks (analyst initials and sample).
3. Apply a thin coating of clear nail polish (Polaroid film coating may also be used).
4. While still wet, position an animal hair in the nail polish. Make sure the entire length of the hair is included.
5. Let the nail polish dry completely.
6. Gently remove the hair to obtain the scale cast.
7. Observe the scale pattern using the compound light microscope. Remember to check the microscope to ensure that samples will be viewed using Köhler illumination.
8. Be sure to observe the entire length of the hair because scale patterns may change from the proximal to distal ends. Draw what you see.
9. Repeat steps 1–8 for additional hair samples.

PART III: ANIMAL HAIR AND HUMAN HAIR CHARACTERISTICS

Procedure

1. Make liquid mounts of the five animal hair samples previously examined.
2. Make liquid mounts of the human hair previously examined and four additional samples.
3. Examine the hairs using the compound light microscope.
4. Document the macroscopic characteristics of each hair: color, shaft form, and diameter.
5. Examine the hairs for any additional microscopic characteristics, which would aid in the identification of the hair type. Document the additional characteristics of each hair: cuticle, cortex and medulla widths, medulla form, root shape, and pigmentation orientation.
6. Measure the diameter of the hair and the medulla for all hairs to calculate the medullary index of each hair. Calculate the medullary index using Equation 15-1 (p. 150).
7. Draw one identifying characteristic for each hair, including information such as sample and magnification.

8. Obtain an unknown hair. Determine if the hair is animal or human. Refer to Table 15-1 (p. 155) as needed. List the macroscopic and microscopic characteristics for the hair on the hair worksheet.

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. Describe each term as it relates to hair: root, cuticle, cortex, medulla, tip. Include drawings and/or examples with your description for each term.
2. List three characteristics that are used to distinguish human hair and animal hair. Give examples of each, and the differences found between human and animal.
3. Describe your examination procedure for the samples you obtained.
4. What did a scale cast help determine? What did the liquid hair mount help determine?
5. Include your documentation and drawings for parts I and II. Make sure your documentation includes the appropriate labeling. (name, sample numbers, lab)
6. Include the unknown hair number and whether you have identified the hair as originating from animal or human. State your reasons to defend your conclusion.

HAIR WORKSHEET

Sample _____

Color _____

Length _____

Shaft Form _____

Diameter _____

Cuticle _____

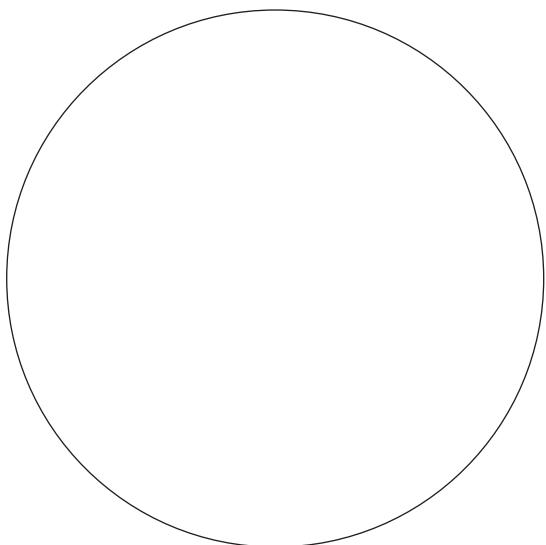
Cortex _____

Medulla _____

Root Shape _____

Pigmentation _____

Medullary Index _____



Sample _____

Color _____

Length _____

Shaft Form _____

Diameter _____

Cuticle _____

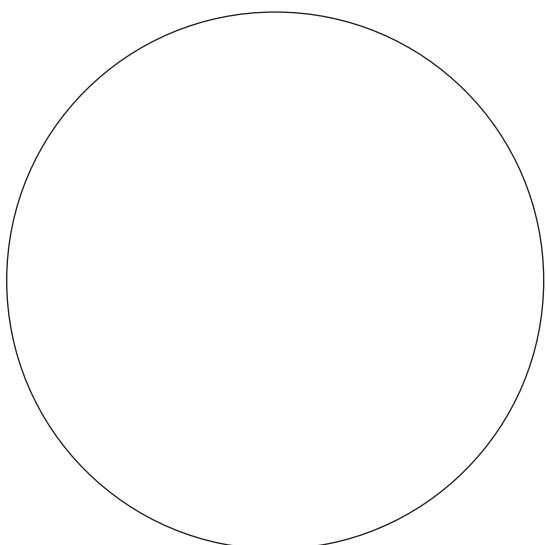
Cortex _____

Medulla _____

Root Shape _____

Pigmentation _____

Medullary Index _____



Experiment 15A: Animal Hair Examinations

Recommended pre-lab reading assignments:

Brunner H. *The Identification of Mammalian Hair*. Melbourne, Australia: Inkata Press, 1974; 1–18.

Spence LE. *Study of Identifying Characteristics of Mammal Hair*. Wildlife Disease Research Laboratory: Wyoming Game and Fish Commission; 1963.

OBJECTIVE

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

1. general characteristics of animal hair
2. use of the stereomicroscope to visualize the characteristics of hair
3. use of the compound or comparison light microscope to identify and visualize the characteristics of hair
4. preparation and observation of scale casts

INTRODUCTION

The Locard Exchange Principle states that whenever two objects come in contact, an exchange of matter occurs. This theory applies to hair evidence. Hair is easily shed and transferred whenever there is contact between two objects, two individuals, or an individual and another object. Pets often shed animal hairs, and these may be easily transferred to clothing or other objects. Animal hair may also be present from clothing or textile products made from animal hair (i.e., wool sweaters, blankets). These hairs may also be transferred to individuals, clothing, or other objects. Therefore, animal hair is often used to associate people with items or scenes. This makes animal hair an important source of information in forensic investigations.

The forensic scientist uses various characteristics to identify animal hair. Animal hair possesses various macroscopic and microscopic characteristics that make identification and further association with a particular species sometimes possible. Three types of animal hair may be encountered in casework: fur, tactile, and guard. Fur hair is a fine inner coat of hair, which is used by the animal for warmth and insulation. Tactile hair is found on the head of the animal and provides sensory functions for the animal. Guard hair is the outer coat of hair and used by the animal for protection. These hairs are generally characteristic of the family or species and may be used for identification.

Dividing animal hair into three ‘families’ provides easier classification of the macroscopic and microscopic characteristics of animal hair. Numerous characteristics are used to divide animal hair into these three groups. Characteristics of a hair such as color, pigment distribution, cuticle patterns, cortex and medulla widths, medulla form, root shape (see Figure 15A-1), and overall diameter of the hair are taken into consideration when classifying a hair to a particular ‘family’ or species.

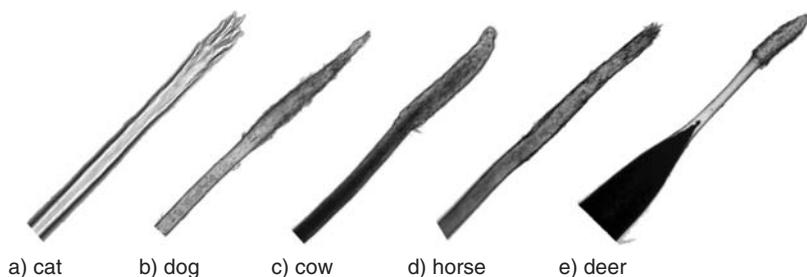


Figure 15A-1 Root shapes are distinctive characteristics for some animals. a) Cat hair has a ‘paint-brush’ shaped root. b) Dog hair has a spade shaped root. c) Cow hair has an elongated root with the medulla present into the root portion. d) Horse hair has an elongated root. e) Hair from animals included in the deer family and antelope have a wineglass shaped root.

Deer Family and Antelope

This group of animal hair can be easily distinguished by the very coarse diameter, which generally has a slight wave or crimp. The ‘wineglass root’ (see Figure 15A-1e) is also a major characteristic of this group. The medulla is composed of spherical cells that occupy the entire hair. Scale cast patterns, in addition to other microscopic characteristics and color banding patterns, can be used to distinguish hair within the deer family and/or antelope. Distinct scale patterns for animals within the deer family and antelope are shown in Figure 15A-2.

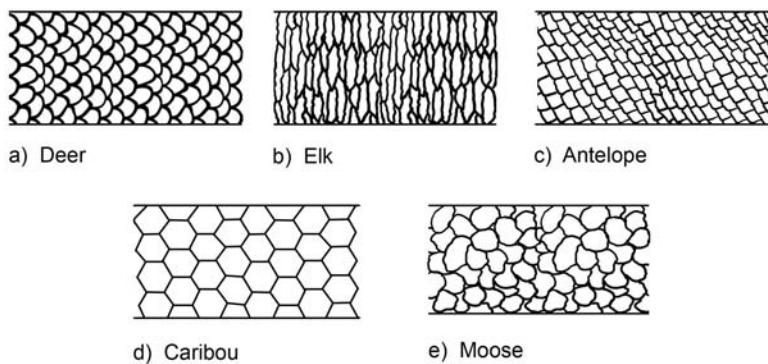


Figure 15A-2 Scale patterns for animals in the deer family and antelope.

Each of these animals also displays specific color banding patterns on their hair. Deer hair begins with an area of white or light gray at the root end. It then proceeds from this color to a gray-brown, then an area of yellow followed by a black tip end. Elk hair begins with an area of brown-gray at the root end, then turning yellow and with a black tip end. Antelope hair starts with an area of brown-gray, followed by an area of brown-yellow and a dark brown tip end. Caribou hair has a white root end followed by a light tan area and a black tip end. Moose hair begins with a white root area then proceeds to a brown-gray color. It then has areas of black, followed by light gray, brown-gray, and a black tip end.

Fur Bearing

This group of animal hair can be easily distinguished by the fine overall diameter. There is also a wide variation in diameter within the hair. Characteristic medullary patterns can be used to distinguish within this group. Scale cast patterns, in addition to other microscopic characteristics, can be used to distinguish some fur-bearing animals. These animals also show distinct color banding patterns. Some specific identifying characteristics are listed in Table 15A-1 for members of the fur-bearing group.

Table 15A-1 Important identifying characteristics for the fur-bearing group.

Animal	Characteristics
Rabbit	Scale cast pattern: irregular-waved mosaic-chevron/irregular wave mosaic Medulla pattern: multi-serial ladder, MI > 0.9
Mink	Color banding: dark shades/lighter shades Scale cast pattern: diamond petal/irregular waved mosaic Medulla pattern: amorphous/lattice Shaft shape: prominent shield
Muskrat	Color banding: reddish brown/dark brown Scale cast pattern: irregular-waved mosaic, single chevron Medulla pattern: multi-serial ladder/lattice, 1/2 width Shaft shape: prominent shield
Chinchilla	Medulla pattern: uniserial ladder Shaft shape: very fine diameter and usually clumped
Raccoon	Color banding: white/dark brown/yellow/black Scale cast pattern: diamond petal/irregular waved mosaic Medulla pattern: amorphous, cellular, 1/2 to 3/4 width
Fox:	Scale cast pattern: mosaic/diamond petal Medulla pattern: unbroken, amorphous or vacuolated, 3/4 width
Red fox	Color banding: dark brown/yellow/red brown
Gray fox	Color banding: white/dark-brown-black/light tan/black
Beaver	Medulla pattern: fur hair-continuous cellular with a beaded-look, thin
Seal	Scale pattern: petal Medulla pattern: absent
Otter	Color banding: light brown-gray/dark brown Scale cast pattern: diamond petal/irregular-waved mosaic Shaft shape: prominent shield Medulla pattern: lattice, wide
Sheep	Scale cast pattern: mosaic Medulla pattern: absent Shaft shape: usually crimped, fur hair curly
Camel	Scale cast pattern: mosaic Medulla pattern: amorphous

Table 15A-1 (*continued*)

Animal	Characteristics
Sable	Scale pattern: petal Medulla pattern: lattice, fine, MI > 0.5
Bear	Scale cast pattern: very large irregular-wave Medulla pattern: amorphous, vacuolated and thin, MI < 0.5 Shaft shape: very wide
Ermine	Color banding: brown gray/light brown Scale cast pattern: irregular petal/irregular waved mosaic Medulla pattern: multi-serial ladder Shaft shape: prominent shield area
Lynx	Color banding: gray/dark brown/white/black or light brown/dark brown/yellow/black Scale cast pattern: petal Medulla pattern: unbroken cellular

Domestic

This group of animal hair can be easily distinguished by the medium overall diameter with only moderate variation along the hair. Medullary patterns are generally amorphous. Characteristic root shapes may also be used to distinguish between some domestic animal hair. Scale cast patterns and other microscopic characteristics can be used to distinguish hairs from the domestic group. Most hair in this group generally does not have color banding patterns. Some specific identifying characteristics for domestic animals are listed in Table 15A-2.

Table 15A-2 Important identifying characteristics of domestic and other animal hair.

Animal	Characteristics
Dog	Medulla pattern: vacuolated or amorphous, 1/2 diameter of overall hair Root shape: spade-like (see Figure 15A-1b) Shaft shape: may have barrel shape
Cat	Scale cast pattern: petal, usually prominent Medulla pattern: uniserial ladder, wide Root shape: elongated and fibrils frayed at base (see Figure 15A-1a) Shaft shape: fine
Cow	Medulla pattern: amorphous and thin Root shape: elongated with pigment into root area (see Figure 15A-1c) Shaft shape: coarse diameter Special characteristics: ovoid bodies prominent with coarse streaky pigment

Table 15A-2 (*continued*)

Animal	Characteristics
Horse	Medulla pattern: amorphous, cellular, MI > 0.5 Root shape: elongated bulb-like shape (see Figure 15A-1d) Shaft shape: coarse diameter Special characteristics: even, fine pigment
Ferret	Scale cast pattern: mosaic/petal/diamond Medulla pattern: uniserial and lattice Shaft shape: shield
Hog	Shaft shape: very coarse, stiff, even diameter Special characteristics: split tips
Goat	Scale cast pattern: irregular-waved mosaic Medulla pattern: lattice $\frac{1}{2}$ to $\frac{3}{4}$ diameter of overall hair Root shape: wineglass Shaft shape: coarse Special characteristics: cortex visible
Opossum	Color banding: white/black Scale cast pattern: mosaic/irregular waved mosaic Medulla pattern: vacuolated and amorphous, $\frac{1}{2}$ diameter of overall hair Shaft shape: fine diameter
Skunk	Scale cast pattern: irregular waved mosaic Medulla pattern: amorphous
Squirrel	Scale cast pattern: irregular wave Medulla pattern: vacuolated, fine
Coyote	Color banding: white/dark gray brown/white/black Scale cast pattern- mosaic/diamond petal/irregular-wave Medulla pattern- vacuolated, $\frac{1}{2}$ to $\frac{3}{4}$ diameter of overall hair
Mountain lion	Color banding: gray brown/black/light brown/black Scale cast pattern: mosaic/irregular-waved mosaic Medulla pattern: vacuolated, very wide
Wolf	Medulla pattern: thick amorphous or vacuolated Special characteristics: little to no pigment in the cortex
Ground Hog	Color banding: dark/white/black Scale cast pattern: irregular-waved mosaic Medulla pattern: vacuolated Special characteristics: red pigment near medulla

An understanding of the macroscopic and microscopic characteristics found in animal hair, allows the forensic scientist to perform animal hair examinations. Animal hair examinations involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the hair for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine the microscopic characteristics of the hair sample. To do this, a scale cast and a semi-permanent or permanent mount is used. When a conclusion is reached as to a possible species origin of the hairs, it should be pointed out that

the animal hairs do not possess enough individual characteristics to be identified to a particular animal to the exclusion of other animals of the same species.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media (a colorless mounting medium in the refractive index range of 1.5–1.60)

Clear nail polish and/or Polaroid film coating

Animal hair

Unknown hair samples

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: ANIMAL HAIR EXAMINATION

Procedure

Complete the hair worksheet located at the end of this experiment and available at <http://www.wileyeurope.com/college/wheeler>.

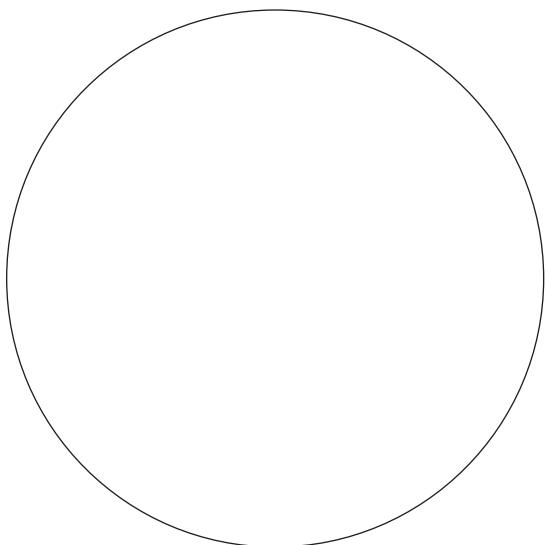
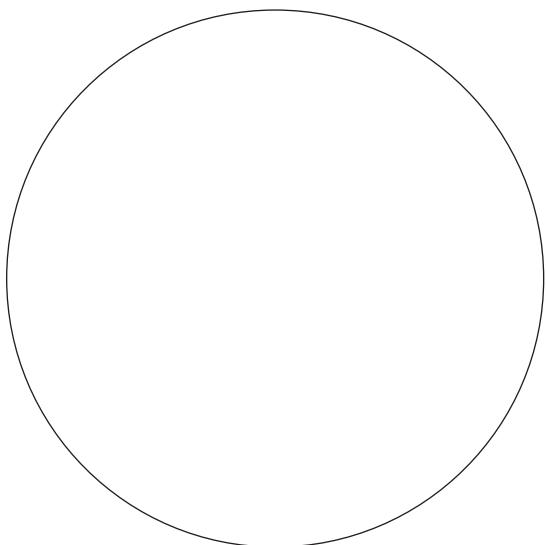
1. Obtain an animal hair from each group (deer and antelope, fur-bearing, domestic).
2. Examine the first hair with the stereomicroscope.
3. Measure the length of the hair. Record the value in centimeters.
4. Note the color and any banding patterns that may be present.
5. Note the form of the hair.
6. Note the diameter of the hair.
7. Label a microscope slide with identifying marks (analyst initials and sample).
8. Make a scale cast by applying a thin coating of clear nail polish to the microscope slide. Polaroid film coating may also be used instead of clear nail polish.
9. While still wet, position the hair in the nail polish. Make sure the entire length of the hair is included.
10. Let the nail polish dry completely.
11. Gently remove the hair to obtain the scale cast. This hair will be used for the liquid mount.
12. Using the scale cast, observe the scale pattern using the compound light microscope. Remember to check the microscope to ensure that samples will be viewed using Köhler illumination.
13. Be sure to observe the entire length of the hair because scale patterns may change from the proximal to distal ends. Draw what you see.
14. Make a liquid mount of the hair.
15. Examine the hair using the compound light microscope.
16. Document the microscopic characteristics of the hair: color, form, and diameter.
17. Examine the hair for any additional microscopic characteristics, which would aid in the identification of the hair type. Document the additional characteristics of each hair: cuticle, cortex and medulla widths, medulla form, root shape, and pigmentation orientation.
18. Measure the diameter of the hair and the medulla for all hairs to calculate the medullary index of each hair using Equation 15-1 (p. 150).
19. Draw one identifying characteristic for each hair, including information such as sample and magnification.
20. Do your results agree with the known characteristics for the animal? List the identifying macroscopic and microscopic characteristics for the hair.
21. Repeat steps 1–20 for hairs from the other groups.
22. Obtain an unknown hair.
23. Repeat steps 1–20 to identify the hair.
24. Describe your examination procedure for the unknown sample.
25. Include the unknown hair number and the species that you identified the hair to be. State your reasons to defend your conclusion.

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. Describe each term as it relates to animal hair: root, cuticle, cortex, medulla, and tip. Include drawings and/or examples with your description for each term.
2. What are the main divisions for animal hair? Pick a species from each division and describe the microscopic characteristics that are used for its identification.
3. Why are scale casts used for animal hair identifications?
4. Can animal hair be identified without a scale cast?
5. An animal hair was found with a medulla that looks like corn on the cob. What species could it be?
6. Dog and cat hair are the most commonly identified animal hairs in forensic cases. What are the identifying characteristics for dog hair?
7. What are the identifying characteristics for cat hair?
8. Can dog and cat hairs be compared?
9. Some animal hairs are used for fibers. How can you determine if it is a hair or a fiber?

HAIR WORKSHEET**Sample** _____**Color** _____**Length** _____**Shaft Form** _____**Diameter** _____**Cuticle** _____**Cortex** _____**Medulla** _____**Root Shape** _____**Pigmentation** _____**Medullary Index** _____**Sample** _____**Color** _____**Length** _____**Shaft Form** _____**Diameter** _____**Cuticle** _____**Cortex** _____**Medulla** _____**Root Shape** _____**Pigmentation** _____**Medullary Index** _____

Experiment 15B: Determination of Racial and Somatic Origin Characteristics of Human Hair

Recommended pre-lab reading assignments:

Robertson J. Forensic and Microscopic Examination of Human Hair. In: Robertson J ed. *Forensic Examination of Hair*. London, England: Taylor & Francis; 1999; 79–153.

Ogle RR. *Atlas of Human Hair Microscopic Characteristics*. Boca Raton, FL: CRC Press, 1998.

OBJECTIVE

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

1. general racial characteristics of human hair
2. general somatic region characteristics of human hair
3. use of the stereomicroscope to visualize the characteristics of hair
4. use of the compound or comparison light microscope to visualize the characteristics of hair
5. preparation and observations of hair cross-sections
6. identification of human hair

INTRODUCTION

Like animal hair, human hair is also easily shed and transferred whenever there is contact between two objects, two individuals, or an individual and another object, and like animal hair, many factors can affect whether or not a hair is shed or transferred. The growth stage of the hair is one determining factor. More importantly, any activity of the individual and/or other individuals and objects involved will affect the shedding or transfer of hair. The average person loses approximately 100 hairs each day. These can be shed or transferred through contact. This contact can occur through daily activities, such as combing hair, or by violent contact, such as an assault or rape. Human hair may be easily transferred to clothing or other objects. Therefore, human hair is often used to associate people with items or scenes. This makes human hair an important source of information in forensic investigations.

Like animal hair, the forensic scientist uses several hair characteristics to identify human hair. Human hair possesses many macroscopic and microscopic characteristics, which make identification and comparisons sometimes possible. These characteristics were discussed in Experiment 15. Because of this fact, human hair is routinely examined and compared in forensic casework.

In some instances, a possible racial determination for a head hair can be accomplished. There are different characteristics associated with one of the three generally accepted anthropological groups: Caucasian, Negroid, and Mongoloid (see Figure 15B-1). Caucasian is an anthropological term designating the peoples originating from Europe and the Indian sub-continent. Negroid is an

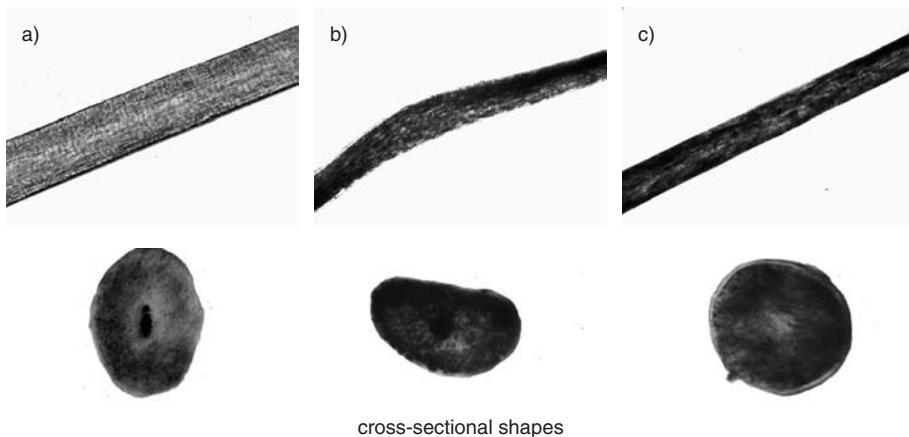


Figure 15B-1 Some hair characteristics are associated with anthropological groups: a) Caucasian; b) Negroid; and c) Mongoloid. A full-color version of this figure can be found in the color plate section of this book.

anthropological term designating most of the peoples originating from Africa. Mongoloid is an anthropological term designating peoples originating from Asia, excluding the Indian sub-continent but including the Native American Indians.

Hairs that are characterized as Caucasian exhibit an overall shaft diameter that is generally moderate with little variation. Pigment granules are fairly evenly distributed, light to heavy in density, and the cross-sectional shape is oval (see Figure 15B-1a). Hairs that are characterized as Negroid exhibit an overall shaft diameter that is fine to moderate with considerable variation. Pigment granules are densely distributed and somewhat clumpy. The cross-sectional shape is flattened with areas of buckling, curling, and twisting (see Figure 15B-1b). And finally, for hairs that are characterized as Mongoloid, the overall shaft diameter is coarse with little to no variation. Pigment granules are densely distributed and streaky. A prominent medulla is generally present with a well-defined thick cuticle. The cross-sectional shape is round (see Figure 15B-1c). Racial group microscopic characteristics are summarized in Table 15B-1. Sometimes, a possible racial group cannot be determined. This may be due to racial mixes. It is important to note when characteristics within the sample are inconsistent, poorly defined, or have only a limited number within the sample, not allowing for one specific racial determination.

Table 15B-1 Racial group microscopic characteristics.

Racial group	Diameter	Cross sectional shape	Pigment	Cuticle	Undulation
Negroid	60–90 μm	flattened	dense and clumped	varies	prevalent
Caucasian	70–100 μm	oval	even	varies	varies
Mongoloid	90–120 μm	round	dense and streaky	thick	none

The somatic region from which a hair was shed can also sometimes be determined. Head hairs are generally long in length with moderate shaft diameter and variation. A thin medulla may be present or absent. For head hair the distal ends can be cut, worn, or split. Pubic hairs are generally coarse with wide variations in diameter and somewhat prominent buckling. The medulla is usually thick, present, and continuous. Large root ends generally have follicular tags. Pubic hair distal ends are tapered, cut, or worn, and sometimes bleached from urine.

Hairs that are generally fine with little variation are limb hairs. Their overall shape is short and arc-like. The medulla is usually broad and discontinuous. Limb hair distal ends are usually worn. Facial hairs are generally coarse and irregular, with a triangular cross-section. The medulla is usually broad and continuous. Some facial hair may exhibit multiple medullas. Facial hair distal ends are generally cut, sometimes angular. Chest hairs are generally moderate in diameter. The overall shape is long and arc-like. Chest hair distal ends are usually worn or tapered. Hairs that are generally moderate and resemble pubic hair with less buckling are known as underarm or axillary hairs. Their distal ends are usually long and fine, being frequently bleached. Eyebrow and eyelash hairs are generally short and stubby with little shaft diameter variation. The overall shape of these hairs is saber-like. Hairs that are generally a mix of characteristics from two body areas are transitional hairs. An example of transitional hair is sideburn hair, which may possess a combination of head and facial characteristics. Somatic region microscopic characteristics are summarized in Table 15B-2.

An understanding of the macroscopic and microscopic characteristics found in human hair allows the forensic scientist to perform human hair examinations. Human hair examinations involve the

Table 15B-2 Somatic region microscopic characteristics.

Hair	Length	Diameter	Texture	Tip end	Medulla	Other
Head	long, varies	moderate, shaft varies	soft	Cut or split	narrow	
Pubic	short	coarse, wide variation with buckling	stiff	tapered, cut, or rounded	thick	
Facial	varies	coarse, with variation	stiff	cut or rounded	broad, doubled	triangular cross sectional shape
Limb	varies	fine, with variation	soft	tapered, rounded, or cut	discontinuous	arc-like shaft form
Chest	varies	moderate with variation	stiff	tapered, rounded, or abraded, long and fine	granular	arc-like shaft form
Axillary	varies	moderate with variation	stiff	tapered, rounded, cut, or abraded, long and fine	discontinuous	bleached ends
Eyebrow/ eyelash	short	slight fluctuation	stiff	tapered		saber-like shaft form

use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the hair for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine the microscopic characteristics of the hair sample. To do this, a cross-section, and either a semi-permanent or permanent mount is used.

A cross-section is a perpendicular sampling of a hair. The cross-section is then mounted in a semi-permanent or permanent mount to observe its shape. Cross-sections of a hair sample provide information about characteristics that are associated with determining racial and somatic origins. At times, the cross-sectional shape may also be determined by viewing the hair sample longitudinally. However, more detail can be obtained by preparing an actual cross-section of the sample. Semi-permanent and permanent mounts are used to visualize other microscopic characteristics of a hair. Once a hair has been identified as human, further characterization of the hair can be done.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media (a colorless mounting medium in the refractive index range of 1.5–1.60)

Polyethylene or plastic sheets

Hot plate

Pipette tips

Microtome

Thread

Nail polish

Human hair

Unknown hair samples

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: HUMAN HAIR EXAMINATION

Procedure

Complete the human hair worksheet located at the end of this experiment and available at <http://www.wileyeurope.com/college/wheeler> for each hair sample.

1. Obtain samples of human hair from each group (Caucasian, Negroid, Mongoloid, head, pubic, facial, chest, axillary, eyebrow, eyelash).
2. Examine the first hair with the stereomicroscope for macroscopic characteristics.
3. Measure the length of the hair. Record the value in centimeters.
4. Record the color that may be present. It is important to note if the color changes along the length of the hair.
5. Record the shaft form of the hair. Examples are: straight, waved, curled, kinked, or curved.
6. Set up Köhler illumination on a compound light microscope. Using a calibrated ocular micrometer measure the diameter of the hair.
7. Determine the cross-sectional shape of the hair (round, oval, flattened, triangular). Choose one of the cross-sectioning methods described below or determine the cross-section by a longitudinal examination once the liquid mount has been made. Draw what you see.

A: Cross-sectioning by sandwiching the sample

- A1. Using either microscope slides or sheets of plastic polyethylene film, ‘sandwich’ the sample. Some laboratories use thick polyethylene sheets, which can be melted to enclose the sample.
- A2. Align the blade of a scalpel or razor perpendicular to the sample.
- A3. Cut a thin cross-section.
- A4. Mount the sample in a suitable mounting media for viewing on the microscope.

B: Cross-sectioning by imbedding the sample

- B1. Fill a pipette tip with a small amount of mounting medium that will harden (Flo-texx™ or Norland 65™).
- B2. Insert sample into tip.
- B3. Allow the mounting media to cure until hardened. This may involve heating in the oven, drying at room temperature, or the use of UV light.
- B4. Align the blade of a scalpel or razor perpendicular to the sample.
- B5. Cut a thin cross-section.
- B6. Mount the sample in a suitable mounting media for viewing on the microscope.

Or

- B1. Using several layers of tape, create a small form on a microscope slide.
- B2. Fill the area with a mounting media that will harden (Flo-texx™ or Norland 65™).
- B3. Insert the sample.
- B4. Allow the mounting media to cure until hardened. This may involve heating in the oven, drying at room temperature, or the use of UV light.
- B5. Align the blade of a scalpel or razor perpendicular to the sample.
- B6. Cut a thin cross-section.
- B7. Mount the sample in a suitable mounting media for viewing on the microscope.

C: Cross-sectioning using a microtome

- C1. Facing the microtome, turn the dial counterclockwise to lower the plunger.
- C2. Loosen the small right screw on the top moveable plate and push plate towards the back.
- C3. Unscrew and remove the two larger screws and remove plate.
- C4. Place 1 drop of microtome oil between the base plate and the top moveable plate.
- C5. Take approximately 12 inches (30 cm) of thread (white or black for contrast with the sample) and double the thread until it forms a small bundle.
- C6. Place the sample in the center of the bundle.
- C7. Place the bundle in the larger hole in the moveable plate and slide the bundle back into the small plunger hole.
- C8. Push the plunger up against the bundle and tighten the right screw.
- C9. Using a razor, evenly slice the top of the sample protruding from the plunger hole.
- C10. Set the moveable plate back on the microtome and secure using the large screws.
- C11. Slightly loosen the small right screw and push back to tighten the packing on the bundle. Tighten screw.
- C12. Turn the microtome dial clockwise, watching carefully, until the bundle begins to move upward.
- C13. Cut the protruding sample off smoothly and evenly with a razor blade.
- C14. Dial the microtome up 1 or 2 additional notches.
- C15. Brush a thin film of nail polish over the sample. Allow to dry.
- C16. Carefully remove the sample and nail polish using a razor blade and a gentle sawing motion.
- C17. Repeat steps 14–16 as necessary.
- C18. Mount the sample in a suitable mounting media for viewing on the microscope.

After cross-sectioning, continue the main procedure following on from point 7.

8. Make a liquid mount of the hair sample. If a cross-section was not made, examine the hair to determine the variation of the diameter along the shaft, so that a cross-sectional assessment can be made of the longitudinal view.
9. Examine the hair using the compound light microscope.
10. Document the macroscopic characteristics of the hair: color, shaft form, and diameter.
11. Examine the hair for any additional microscopic characteristics that would aid in the identification of the hair type. Document the additional characteristics of each hair: pigmentation distribution and density, root shape, cuticle, medulla width and distribution, and distal end condition.
12. Draw one identifying characteristic for each hair, including sample information and magnification.
13. Do your results agree with the known characteristics for the racial group or somatic region for your hair sample? List the identifying macroscopic and microscopic characteristics for the hair that agree.
14. Repeat steps 1–13 for hairs from the other groups.
15. Obtain an unknown hair.
16. Repeat steps 1–12 to identify the unknown hair. Complete the Human Hair Characteristics Worksheet for an unknown sample. In addition to the worksheet include your unknown hair number and what you identified the hair to be. State your reasons for your decision and defend your conclusion.

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. Describe each term as it relates to human hair: root, cuticle, cortex, medulla, distal tip. Include drawings and/or examples with your description for each term.
2. Describe your examination procedure for the unknown sample.
3. List all the macroscopic characteristics of human hair. Give an example of each.
4. List all the microscopic characteristics of human hair. Give an example of each.
5. What is a cross-section of human hair? How is it prepared?
6. List the macroscopic and microscopic characteristics of hair that are used to classify hair into the three racial groups. Give an example of why a racial group might not be able to be determined from a single questioned hair.
7. Does hair have enough identifying characteristics that allow it to be individualized to a person?
8. A hair examiner has examined an unknown hair and a set of known head hairs in a case. If all the characteristics examined are similar, write a statement that the examiner can use in reporting the results of the comparison.

HUMAN HAIR CHARACTERISTICS WORKSHEET**KNOWN SAMPLE** _____**Macroscopic:**

Color_____
Length_____
Shaft form_____
Diameter_____

Microscopic:

Cuticle appearance:_____

Cortex:

Pigmentation color_____
Pigmentation distribution_____
Pigmentation density_____
Other characteristics_____

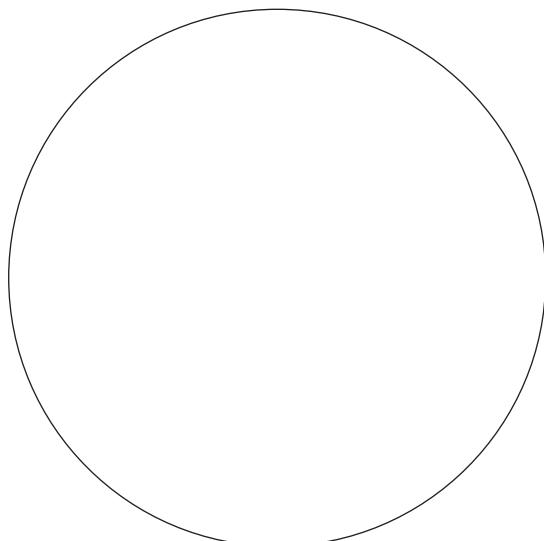
Medulla:

Appearance_____
Distribution_____

Root appearance:_____

Tip appearance:_____

Special characteristics:_____



HUMAN HAIR CHARACTERISTICS WORKSHEET**UNKNOWN SAMPLE NUMBER** _____**Macroscopic:**

Color_____
Length_____
Shaft form_____
Diameter_____

Microscopic:

Cuticle appearance:_____

Cortex:

Pigmentation color_____
Pigmentation distribution_____
Pigmentation density_____
Other characteristics_____

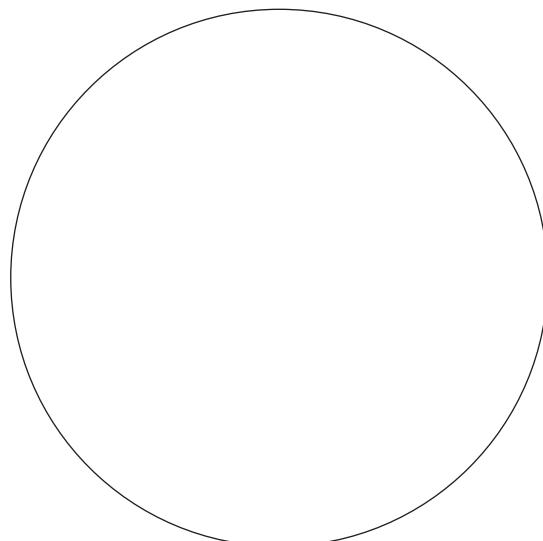
Medulla:

Appearance_____
Distribution_____

Root appearance:_____

Tip appearance:_____

Special characteristics:_____



Experiment 15C: Human Hair Examinations and Comparisons

Recommended pre-lab reading assignments:

Barnett PD, Ogle RR. Probabilities and Human Hair Comparisons. *Journal of Forensic Science*. 1982; 27(2): 272–8.

Deadman HA. Human Hair Comparisons Based on Microscopic Characteristics. *Proceedings of the International Symposium on Forensic Hair Comparisons*. 1985; 45–9.

Gaudette BD. *Forensic Hair Comparison*. Crime Laboratory Digest. 1985; 12: 44–59.

OBJECTIVE

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

1. general characteristics of human hair
2. use of the stereomicroscope to visualize the characteristics of hair
3. use of the compound or comparison light microscope to visualize the characteristics of hair
4. cross-sections of human hair
5. comparison of human hair

INTRODUCTION

Human hair comparisons are performed to determine possible associations between people, places, and objects. This association is accomplished through the comparison of macroscopic and microscopic characteristics. Learning to recognize the characteristics and the range found within an individual's known hair sample allows the forensic scientist to identify and compare human hair. Careful examination of known samples and unknown hair can provide information as to similarities or dissimilarities for an investigation.

Since such a variety of macroscopic and microscopic characteristics are found among hairs within a single source, it is important to obtain a suitable sample when performing comparisons. Known samples should be collected from each somatic region so that hair comparisons might be performed. So, if the unknowns to be compared are both head and pubic hairs, a known sample should be collected from each region. It is also necessary to have a known sample that contains the range of characteristics found within a particular source. This should include hairs from all growth phases and from several locations from that particular body area. For example, head hair should be collected by combing and pulling to obtain all growth phases and from five different areas of the head (center, back, front, and both sides). This should provide samples containing the range of characteristics that might be found from that source. In most instances, suitable known samples are found if the quantity of the known sample is sufficient. This is usually 50–100 hairs for head hair and 25–50 hairs for pubic hair. However, in some instances more or less can be used. Once

a suitable known sample has been obtained, the macroscopic and microscopic characteristics are determined.

Unknown hairs are examined next to identify the range of macroscopic and microscopic characteristics displayed by that particular sample. Comparison of these characteristics to the range found within the known sample can then be performed.

A large number of macroscopic and microscopic characteristics should be considered when performing human hair examinations and comparisons, and many of these were discussed in Experiments 15 and 15B. Environmental characteristics, acquired traits, artificial treatments, and diseases provide additional characteristics that can individualize a hair sample. Environmental traits

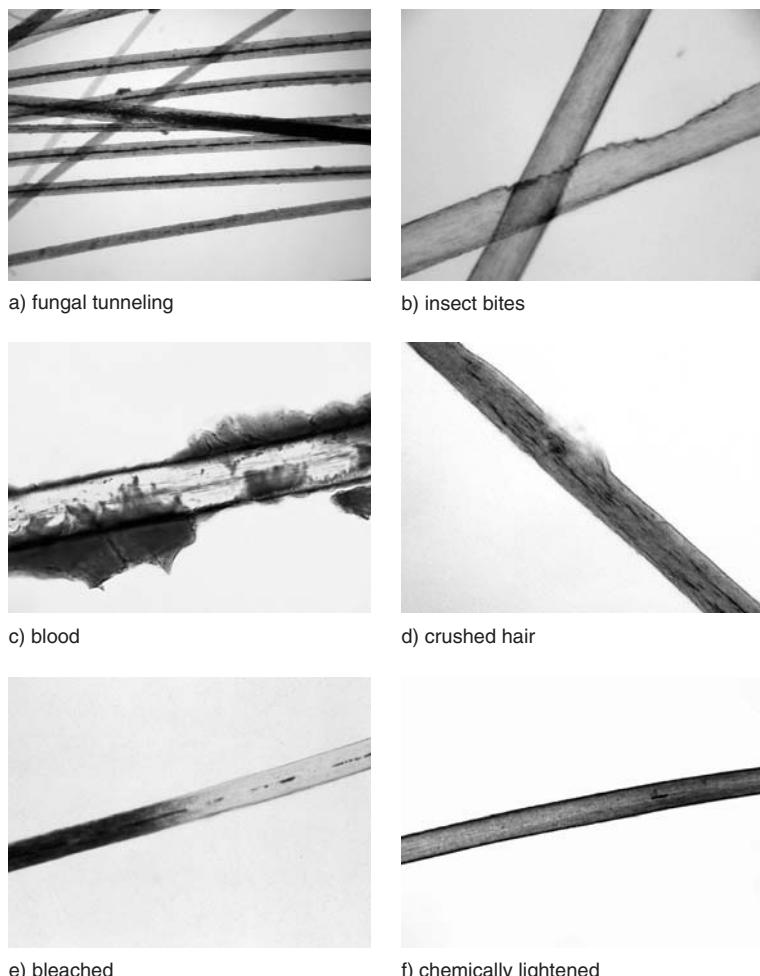


Figure 15C-1 Acquired hair characteristics may be obtained through environmental conditions or by artificial treatments: a) fungal tunneling (viewed as dark irregular spaces) are air pockets that are caused by fungal growth; b) insect bites are found on hair that has been discarded for a time period (i.e., dustballs); c) blood can leave colored material along the hair shaft; d) the type of damage to a hair can also sometimes be determined, for example, this hair has a crushed portion; the color of hair can be chemically changed by e) bleaching or f) dying. A full-color version of this figure can be found in the color plate section of this book.

are characteristics, which the hair obtains such as fungal growth or insect bites (Figure 15C-1a and b). Acquired traits usually involve changes or damage to the hair. In many situations, blood may be visible on the hair shaft (Figure 15C-1c). Various types of damage may also be visible on the hair. Most of the time, this involves the tip end of the hair; however, some characteristics, such as singeing and crushed areas (Figure 15C-1d), may be found along the entire hair shaft. Artificial treatments are chemical actions done to the hair. These may be semi-permanent or permanent (Figure 15C-1e and f). Disease characteristics are more rarely viewed; however, they can be important characteristics when identified. Table 15C-1 contains a summary of human hair characteristics and possible descriptions for each.

Table 15C-1 Summary of macroscopic and microscopic characteristics of human hair.

Macroscopic characteristics	Descriptions
Color	white/gray, blonde, red, brown, black, other
Length	this is usually measured in centimeters or inches
Shaft form	straight, waved, curled, kinked, curved
Diameter	fine, medium, coarse, variations
Microscopic characteristics	Descriptions
Color	colorless, yellow, red, brown, black, opaque, other
secondary color	gray, yellow, red
intensifier	light, dark
Pigmentation distribution	uniform, peripheral, one-sided
Pigmentation granule color	red, brown
Pigmentation granule size	small, medium, large
Pigmentation granule density	light, medium, heavy
Pigmentation granule aggregation size	small, medium, large
Pigmentation aggregation shape	streaked, clumped, patchy
Pigmentation aggregation density	light, medium, heavy
Shaft shape	buckled, shouldered, convoluted, undulated, twisted, uniform
Cross-sectional shape	round, oval, flattened, triangular
Root characteristics	anagen, catagen, telogen, sheathed, follicular tag, post mortem changes
root present	square cut, angular cut, angular cut with tail, crushed, broken, singed, post mortem changes
root absent	thin, moderate, thick
Shaft characteristics	transparent, translucent, yellow
cuticle thickness	smooth, flattened, serrated, looped, ragged, cracked
cuticle color	distinct, pigment gapping
cuticle outer margin	apparent, not apparent, extreme
cuticle inner margin	size, distribution, abundance
Cortex characteristics	size, shape, location, distribution, abundance
cellular texture	absent, continuous, discontinuous
ovoid bodies	opaque, translucent, absent
cortical fusi	fine, moderate, coarse, double
Medulla characteristics	
medulla continuity	
medulla appearance	
medulla width	

Table 15C-1 (*continued*)

Microscopic characteristics	Descriptions
Tip characteristics	tapered, rounded, square cut, angular cut, angular cut with tail, frayed, split, crushed, broken, singed, abraded
Artificial treatments	bleaching, dying (permanent, semi-permanent, color rinses, color rinses), permanent waves, relaxers, hair sprays and gels, crème rinses
Environmental or acquired characteristics	lice, mold, fungal tunneling, insect bite marks, debris, blood
Diseases	Pili Annulati, Trichoschisis, Monilethix, Trichorrhexis Nodosa, Trichorrhexis Invaginata, Pili Torti, Trichonodosis, Cartilage Hair Hypoplasia, Trichoptilosis

An understanding of the macroscopic and microscopic characteristics found in human hair allows the forensic scientist to perform human hair comparisons. Human hair comparisons involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the hair for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine the microscopic characteristics of the hair sample. To do this, a cross-section, and either a semi-permanent or permanent mount is used. Whereas most head and pubic hair contain characteristics worthy of further comparison, hairs from other somatic regions are generally identified but not compared to known samples. Once the known sample has been examined, characteristics can then be compared for the unknown sample. This is usually performed on a comparison compound microscope (see Figure 15C-2).

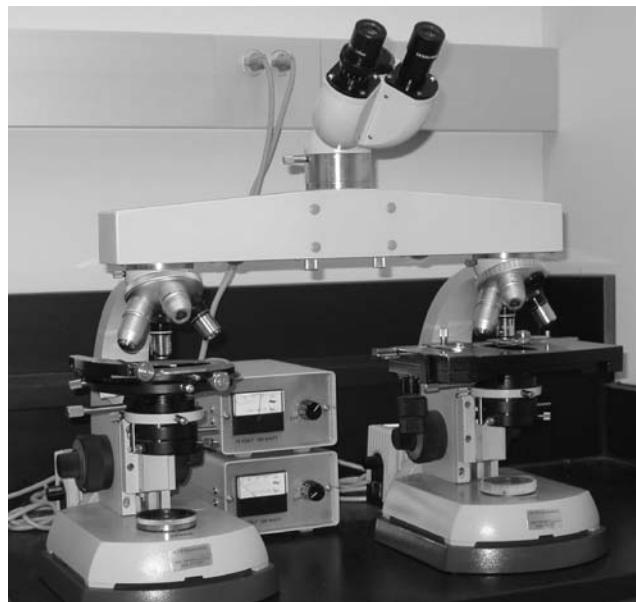


Figure 15C-2 The basic Zeiss™ comparison compound microscope contains two compound microscopes connected by an optical bridge. This allows viewing and comparison of two samples within one field of view.

A comparison compound microscope is composed of two compound microscopes that are connected by an optical bridge. The bridge contains prisms and mirrors that are used to direct the light to a common set of oculars. A set of knobs is used to adjust the field of view, so that items can be viewed from either microscope independently or combined. In the combined view the field of view is split into side-by-side views of portions from each microscope. This allows the examiner to view two items side-by-side on a microscopic scale (see Figure 15C-3). The comparison compound microscope usually uses a translational stage instead of a rotating stage, which allows for precise alignment of two hairs.



Figure 15C-3 The split field of view as seen on the comparison microscope. This allows comparison of known and unknown samples within the same viewing field. A full-color version of this figure can be found in the color plate section of this book.

If all the characteristics displayed by the unknown hair are found within the known sample, an association may be reached. However, when an association is reached as to a possible origin of the hair, it should be pointed out that a similarity to a known sample does not exclude the possibility of another similar source.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Comparison microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media (a colorless mounting medium in the refractive index range of 1.5–1.60)

Nail polish and/or Polaroid film coating

Known human hair samples

Unknown hair samples

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: HUMAN HAIR EXAMINATIONS

Procedure

Complete a known human hair worksheet located at the end of Experiment 15B and available at <http://www.wileyeurope.com/college/wheeler> for each sample.

1. Obtain two known samples of human hair.
2. Examine the first sample with the stereomicroscope.
3. Measure the range of length of several hairs in the sample. Record the value in centimeters or inches.
4. Record the colors that may be present. It is important to note any color changes.
5. Note the shaft form of the hairs.
6. Describe the diameter of the hairs.
7. Using a compound microscope set up with Köhler illumination, determine the cross-sectional shape. This can be done by making a cross-section (refer to Experiment 15B if necessary) or from a longitudinal liquid mounted hair.
8. Make a liquid mount of the hair sample. Remember, you want to obtain the range found within the sample, and so more than one hair should be examined.
9. Examine the hairs using the compound light microscope.
10. Document the microscopic characteristics of the hair: color, shaft form, diameter, cross-section, root, cuticle, cortex, medulla, and any special characteristics.
11. Draw four identifying characteristics for the hair sample, including information such as sample and magnification.
12. Examine the second known sample following steps 2–11.

PART II: HUMAN HAIR COMPARISONS

Complete an unknown human hair worksheet located at the end of Experiment 15B and available at <http://www.wileyeurope.com/college/wheeler>

1. Obtain an unknown hair.
2. Repeat steps 2–11 of Part I with the unknown hair. It will be necessary to first determine if the sample is a hair, and if it is animal or human.
3. Compare the macroscopic and microscopic characteristics that you have documented to the known samples analyzed in Part I. Is the unknown hair sample similar to one of the known hair samples?
4. If possible, use a comparison microscope to examine both samples simultaneously. If you believe that the samples are similar, find areas in the known sample that have similar characteristics to that in the unknown hair. Draw what you see for the root end, tip, and sections of the shaft showing cuticle, cortex, and medulla similarities.
5. Include the unknown hair number and state the result of the hair comparison. Fully defend your conclusion.

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. Why is it possible to perform human hair comparisons?
2. How is a human hair comparison conducted?
3. What percentage of the population would have similar characteristics?
4. Can a hair be associated with only one person? Explain your answer.
5. How many head hairs must be taken and examined in order to determine if a questioned hair sample could have come from an individual? Why is the number so large?

6. How many pubic hairs must be taken and examined in order to determine if a questioned hair sample could have come from an individual? Why is the number so large?
7. Is it possible for an unknown hair from a murder scene to be similar to a known sample, but for that person to have no involvement in the crime? Explain your answer.
8. Is it possible for an unknown hair from a murder scene to be dissimilar to a known sample, but for that person to be involved in the crime? Explain your answer.

Experiment 15D: Evaluation of Human Hair for DNA

Recommended pre-lab reading assignments:

Linch C, Smith S, Prahlow J. Evaluation of the Human Hair Root for DNA Typing Subsequent to Microscopic Comparison. *Journal of Forensic Sciences*. 1998; 43(2): 305–14.

Dizinno J, Wilson M, Budowie B. Typing of DNA Derived from Hairs. In: Robertson J, ed. *Forensic Examination of Hair*. London: Taylor & Francis; 1999; 155–74.

OBJECTIVE

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

1. development of the hair follicle
2. hair growth and hair growth phases
3. basic root characteristics of the hair growth phases (anagen, catagen, and telogen)
4. use of the stereomicroscope to visualize the roots of the hair
5. use of the compound light microscope to visualize the roots of the hair

INTRODUCTION

With the advent of DNA, hair examinations have taken on additional importance. Whereas microscopic examinations provide physical, phenotype, variable, and qualitative information, DNA provides genetic, genotype, invariant, and quantifiable information. These are complementary techniques and should both be used in forensic work.

To evaluate hair roots for DNA, one must understand the growth of the hair follicle and the hair. The hair follicle develops through several stages. Pre-germ stage takes place approximately eight weeks after conception. In this stage, the cells begin to cluster in the basal layer of the epidermis. This begins the development of a protrusion on the underside of the epidermis that becomes the hair germ, which is the next stage. The stage begins around 11 weeks. The epidermal basal cells begin to become columnar, with elongated nuclei, perpendicular to the skin. This produces a bulge in the external epidermis and also a downward growth in the dermis. Additional cells begin to accumulate, causing the formation of the dermal papilla.

In the hair peg stage, continued cell division causes the hair germ to elongate further into the dermis as a solid column of epithelial. The cells advance longitudinally in a manner that creates a concave end. The cells at the leading edge of the hair peg push further down, creating the dermal papilla. The outer layer of the elongated hair germ is continuous with the basal layer, forming the sheath. Tissue differentiation begins at the bulbous peg stage. Two epithelial swellings develop along the posterior side of the follicle. The lower swelling is the site of the bulge, where the arrector pili muscle attaches. The upper swelling contains lipids and is the site of the sebaceous gland. The advancing end of the developing follicle broadens, enclosing the dermal papilla, forming the base of the hair bulb. The central cells in the peg begin to elongate backwards forming the hair

canal. Now that the follicle has been formed, the cells in the hair bulb now begin to divide. The inner root sheath differentiates, forming the hair cone. The hair cone elongates, pushed upward by the dividing cells below. When the tip of the hair cone is about halfway up the follicle, it begins to harden. The new hair finally breaks through the skin around 19–21 weeks. The initial hairs developed are called lanugo hairs. These hairs are shed prior to birth and replaced by new lanugo or vellus hairs. Vellus hairs are extremely soft and fine and replaced by terminal hairs. Terminal hairs are commonly examined in casework.

Once the hair follicle has developed, hair growth begins a continuous process of cell proliferation, cell differentiation, and cell synthesis. This continuous growth process occurs in several zones of the hair follicle. The structure and development of these zones are attributed to several regions of the hair follicle. The root is the region of active cell division. The greatest cell activity is at the widest portion of the dermal papilla. The cells in this area are undifferentiated and characterized by a high nuclear to cytoplasmic ratio. These cells actively divide. They are several layers thick and begin to give way to concentric layers that will form the follicle and hair shaft. Twenty percent of these cells differentiate into the hair fiber while the remaining cells make up the sheath. Changes can be seen in the medulla cells as they begin to move upward from the dermal papilla. Differentiation begins and amorphous granules appear. The granules vary in size and become irregular as the nuclei degenerate with the upward movement. The granules fuse, giving rise to hardened proteins. With the fusing, intercellular spaces are created forming the central core of the hair fiber. Cortical cells are derived from further cell division around the bulb. As cells move upward surrounding the medulla cells, they elongate into filaments and align themselves with the axis of the hair fiber. Cortical cells harden and dehydrate as they reach the keratogenous zone. Cuticle cells arise from a single layer surrounding the cortex cells. The cells undergo change as they move upward from the bulb creating an elongated flattened cell. The inner and outer root sheathes are layers surrounding the cuticle cells. Maturation and hardening of the inner root sheath forms and shapes the hair fiber. The outer root sheath is the outer most layer of the follicle. Once the follicle has developed a hair fiber, the follicle continues this process of on-going hair growth in a cycle. The cycle is composed of the anagen, catagen, and telogen phases.

The anagen phase is a period of high metabolic and mitotic activity. This phase will last approximately 17 weeks to 8 years. 80–90 % of the hair on a head is in the anagen phase. Because hair in the *anagen* phase is alive, it is strongly attached to dermal papilla so the root will be fleshy and sheath material may be present. Since it is actively growing, the root will be pigmented (see Figure 15D-1a and b).

The catagen phase is a period of transition. The follicle undergoes morphological changes and stops growing. This phase will last approximately 2–3 weeks. Approximately 2 % of the hair on a head is in the catagen phase. Because hair in the *catagen* phase is transitional, the root sheath is disintegrating so it will appear brush-like. Pigmentation is no longer being produced so the root will be lacking in color. The epithelial sac may be visible but no sheath material will be present (see Figure 15D-1c).

The telogen phase is a period of resting. This phase will last approximately 3–4 months. Approximately 8 % of the hair on a head is in the telogen phase. Because hair in the *telogen* phase is resting, the root shows no pigment or coloration. The medulla, when present, is distant from the root and no sheath material will be attached (see Figure 15D-1). As a hair reaches the telogen phase, it rests. After a short period of time, a new hair begins an anagen phase. This action helps to push out the telogen hair naturally.

DNA can be analyzed from hair using two techniques: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). For successful DNA results, the type of DNA technique used on human hair is

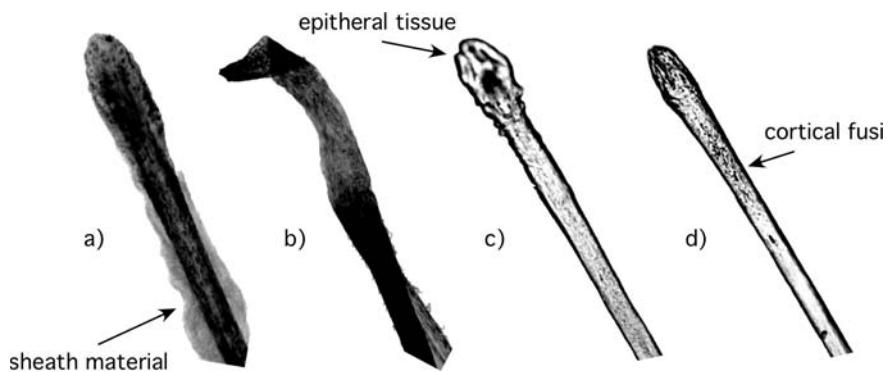


Figure 15D-1 a) Hair root in the anagen phase; the root end is heavily pigmented and a large amount of sheath material is present. b) Hair root in the anagen phase; the shape of this root is distorted because of the force used to dislodge the hair, however, the root end still has heavy pigmentation and a slight amount of sheath material present. c) A hair root in the catagen phase; note the epithelial tissue present and lack of pigmentation in the root area. d) A hair root in the telogen phase; the hair contains no tissue or pigmentation in the root end, however, the formation of cortical fusi has begun.

dependent on the growth phase of the hair, thus the need for understanding the growth phases. If a hair is in the anagen stage, nDNA is contained with the root and sheath material. If the hair is in the catagen or telogen stage, and if there is a good amount of follicular tag, nDNA may be possible. If the hair does not have sheath material or a follicular tag or does not have a root, mtDNA may be possible. The length of the hair is then important because this type of analysis generally takes approximately 1 cm of hair. Table 15D-1 compares the parameters used when determining the extraction techniques for nuclear DNA and mitochondrial DNA.

Table 15D-1 Criteria used when deciding on DNA technique for extraction.

nDNA	mtDNA
<ul style="list-style-type: none"> ● Sheath material present ● Anagen or early catagen phase hair (cells in the root area are more easily extracted than differentiated cells further down the shaft) ● Large root diameter ● Length of hair, 2 cm 	<ul style="list-style-type: none"> ● Telogen phase hair ● Length of hair, 1 cm

Hairs that are in the anagen phase have root sheath attached; however, this may become dislodged as it dries. Roots appear fleshy, and at times a portion of the dermal papilla may be attached. Pigment granules will be present in the roots of anagen phase hairs. The hair shaft may be twisted, stretched, or broken because force is required for removal. Hairs that are in the catagen phase may contain dried root sheath. The root may appear club shaped, and generally the root sheath is dried up. Pigmentation in the root area is diminishing and, when present, is located more distally. Telogen phase hairs have no sheath material around the root area. There is no pigmentation close

to the root area and likewise, no medulla close to the root area. Cortical fusi will be present near the root.

The average person has approximately 2 million hair follicles. Approximately 100,000 of these hair follicles are on the head. The average person will lose approximately 100 hairs per day. These are normally hairs that are in the catagen/telogen phase because they are ready to be shed. However, in addition to these hairs, other hairs may become evidence through transfer. A stereomicroscope provides low-level magnification, which allows for viewing of the initial characteristics of the root of a hair. Further examination can be performed using the compound light microscope for more detail. The microscopic assessment of the hair will determine which type of DNA analysis should be used for the best results.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and calibrated focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media (a colorless mounting medium in the refractive index range of 1.5–1.60)

Human hair

Unknown hair samples

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

PART I: EVALUATION OF HUMAN HAIR FOR DNA

Procedure

1. Mount hair samples onto microscope slides using techniques previously learned (or you can use previously mounted hair).
2. Examine the hair using the stereomicroscope.
3. Note the characteristics of each root if present and the length of the hair.
4. Next examine the same hair using the compound light microscope. Remember to check the microscope to ensure that samples will be viewed using Köhler illumination.
5. Examine the hair for microscopic characteristics of each root/hair. Using the circle template located in Appendix F draw what you see.
6. Observe several hair samples using steps 1–5.

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. Describe each term as it relates to hair: anagen, catagen, and telogen. Include drawings and/or examples with your description for each term.
2. List three characteristics that are used to distinguish anagen hairs from telogen hairs.
3. Why is it necessary to determine the growth phase of hair prior to DNA extractions?
4. What are the criteria for nDNA hair samples?
5. What are the criteria for mtDNA hair samples?

RECOMMENDED AND FURTHER READING

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Glass Examinations

Experiment 16: Glass Examinations

Recommended pre-lab reading assignments:

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OBJECTIVE

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

1. examination of glass fragments using the immersion method:
 - a) Becke Line method
 - b) Oblique illumination method
2. density comparisons of glass fragments

INTRODUCTION

Glass is one of the most common types of evidence examined with a microscope. The main components of synthetic glass are formers (usually silicon oxide or boric oxide), fluxers (usually soda ash or potash), and stabilizers (usually calcium or magnesium). Metal oxides are sometimes added to increase durability, hardness and refractive index (lead), chemical corrosion resistance (alumina), and temperature change resistance (boron). The color of the glass is controlled by additional elements such as copper (blue), chromium (green), manganese (violet), Fe^{3+} (yellow-brown), and Fe^{2+} (bluish green). It is easy to see then that glass has great variability in its composition.

Table 16-1 Refractive indices for five common types of glass.

Glass Type	n_D^{25}
Fused silica quartz	1.459
Borosilicate	1.479
Soda lime	1.512
Alumina silicate	1.530
High lead glass	1.693

One optical characteristic of glass that can be used to compare and distinguish glass fragments is refractive index. Glass types can easily be distinguished by measurement of their refractive index using a compound or phase contrast microscope. Table 16-1 contains a list of five common types of glass and their average refractive index at 25°C. Determining the refractive index of glass using the Becke Line method was discussed in Experiment 2D in Chapter 2. When using this method, the glass fragment is immersed in an oil and the Becke Line is observed when increasing the stage to objective distance, often called ‘raising the focus.’ The Becke Line moves to the medium with the higher refractive index when the focus is raised. Refractive index is known to vary with wavelength and temperature. The variation with wavelength is known as the dispersion. Refractive index measurements of glass are typically recorded at sodium’s D line (589 nm, yellow-orange). For dispersion measurements refractive index is also commonly measured at hydrogen’s C (656 nm, red) and F lines (486 nm, blue). The dispersion of refractive index is then determined either graphically by plotting refractive index vs. wavelength or by Equation 2D-3. Since refractive index also varies with temperature, it is important that temperature control be maintained. If temperature control is not possible, a temperature correction must be applied. This is done by taking into consideration the temperature coefficient of the immersion oil. The change per degree centigrade, $-dn/dT$, is called the temperature coefficient. Commercially available Cargill™ refractive index liquids have their $-dn/dT$ marked on each bottle. Refractive index is commonly reported at 25°C, whereas the immersion measurements often occur at room temperature, 23°C. To perform a temperature correction, the following formula is applied:

$$n^{25} = n^{23} - (25 - 23) \frac{dn}{dT} \quad (16-1)$$

where n^{25} is the index of refraction at 25°C, n^{23} is the index of refraction obtained at the measurement temperature of 23°C, and dn/dT is the positive value of the temperature coefficient of refractive index for the liquid found on the immersion oil bottle.

Since temperature and refractive index are inversely related it is important to check your answer using the rule that as temperature increases refractive index decreases and vice versa. A similar relationship exists for dispersion of refractive index. Normal dispersion is exhibited when an increase in refractive index occurs at shorter wavelengths.

An older method of refractive index determination that still has some value in forensic examinations is the oblique illumination method. Oblique illumination is obtained by covering one side of a fully opened substage aperture with an opaque card such as a business card. When the condenser and shade are properly positioned about half of the field appears dark while the other is light. It is also possible to mimic this effect by partially inserting an accessory plate. A glass particle that has a higher refractive index than the liquid will be shaded on the dark side of field of view.

Table 16-2 Information provided by color fringes in oblique illumination method.

Fringes	Information to be gained
Blue, green, violet	Ignore these colors: They are difficult to interpret.
Yellow	The glass particle has a higher refractive index than the oil.
Brown	The glass particle has a lower refractive index than the oil.
Orange-red	The glass particle has the same refractive index as the oil.
Bright fringes	The glass particle has a lower refractive index than the oil.
Dim fringes	The glass particle has a higher refractive index than the oil.

The color fringes that appear on the edges of the glass particles provide additional information summarized in Table 16-2.

A physical property of glass that is sometimes used to compare and identify glass fragments is density. Density is defined as mass per unit volume:

$$D = \frac{\text{mass}}{\text{volume}} \quad (16-2)$$

For example, lead has a density of 11.3 g per cubic centimeter while table salt (sodium chloride) has a density of 2.2 g per cubic centimeter. Hence a given volume of lead weighs 5.1 times as much as the same volume of salt. Different types of glasses also have distinctly different densities in the range of 2.2–2.6 g per cubic centimeter. Density can be used as a screening technique where large numbers of fragments are encountered. The density of glass fragments varies with the chemical composition and the thermal history of glass. Thermal history must be considered when measuring density of glass from an arson case. Absolute measurements of density report a numerical value for the density of the glass fragment whereas comparative measurements of density are used to determine if two glass fragments have the same or different densities. Absolute measurement of density can be accomplished by several techniques such as: 1) the sink float method; 2) density gradient tubes; or 3) use of a density meter. The sink-float method requires placing a glass fragment in a liquid, whose density is adjusted to obtain a ‘glass suspension point.’ It can then be concluded that the glass particle has the same density as the liquid. For absolute density measurements the density of the liquid that suspends the sample should then be accurately determined using a calibrated plummet. Density gradient tubes require that specific liquids be layered so that various density levels can be determined, and the density of the position at which the glass is suspended can be identified. Both of these methods can be used for absolute or comparative density determinations. For comparative examinations, two fragments are easily distinguished by their relative position in the liquid.

Density is temperature dependent. As the temperature increases generally the density of a substance decreases. However, there are exceptions to this generalization. For example, the density of water increases between its melting point at 0°C and 4°C and similar behavior is observed in silicon at low temperatures. Comparison of densities can be made at the same room temperature without temperature control. However, if the absolute density of a glass fragment is being measured, it is important to control the temperature and record the temperature.

An understanding of the physical and optical properties of glass allows the forensic scientist to perform complete glass examinations. Since glass manufacturing has stringent quality control guidelines, the refractive index and density of glass samples are becoming less variable and therefore less of a probative factor for comparison of unknown and known samples. However, many glass manufacturers now use a variety of chemical components to develop specific properties

for their glass products. This variety of components yields a diversified elemental composition for each glass produced. With enhanced instrumental techniques, elemental compositions are now becoming the most discriminating method for glass examinations.

EQUIPMENT AND SUPPLIES

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X)
Accessory plates
Cargille™ refractive index liquids
Density liquids
Bromoform
Methanol
Poly-Gee™ Brand; Sodium Polytungstate from Geoliquids, Inc.
(sodium polytungstate saturated solution)
Distilled water
Micro kit
Cover slips
Microscope slides
Glass fragments of known refractive index
Glass fragments of known density
Glass fragments of 12 unknowns
Disposable beakers

SAFETY

Use standard laboratory safety procedures as described in rules set by your instructor. Be cautious of microscope light levels to avoid eye damage. Bromoform is **extremely toxic and a suspected carcinogen**. Use this chemical in a hood and wear protective eyewear and gloves for this procedure.

PART I: REFRACTIVE INDEX BY IMMERSION METHOD

Becke Line Method

1. Choose a glass particle of known refractive index (RI) and immerse it in
 - a) a Cargille™ oil of *higher* RI
 - b) a Cargille™ oil of *lower* RI

Example: Glass with RI of 1.52 in oil of 1.500 and 1.540
2. With the microscope set up and adjusted for Köhler illumination, focus on the edges of a representative particle using the 10X objective. Use the orange filter to obtain monochromatic light. This will give you values for the sodium D line.

3. Close the substage condenser to the minimum setting. Using the glass particle in the lower RI oil, observe the Becke Line and then focus ‘upward’ and ‘downward’ to determine whether the glass fragment or the solution has the higher RI. Remember that as you focus up (raise the microscope lens away from the specimen), the Becke Line will move toward the substance with the higher index. When the RI of the glass and the immersion liquid are the same, the chip will become almost invisible, and it may be difficult to find and bring the edges of the chip into focus. Draw what you observe. What is your conclusion?
4. Repeat the steps above with a glass particle in a higher RI. What did you observe? What are your conclusions?
5. Repeat the above with another known glass particle. What did you observe? What are your conclusions?
6. Choose one of the unknown glasses (1–12).
7. Select an oil in the middle of the range of the unknowns provided by the instructor. Prepare a liquid mount.
8. Focus on the glass particle using the 10X objective. Be careful not to get any oil on the objective. If oil does get on the objective your instructor will show you how to clean it with xylene and lens paper.
9. Using the coarse focus adjustment, raise the focus (increase the distance between the stage and the objective). Watch the Becke Line movement. The Becke Line moves to the medium with the higher RI.
10. Repeat the previous step with an oil of a higher or lower RI.
11. Continue to try new oils until you obtain minimum relief. If you cannot obtain a good match with a single oil, mix two oils by adding a drop of each to the glass particles and dropping a cover slip on top.
12. Using the circle template located in Appendix F, draw what you observe once you have determined the RI.
13. Using a thermometer measure the room temperature. If necessary, correct the RI determined for the glass by using Equation 16-2 to calculate the RI of the glass at 25°C. The temperature coefficient of the RI can be found on the Cargill™ oil that was found to be a match to your glass fragment’s RI.
14. Report the RI of the matching oil and unknown number on a circle template.

Oblique Illumination Method

1. Choose glass particles of known RI. Immerse the particles in an oil of higher RI. With the accessory plate inserted into its slot, darken half of the field of view and examine how the bright and dark shadows appear on the glass particles. Are they towards the bright or dark sides of the field? Repeat with an oil of lower RI than the glass particles. Draw what you observe in both cases.
2. Obtain an unknown glass sample. Using the rules developed in step 1, determine the RI by using different oils until you obtain minimum relief. Remember that you are looking for an orange/red fringe for the match point. Use the brown and yellow fringe colors to enable you to choose your oils according to Table 16-2. Draw what you observe when you find the match point.
3. Checked the value by the Becke Line method.

4. Using a thermometer measure the room temperature. Correct the determined RI by using Equation 16-2 to calculate the RI of the glass at 25°C. The temperature coefficient of RI can be found on the Cargille™ oil that was found to be a match to your glass fragment's RI.

PART II: DENSITY BY SINK FLOAT METHOD

This method determines if a glass particle will float, sink, or be suspended in a liquid. Before making a determination of floating make sure the glass fragment has broken the surface tension of the liquid. A fragment that never breaks the surface tension of the liquid may appear to float when it is actually the same density and would be suspended. A suspended particle may be near the top or near the bottom. As long as it does not sink all the way to the bottom or float on the surface it can be determined to be suspended. These experiments are performed at room temperature so it is important not to heat up the test tubes unnecessarily. Slight warming can cause a glass fragment to sink whereas slight cooling can cause it to float. Holding the test tube at the top will prevent unnecessary warming. Although not practical in most educational settings, but required in a forensic laboratory, the use of temperature controlled equipment should be used to determine accurate measurements of absolute density.

Comparative Density Determination of Glass Fragments by the Sink/float Method

Additional Note:

This procedure must be performed in the hood because bromoform is **extremely toxic and a suspected carcinogen**. Wear protective eyewear and gloves for this procedure.

The instructor may choose to substitute sodium polytungstate and water for this procedure.

1. Samples of glass of known density will be available. Obtain a clean dry piece of density standard. Examine it carefully under the microscope and note any identifying features. Make a sketch of the piece of glass. Repeat this for each density standard.
2. Obtain your unknown glass fragment. Examine it carefully under the microscope, note any identifying features, and make a sketch of the piece of glass. It is important to be able to recognize each piece of glass separately as they will at times be placed together in the same test tube. Discrimination of the glass samples will be possible using the sketches and the stereomicroscope. The unknown and density standards should be approximately the same size.
3. Place the first density standard into a test tube containing a small amount of bromoform (diiodomethane). If the glass is less dense than the solution it will float on the surface of the liquid. If this occurs, slowly add a drop of the less dense methanol. (*Hint:* to ensure that the solution is uniform, mix thoroughly). Mix the solution and observe the position of the glass fragment. Continue to add bromoform or methanol liquid until the particle is suspended. Between additions of liquids it is important to mix the solution thoroughly.
4. Add a similar size, clean, dry fragment of unknown glass, which has been microscopically examined and for which an identifying sketch has been made. If the two fragments are the same density they will both remain suspended in the liquid. If not, one will float or sink relative to each other. Record your observations.

5. Remove your unknown glass particle and clean it with acetone. Dry the unknown glass particle and repeat steps 3 and 4 for the remaining density standards.
6. The standard that matches the unknown should react in exactly the same manner as the unknown. It is possible to have an unknown that does not match any of the standards.
7. Report the density of the unknown if it is found to match one of the standards. If the unknown does not match any of the standards report the density range (i.e., higher than standard 3, in between standard 2 and 3 or lower than standard 1). Write a paragraph defending your conclusions. Be sure to include your identifying sketches.

Absolute Density Determination of Glass Fragments by the Sink/float Method

Additional Note:

This procedure uses Poly-Gee™ (sodium polytungstate solution) and water.

The instructor may choose to substitute bromoform and methanol for this procedure. Bromoform is extremely toxic and a suspected carcinogen and should be used in a hood, with protective eyewear and gloves.

1. Obtain a clean dry piece of glass of known density. Examine it carefully under the stereomicroscope and note any identifying features.
2. Place the glass sample into a test tube containing a small amount of Poly-Gee™ (saturated sodium polytungstate solution). The glass will most likely float on the surface of the liquid. Slowly add water, which is less dense, drop wise with stirring, until the particle is suspended in the middle of the liquid. (*Hint:* to ensure that the solution is uniform, mix thoroughly). If too much water is added so that the glass fragment sinks, slowly add more of the denser Poly-Gee™ liquid.
3. Determine the density of the glass fragment. Since the glass fragment is suspended, it has the same density of the liquid. To determine the density of the liquid, accurately pipet a known volume of liquid into a small plastic beaker that has been weighed on an analytical balance. Next weigh the beaker and liquid on the same balance. Subtracting the weight of the beaker, use Equation 16-1 to determine the density of the liquid. Report the density of the known glass sample to the appropriate number of significant figures. Record the room temperature at the time of this measurement.
4. Examine the unknown glass sample using the same procedure as performed with the known glass. Recheck the room temperature.
5. Report the density of each piece of glass to the appropriate number of significant figures. You will be provided the density of the known sample. Calculate the percent error in the density you obtained according to the following equation:

$$\% \text{ error} = \left| \frac{\text{known} - \text{measured}}{\text{known}} \right| \times 100 \quad (16-3)$$

As part of your lab report discuss the accuracy of this method using the percentage error for the known glass sample. Discuss possible sources of error with the procedure.

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 main ingredients used to produce glass?
2. How would changes in the amount of these ingredients affect the refractive index of various glass types; give at least one example?
3. How would changes in the amount of these ingredients affect the density of various glass types; give at least one example?
4. Why does leaded glass sparkle? Explain why leaded glass has a high refractive index.
5. What is the density range for glass samples in the following categories: borosilicate glass, leaded glass, soda lime glass.
6. Include the calculations made to determine the density of your known glass sample. What type of glass do you suspect that it might be?
7. Include the calculations made to determine the density of your unknown glass sample. What type of glass do you suspect your unknown glass to be?
8. If your known and unknown glass samples have similar densities, how would you examine the samples further to determine if they have a common origin?
9. Why is it important to use temperature control in absolute density measurements? How could you have controlled temperature in this experiment?

Experiment 16A: Glass Breakage Determinations

Recommended pre-lab reading assignments:

Koons RD, Buscaglia J, Bottrell M, Miller ET. Forensic Glass Comparisons in Forensic Science Handbook.
In: Saferstein R, ed. *Forensic Science Handbook*. 2 ed. Upper Saddle River: Pearson Education; 2002; 188–9.

McJunkins SP, Thornton JI. Glass fracture analysis: A review. *Forensic Science*. 1973; 2(1): 1–27.

OBJECTIVE

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

1. float glass manufacturing method
2. identification of float glass
3. glass breakage and determination of impact points
4. determination of order of impact points
5. determination of direction of force for impact points

INTRODUCTION

Most forensic glass examinations involve flat glass. Flat glass is typically used in windows and any glass products that contain sheet glass (i.e., mirrors, windshields, doors). Most flat glass is now produced by the float method. This process begins by feeding the ingredients into a mixer that feeds into the furnace. Full melting is achieved at approximately 1600°C. Once the now molten glass reaches the end of the furnace, it is fed onto a bath of heated liquid, usually molten tin. Minute amounts of molten tin become mixed with the molten glass. This can be visualized as fluorescence under short-wave ultraviolet light (254 nm). Float glass will exhibit a white fluorescence when illuminated with short-wave light on a non-fluorescent background. Since the liquid is so hot, irregularities are melted out ensuring a flat surface. The molten glass next enters the spreader area, which stretches the glass to the desired width. The thickness of the glass is dictated by the speed of the float and spreader process. Toward the end of the spreader area, the molten glass enters an annealing phase. Annealing is the controlled heating and/or cooling during glass production that reduces the residual strain within the glass. This phase cools the glass to 200°C. Once cooled, the glass finally enters a cutting, stacking, and offloading area.

Some glass goes through additional processes to meet special needs: Two examples are tempered glass and laminated glass.

Tempering is a process that quickly heats the glass to a softening point (700°C) and then quickly cools it with a blast of compressed air. When tempered glass shatters, it breaks into small pebble-like pieces, without sharp edges. Tempered glass has bending and impact strengths that are three to five times higher than those of annealed glass of the same thickness. It is used in for glass doors,

side and rear windows of automobiles, and other applications where safety is an issue. When two or more layers of glass are held together with a plastic interlayer, the glass is called laminated glass. Usually polyvinyl butyral (PVB) is used. The PVB is sandwiched between the glass, which is then passed through rollers to expel any air pockets. The initial bond is formed by the rollers, but ensured when heated to around 70°C in a pressurized oil bath. Laminated glass is referred to as safety glass because it holds together when shattered. It is commonly used when there is a possibility of human impact or where the glass could fall if shattered. In windshields, the tint at the top of some cars windshields is actually in the PVB.

When glass is struck, it will tend to bend on the side opposite to the impact. When stretched too far, it breaks the glass causing radial fractures. Radial fractures start at the impact point and propagate outward like the spokes on a bicycle (Figure 16A-1). As they break, ridges are formed on the broken edge of the crack. These ridges are called Wallner lines or rib markings. (Figure 16A-2) Wallner lines form perpendicular to the side opposite the impact and parallel to the side of impact. As the radial cracks bend away from the force applied, additional cracks will then begin. These are called concentric cracks. Concentric fractures are fractures formed after the radial cracks, in a circular pattern around the point of impact and can be compared to the wheel of the bicycle (Figure 16A-3). Since radial cracks always develop prior to concentric cracks, the

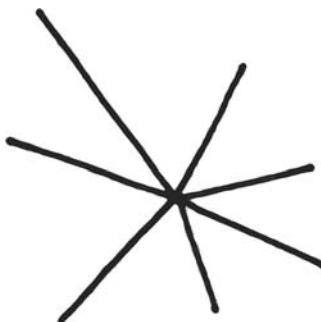


Figure 16A-1 Radial fractures are the first fractures to begin after an impact has occurred.



Figure 16A-2 Wallner lines (rib markings).

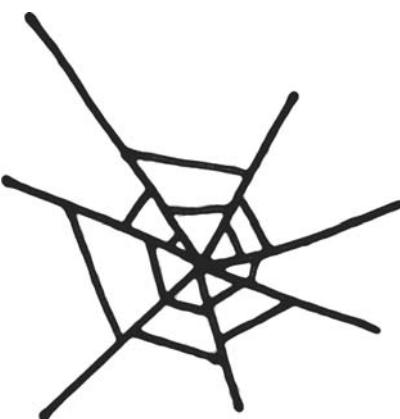


Figure 16A-3 An impact point with radial and concentric fractures.

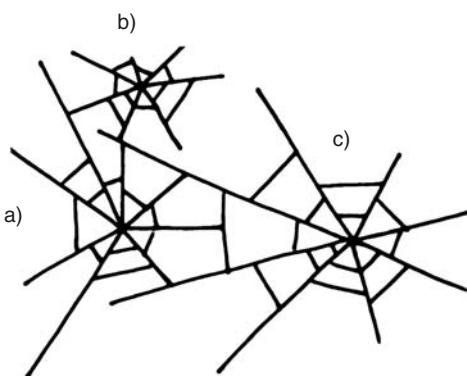


Figure 16A-4 Order of impact determination. The order of impact shown here from first to last is c) first, a) second, and b) third.

order of impact points can sometimes be determined. Figure 16A-4 shows three impact points for which order may be determined.

Sometimes, it may also be important to determine from which side the force causing the breakage was applied. This can be determined by two methods. An examination of the rib markings on fractures can determine the direction of the force breaking the glass. To accomplish this, you must first determine the point of impact. Portions of the broken glass must be put back together enough to determine the point of impact. This allows you to distinguish radial from concentric cracks. Once the point of impact is determined, the 4R rule can be applied. The 4R rule is: **Ridges on Radial fractures are at Right angles to the Rear** (the side opposite the impact), and is demonstrated in Figure 16A-5.

The direction of force can also be determined by examining the impact point. When the glass breaks, some of the glass continues to move forward. With high velocity impacts, a coning or catering effect is seen.

A wider hole is created on the exit side than from which the force was applied (Figure 16A-6).

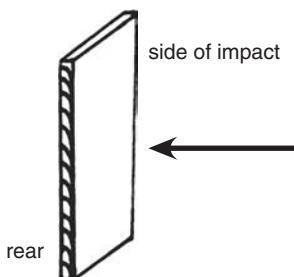


Figure 16A-5 Wallner lines on a radial fracture showing force was applied from the right side.

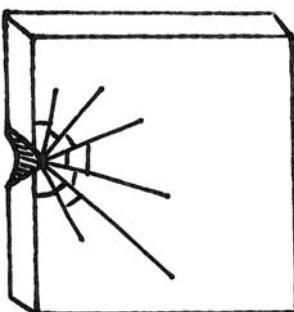


Figure 16A-6 Coning effect on a section of glass.

Exceptions to this examination include tempered glass that shatters, small windows held tightly so that they cannot bend, and windows that are broken by heat or explosion where there is no point of impact.

EQUIPMENT AND SUPPLIES

- UV light box
- Broken float glass samples
- Broken molded glass samples
- Unknown glass samples

SAFETY

Use standard laboratory safety procedures as described in rules set by your instructor. Broken glass samples have sharp edges; use caution when handling. The UV light box can damage the eyes and skin. View samples through the viewer only. Do not turn the box over and look directly at the bulb. Do not keep your hand under the light while the UV light is on.

PART I: FLUORESCENCE OF FLOAT GLASS

The fluorescence test for float glass requires shortwave UV light to excite the tin present in the glass. Observations should be made in darkness if possible.

1. Place a section of known float glass under the UV light box. Illuminate the glass with short-wave light. Observe the first side of the glass sample. Turn the glass over and observe the other side. Did one or both sides fluoresce?
2. Now observe both sides under long-wave UV light. Is there any difference? Alternate between short-wave and long-wave UV light. Different colors of fluorescence have sometimes been observed when switching between the two lights. Record your observations in your report.
3. Repeat step 1 and 2 with a known sample of molded glass. Record your observations in your report.

PART II: RECONSTRUCTION OF GLASS FRAGMENTS

Direction of Force

1. Obtain a plastic bag with broken glass fragments.
2. Examine and carefully piece together the broken windowpane made of float glass. (*Hint:* Use the fluorescence test to determine the tin side of the glass.) Draw the reconstructed windowpane.
3. Determine the point of impact. Label this on your drawing.
4. Identify the radial and concentric cracks. Label a radial and concentric crack on your drawing. Using the 4R rule, determine from which side the force was applied. Draw the radial crack and the Wallner lines used for your determination.
5. Using the ‘coning effect,’ determine from which side the force was applied. Does this conclusion agree with your conclusion from step 4?

Order of Breakage

1. Obtain a plastic bag with broken glass fragments.
2. Examine and carefully piece together the broken windowpane made of float glass. (*Hint:* Use the fluorescence test to determine the tin side of the glass.) Draw the reconstructed windowpane.
3. Determine the points of impact. Label these as ‘A’ and ‘B’ on your drawing.
4. Determine the order in which the impacts occurred. Identify the radial or concentric cracks used in your determination. Label these on your drawing.

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. Describe the portion of glass manufacturing that allows float glass to be easily identified.
2. Which side of float glass is found to fluoresce using short-wave UV light? What component of the glass is being excited?
3. What is a radial crack? How is it formed?
4. What is a concentric crack?
5. What are Wallner lines? Why are they important?
6. Given Figure 16A-7:
 - a) label a radial crack
 - b) label a concentric crack
 - c) place the impacts in order below

1: _____ 2: _____ 3: _____ 4: _____

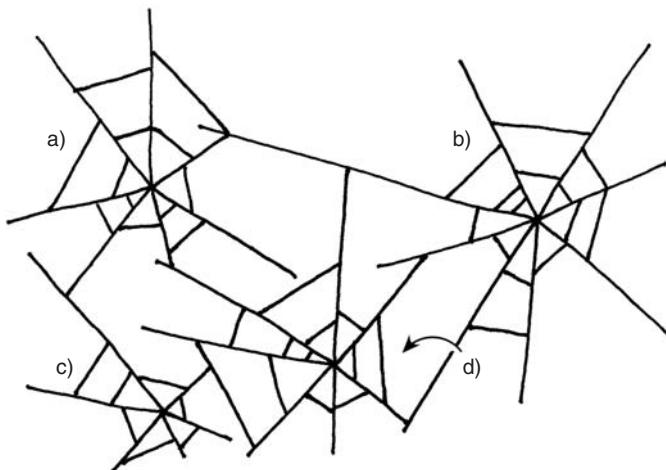


Figure 16A-7 Determine the order of impact for the four impacts.

RECOMMENDED AND FURTHER READING

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- Koons RD, Buscaglia J, Bottrell M, Miller, E. Forensic Glass Comparisons. In: Saferstein R, ed. *Forensic Science Handbook*. 2nd ed. Upper Saddle River, New Jersey: Pearson Education; 2002; 161–213.
- Koons RD, Buscaglia JA. The Forensic Significance of Glass Composition and Refractive Index Measurements. *Journal of Forensic Sciences*. 1999; 44(3): 496–503.
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- Locke J. Improvements in the Use of Silicone Oils for the Determination of Glass Refractive Indices. *Journal of the Forensic Science Society*. 1982; 22: 257–62.
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Fiber Examinations

Experiment 17: Textile Fibers Examinations

Recommended pre-lab reading assignments:

Carroll GR. *Forensic Examination of Fibres*. London, England: Ellis Horwood, Ltd.; 1992; 99–105.
Eyring MB, Gaudette BD. An Introduction to the Forensic Aspects of Textile Fiber Examination. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 245–61.

OBJECTIVE

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

1. general characteristics of a textile fibers
2. use of the stereomicroscope to visualize the characteristics of textile fibers
3. use of the polarizing light microscope to visualize the characteristics of textile fibers
4. preparing and observing cross-sections of fibers
5. refractive indices for fibers
6. sign of elongation
7. dyed and pigmented fibers

INTRODUCTION

Items manufactured from natural and man-made fibers surround us. Therefore, people find themselves in constant contact with fabrics and the fibers of which they are composed. Natural fibers are typically limited in characteristics to determine color and general fiber type. However, man-made fibers possess characteristics that sometimes make identifications and further comparisons possible. Because of this fact, microscopic examination of fibers presents itself as indirect or circumstantial evidence in a court of law. This evidence is generally used in conjunction with other forensic evidence. Fiber examinations are performed to determine possible associations between people, places, and objects.

Fibers are divided into two main types: natural and man-made. Natural and man-made fibers can easily be differentiated by macroscopic and microscopic characteristics. Natural fibers are created from vegetable/plant, animal, or mineral sources. Due to their cellular structures, natural fibers are easily recognized when using the microscope. Man-made fibers are created from raw materials, either natural or chemical based. Natural based man-made fibers are called natural polymers. They may be composed of regenerated cellulose or regenerated protein. Chemical based man-made fibers are referred to as synthetic fibers. They are also easily recognized due to their unique microscopic characteristics.

As already stated, natural fibers are derived from vegetable, animal, or mineral sources. Vegetable fibers originate from any part of a plant; the stem or bast, leaf, fruit, and seed. Therefore, vegetable fibers are easily recognized by the presence of cellular walls, features of the cell walls and the lumen. During microscopic examinations, it is also important to note the appearance or lack of the following: pits and spiral thickenings, convolutions, wall thickness, transverse markings, crystals, bundles, and length. Animal fibers generally originate from the ‘fur hair’ of animals. Because of this, they are also easily recognized by their microscopic characteristics. Scale margin appearance, scale margin distance and scale patterns, along with morphological features can be used to identify animal fibers. Mineral fibers are inorganic fibers and are asbestos or mineral wool. Extinction characteristics make these fibers easy to identify.

Since man-made fibers are generated by mechanical means, certain microscopic characteristics remain consistent. During microscopic examinations of man-made fibers, it is important to note the appearance or the lack of the following characteristics: color, luster, thickness, inclusions, cross-section, and variations in characteristics within the fiber, birefringence, pleochroism, and sign of elongation.

Fiber examinations involve the use of a variety of microscopes, techniques, and instruments. Examinations are usually performed to identify a fiber and then, when necessary, to compare a fiber back to a known fiber source. Initially a stereomicroscope is used to examine the fiber for color and possibly determine whether it is a natural or man-made fiber. The length, crimp, and diameter are also determined.

Further testing can be performed using a polarized light microscope to determine optical properties such as refractive index, birefringence, and sign of elongation. The refractive index of a fiber is a measurement of how much light is slowed as it passes through the fiber. For fibers, there are two refractive indices: parallel and perpendicular to the long axis. The refractive indices are determined by examining the fiber with orientation of its long axis parallel and perpendicular to the plane of polarization. The Becke Line method is also used to determine the refractive index of fibers. The Becke Line is a bright halo appearing at the boundary of a particle. This halo will move as the focus is adjusted up and down. As the working distance of a microscope is increased, the Becke Line travels towards the medium of higher refractive index.

The birefringence of fibers can also be used to aid in the identification of a fiber. The thickness of the fiber can be measured using a calibrated ocular micrometer. Using these two characteristics and the Michel-Lévy birefringence chart or tabular data, such as that found in Appendix A and B, the identification of the fiber can be determined. With natural fibers, refractive indices are not the most distinguishing characteristic; however, it is still an identifying feature. Refractive indices for common plant and animal fibers are found in Appendix A. For mineral fibers, there may be three refractive indices, depending on the variety. Data for mineral fibers is also contained in Appendix A. With man-made fibers, the refractive index is even more important in that it can be used to distinguish between fibers and manufacturers. Refractive indices for common man-made fibers are found in Appendix B.

Another identifying characteristic is the sign of elongation. If the n-parallel is greater than n-perpendicular, the sign of elongation is said to be positive. If the reverse is true, the sign of elongation of the fiber is negative. Sign of elongation can be determined by two methods: a) when using immersion oils, the sign can be determined by mathematical subtraction – n-parallel minus n-perpendicular; or b) by inserting a compensator into the optical path. When inserting a compensator, the retardation will either be ‘added’ or ‘subtracted.’ This can be determined using the Michel-Lévy birefringence chart as a reference to determine if the retardation adds (increases to higher order) or subtracts (decreases in order).

In addition to the overall color of the fiber, microscopic color assessments should also be made. The color of the fiber may be uniform along its length or it may vary. There may also be variation in colors between fibers within the same sample. A microscopic examination of the fiber will show differences when the fiber is dyed, surface-dyed, and pigmented. Dyed and surface-dyed fibers are chemically colored after the fiber has been manufactured. Since pigmented fibers are colored during the manufacturing process, only man-made fibers can be pigmented. The size, shape, and distribution of pigment granules should be taken into consideration. Some fibers may also contain delustering agents. Delustering agents are pigment granules that are used to dull the luster of a manufactured fiber. Titanium dioxide is commonly used as a delustering agent.

Other microscopic characteristics including cross-sections and solubilities may also be determined during fiber examinations. The cross-sectional shape of a fiber is one characteristic that may be used to help establish a fiber type and usage (Figure 17-1).

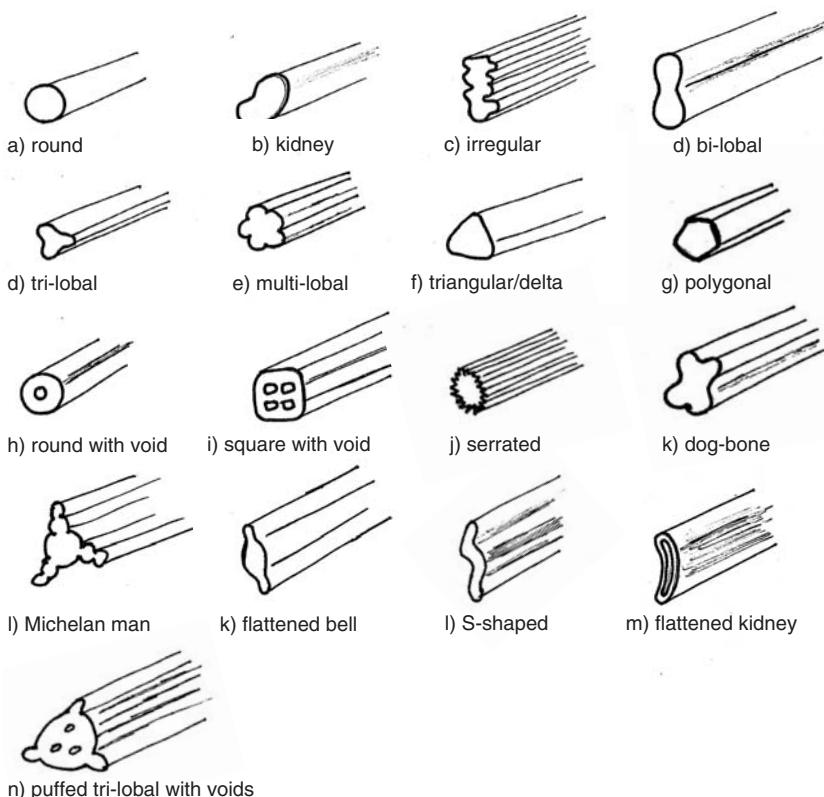


Figure 17-1 Common cross-sections and longitudinal views for fibers.

Natural fibers have very distinct cellular shapes. Man-made fibers have various shapes produced by the manufacturing process. These cross-sections come in a wide variety of shapes: round, bilobal, trilobal, multilobal, bell, dogbone, triangular, hollow, and irregular. Solubility testing of fibers may be a useful technique in the identification of natural and man-made fibers. Solubility testing involves a possible reaction of the fiber with a variety of solvents from an identification scheme. Although this method is destructive, valuable information can be obtained from solubility testing.

Instrumentation may also be used to further identify specific fiber characteristics. Thermal microscopy (Experiment 24), infrared microscopy (Experiment 22), fluorescence microscopy (Experiment 4A), UV-Visible-NIR microspectrophotometry (Experiment 23), and thin layer chromatography are techniques that may be performed on fibers.

Natural and man-made fibers can easily be differentiated by macroscopic and microscopic characteristics. Most natural fibers (vegetable and animal) contain various cellular features that make their microscopic differentiation easy. Mineral wool and asbestos fibers show extinction characteristics, which make their microscopic characterization possible. Man-made fibers have many documented microscopic characteristics, which also make identification possible.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Polarizing light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media (a colorless mounting medium in the refractive index range of 1.50–1.70)

Vegetable fibers (flax, ramie, jute, hemp, sisal, coir, kapok, cotton, akund, abaca, sunn)

Animal fibers (wool, mohair, alpaca, angora, cashmere, camel, llama, silk)

Mineral fibers (asbestos and mineral wool)

Natural based man-made fibers (acetate, triacetate, rayon, lyocell)

Synthetic fibers (nylon, polyester, acrylic, modacrylic, olefin)

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums, and use them with appropriate precautions as set by your instructor. Dispose of glass in an appropriate container.

Special precautions: Ventilation is required when working with asbestos, a human carcinogen. Disposal of asbestos-containing materials should follow United States Environmental Protection Agency (EPA) guidelines.

PART I: EXAMINATION OF FIBERS

Procedure

Use the fiber worksheet located at the end of this experiment and available at <http://www.wileyeurope.com/college/wheeler> for note taking.

1. Examine the fiber with the stereomicroscope. Note the color, length, and longitudinal form of the fiber.
2. Set up a polarized light microscope for Köhler illumination. Determine the cross-sectional shape. This can be done by making a cross-section (procedures for making cross-sections are outlined in Experiment 15B in Chapter 15) and comparing it to cross-sections in Figure 17-2 or viewing a liquid mounted fiber as described in the next step.
3. Using the liquid mounted fiber, examine the fiber under non-polarized light. Determine the diameter and longitudinal shape. Draw what you see. Note any other morphological or microscopic features of the fiber. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Return it to the original position.
4. Examine the fiber under crossed polars. Draw what you see.
5. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Document any further characteristics viewed.
6. Repeat steps 1–5 for using a higher magnification.
7. Repeat steps 1–6 for all fibers assigned.
8. Make a chart of the features you examined for each fiber. Required features are cross-sectional shape, longitudinal shape, diameter, birefringence, and extinction characteristics. Additional characteristics should be noted such as color, striations, and cellular structures.

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 two classifications of fibers?
2. What are the three types of natural fibers? Name one example of each type. Of what are these fibers composed?
3. How are man-made fibers different to natural fibers?
4. What are the types of man-made fibers? Name an example of each type and provide the chemical makeup.
5. What feature is always present in natural fibers?
6. Is asbestos isotropic or anisotropic?
7. What are two microscopic characteristics that could be used to distinguish acrylic fibers from polyester fibers?

FIBER CHARACTERISTICS WORKSHEET**Macroscopic:**

Color _____
Length _____
Longitudinal form _____

Microscopic Morphology:

Color _____
Diameter _____
Longitudinal shape _____
Cross-sectional shape _____
Other _____

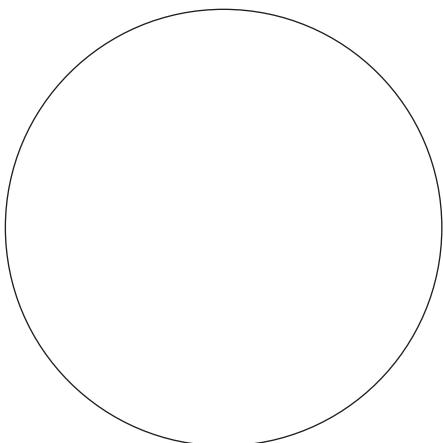
Microscopic Optical Properties:

Refractive Index: n-parallel _____
n-perpendicular _____
Birefringence _____
Extinction _____
Sign of elongation _____
Pleochroism _____
UV fluorescence _____
Melting point _____
Solubilities _____

Fluorescence _____
Other _____

Instrumental Properties:

FTIR _____
Microspec _____



Experiment 17A: Natural Fiber Examinations

Recommended pre-lab reading assignments:

- Textile Institute. *Identification of Textile Materials*. 7 ed. Manchester, England: The Textile Institute; 1975.
- Eyring MB, Gaudette BD. An Introduction to the Forensic Aspects of Textile Fiber Examination. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 263–65.
- Palenik SJ. Microscopical Examination of Fibres. In: Robertson J, Grieve M, eds. *Forensic Examination of Fibres*. London: Taylor and Francis; 1999; 160–76.

OBJECTIVE

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

1. general characteristics of natural fibers: vegetable, animal, mineral
2. use of the stereomicroscope to visualize and identify the characteristics of natural fibers
3. use of the polarizing light microscope to visualize and identify the characteristics of natural fibers
4. determining cross-sectional shape for natural fibers
5. measurement of refractive indices of natural fibers
6. use of the fluorescence microscope to visualize characteristics of natural fibers
7. use of complementary tests for identification of natural fibers:
 - a) twist test
 - b) Billingham test
 - c) Herzog test
 - d) burn test
 - e) solubility tests

INTRODUCTION

The Locard Exchange Principle states that whenever two objects come in contact, an exchange of matter occurs. This theory applies to fiber evidence. Fibers are easily shed and transferred whenever there is contact between two objects, two individuals, or an individual and another object. Therefore, fiber examinations are often used to associate people with items or scenes. This makes fibers an important source of information in forensic investigations.

Natural fibers are derived from plant, animal, or mineral sources and possess various macroscopic and microscopic characteristics that make identifications and further association with a particular source sometimes possible. Because of this fact, natural fibers are routinely examined in forensic casework. A discussion of microscopic characteristics for common plant, animal, and mineral fibers follows.

Plant Fibers

Examinations of natural plant fibers include viewing the microscopic features of the fiber cells. Observing the longitudinal and cross-section properties of the fiber allows a forensic scientist to determine the microscopic features. Characteristics such as the shape of the cells, the relative thickness of the cell wall, the cell length, size, shape and thickness of the lumen, shape of fiber bundles, and the presence of crystals should be noted.

Seed hair fibers

- **Cotton:** Cotton fibers are obtained from the herbaceous shrub, *Gossypium hirsutum*, *barbadense*, *arboreum*, and *herbaceum*. Cotton fibers are essentially 95 % cellulose. They are tapered at each end and twisted or convoluted along the length of the shaft, reversing occasionally, resembling flattened, twisted ribbons. There is generally a central canal, although both the lumen and the convolutions are absent at the tip ends. Cotton fibers are easily distinguished from other natural fibers under crossed polars, in that they do not go to extinction upon stage rotation but remain bright in all orientations (see Figure 17A-1a).
- **Kapok:** Kapok fibers are obtained from the seed pod of a tree of the *Bombaceae* family, *Ceiba pentandra*, or *Bombax malabaricum*. They are tapered at both ends and generally resemble a smooth, cylindrical, hollow, thin-walled fiber that is sometimes flattened. The fibers taper to a point at one end, with the other end having a bulbous base with annular reticular markings.
- **Akund:** Akund fibers are obtained from the seed hair of *Calotropis procera*. They resemble the kapok fiber but do not show the net-like thickenings.

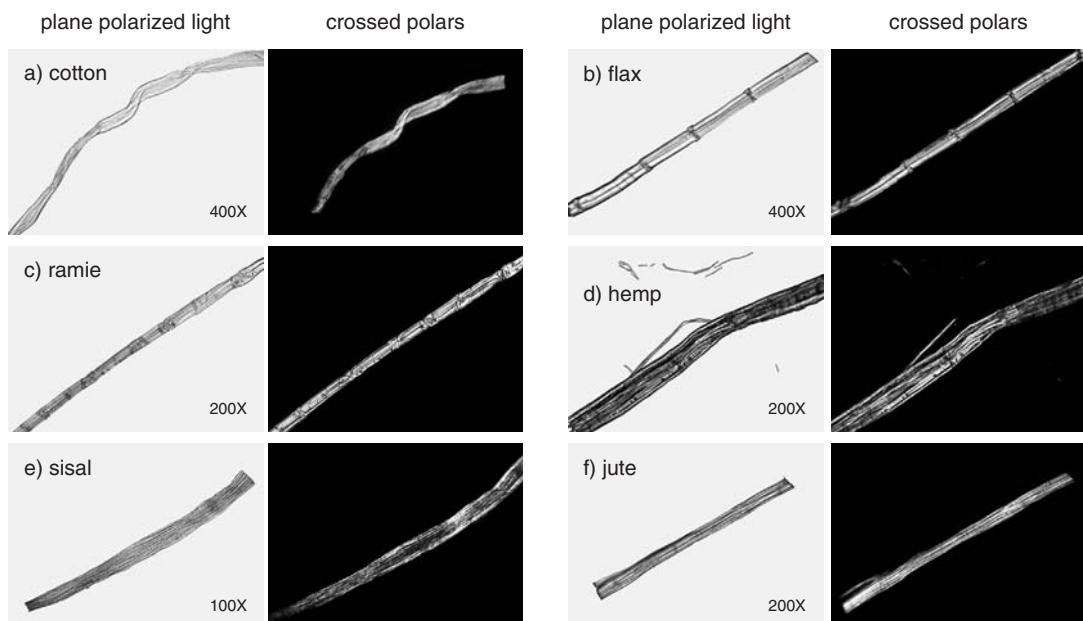


Figure 17A-1 Common natural fibers under plane polarized and crossed polarized light. A full-color version of this figure can be found in the color plate section of this book.

Bast fibers (stem fibers)

- **Flax:** Flax fibers are obtained from *Linum usitatissimum L.* Flax fibers are straight and have a rounded-polygonal cross-section, usually with distinct lengthwise striations. The fibers have a thin, indistinct lumen. Flax is easily distinguished by the slight thickenings at the nodes, which give an identifiable 'X', 'Y', 'V', or 'T' marking when observed under crossed polars (see Figure 17A-1b).
- **Hemp:** Hemp fibers are obtained from the annual plant *Cannabis sativa*. Hemp fibers are colorless cylinders with surface irregularities such as joints and fractures, similar to that of the flax fiber. At times crystals may appear on the surface of untreated bundles. The fibers have a polygonal cross-section with rounded edges. The lumen is generally broad and flattened. The twist test further differentiates between flax and hemp (see Figure 17A-1d).
- **Sunn:** Sunn fibers are obtained from the stem of the legume *Crotalaria juncea L.* The sunn fiber resembles hemp microscopically, with the exception of the presence of crystals found in hemp fibers.
- **Jute:** Jute fibers are obtained from the annual herbaceous plant *Corchorus, C. capsularis, or Corchorus C. olitorius*. Jute fibers taper at both ends and are generally smooth straight cylinders with occasional markings. The cell walls are thick and the lumen irregular with constrictions (see Figure 17A-1f).
- **Ramie:** Ramie fibers are obtained from the stem of *Boehmeria nivea*. Ramie fibers are cylindrical and have node-like ridges and longitudinal striations. The cell wall is thick, with a well-defined lumen (see Figure 17A-1c).

Leaf fibers (hard fibers)

- **Sisal:** Sisal fibers are obtained from the plant *Agave sisalana*. Sisal fibers are cylindrical with broadening toward the middle. Ends are blunt and thick. Cell walls are thick, with a rounded polygonal lumen that is large but not prominent. Rod-like crystals may also be present (see Figure 17A-1e).
- **Abaca (manila):** Abaca fibers are obtained from the plant *Musa textilis*. The abaca fiber is identified by the characteristic stegmata (small silica cells) that are present in the longitudinal files adjacent to the fibers.

Other

- **Coir:** Coir fibers are obtained from the fruit or husk of the *Cocos nucifera L* plant. The coir fibers are short and have distinct wavy outline.

Animal Fibers

Morphological features that should be noted for animal fibers include the root, medulla, and cuticle. Shield size and the shape may also be helpful characteristics. Scale casts can be useful in distinguishing some animal fibers.

- **Wool:** Wool fibers are obtained from several species of sheep. Wool fibers are colorless cylinders with visible, prominent, overlapping scale structure. The fiber edges appear serrated and wavy (see Figure 17A-2b).

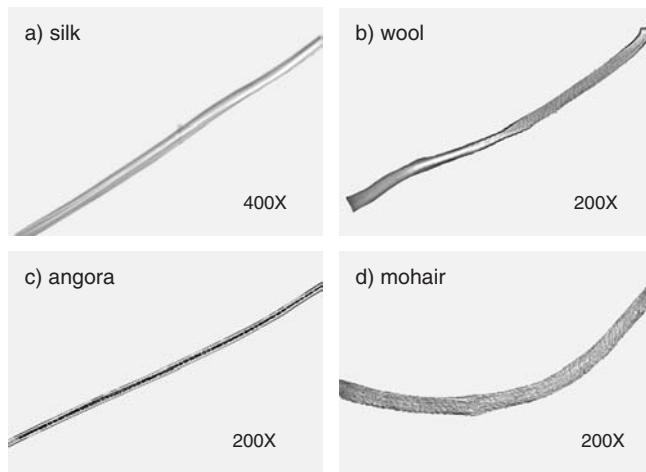


Figure 17A-2 Common animal fibers under plane polarized light.

- **Mohair:** Mohair fibers are obtained from the Angora goat. The scale structure found on mohair fibers is a faint, irregular-wave mosaic pattern (see Figure 17A-2d).
- **Cashmere:** Cashmere fibers are obtained from the Cashmere goat. Cashmere fibers are generally longer and more uniform than the mohair fiber. The scale structure is a wavy, irregular pattern.
- **Camel:** Camel fibers are obtained from two areas of the camel: the outer coarse hair and the fine fur hair. Camel fibers have faint scales, which project slightly from the edge of the fiber. They are generally round to slightly oval in cross-section.
- **Llama:** Llama fibers are obtained from the alpaca llama. Guanaco and vicuna hair are also used for llama fibers. Llama fibers are generally smooth and have fine diameters.
- **Silk:** Silk fibers are obtained from the cocoon of *Bombyx mori*. Silk fibers are generally continuous ribbons with small wedge-shaped or polygonal cross-sections. The fibers may exhibit oblique depressions at intervals (see Figure 17A-2a).
- **Rabbit:** Rabbit fibers are obtained from two portions of the rabbit: the guard and finer fur hairs. The scale pattern changes along the length of the rabbit hair. The medulla pattern is characteristic (see Figure 17A-2c).
- **Horse:** Horse fibers are obtained from the mane and the tail hair. They can be distinguished by the coarseness and coronal scale pattern.

Mineral Fibers

Mineral fibers or inorganic fibers are generally considered asbestos. Isotropic characteristics make these fibers easy to identify.

- **Asbestos:** This is a general term used for several types of natural fibers that are commonly occurring crystalline inorganic silicates. These fibers can be distinguished by their isotropic or low birefringence. There are many varieties of asbestos fibers, several of which are shown in Figure 17A-3. Chrysotile is the most common asbestos.

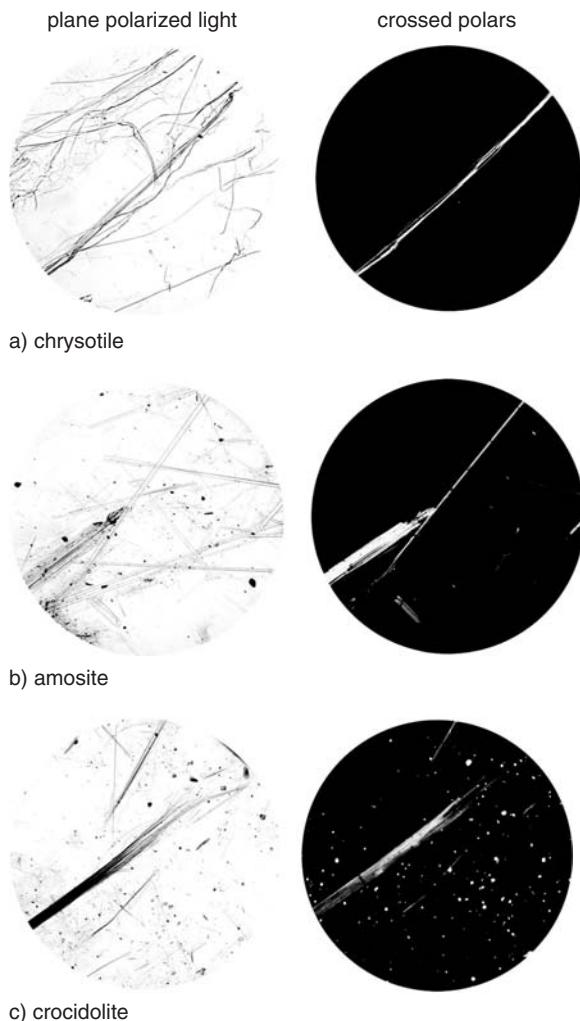


Figure 17A-3 Common asbestos fibers under plane polarized and crossed polarized light. A full-color version of this figure can be found in the color plate section of this book.

An understanding of the macroscopic and microscopic characteristics found in natural fibers, allows the forensic scientist to perform natural fiber examinations. Fiber examinations involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine the microscopic characteristics of the fiber sample.

Two of the identifying characteristics of all fibers are the refractive indices and the birefringence. With natural fibers, these are not the most distinguishing characteristics, however, they are still an important feature. The refractive index is usually determined by the Becke Line immersion method (see Experiment 3B in Chapter 3) or dispersion-staining (Experiment 2D in Chapter 2). The birefringence of fibers can also be used to aid in the identification of a fiber (Experiment 3B).

Once the birefringence is determined, the thickness of the fiber can be measured (Experiment 2B). From the thickness and birefringence, the Michel-Lévy chart (Appendix C) and tabular data such as that found in Appendix A and B can be used for identification.

Another identifying characteristic is the sign of elongation (Experiment 3C). If the refractive index for light parallel to the fiber exceeds that of the light perpendicular to the fiber, the elongation is said to be positive. If the reverse is true, the fiber is negative.

With natural fibers, many of the other microscopic characteristics are better identifiers. Characteristics such as the shape of the cells, the relative thickness of the cell wall, the cell length, size, shape, and thickness of the lumen, shape of fiber bundles, and the presence of crystals for plant fibers and various morphological features for animal fibers are good identifying characteristics. Fluorescence of optical brighteners, dyes, and contaminants such as oils, fats, adhesives, pigments, toothpaste and denture cleaners, food products, cosmetics, paints, and soil may also be observed. To look for these characteristics, a cross-section and either a semi-permanent or permanent mount are used.

Tests that may also be performed during natural fiber examinations are the twist test, Billingham test, Herzog test, burn test, or solubility test. The twist test is used to distinguish S and Z twist fibers, and relies on heating wet fibers quickly over a hotplate and watching them untwist. With this test, S twist fibers (flax, ramie) turn clockwise whereas Z twist fibers (hemp, jute, abaca, sisal) turn counterclockwise. Cotton and coir react irregularly.

The Billingham test is a chemical test using sodium hypochlorite to stain fibers. It is usually used to distinguish sisal and abaca fibers. Abaca fibers will be stained orange, whereas all other leaf fibers are stained pale yellow. This test should only be performed on undyed fibers, otherwise the results may be confusing.

Another test that can be used to determine twist is the Herzog test. This test determines addition or subtraction of retardation with a fiber oriented in the E–W direction under crossed polars. The S twist fibers (cotton, flax, and ramie) display a slight additive effect, whereas Z twist fibers (jute, hemp, sisal, and abaca) display a slight subtractive effect.

Table 17A-1 Common uses of natural fibers.

Fiber type	Common uses
Cotton	Numerous types and grades of textile products, cordage
Kapok	Stuffing for pillows, mattresses, life jackets, life belts, buoys, and other water-related safety items
Akund	Similar to kapok
Flax	Primary use in textile products, cordage, or paper making
Hemp	Twine and thin ropes, fabrics, canvas, sacking and paper making
Sunn	Cigarette papers
Jute	Coarse woven fabrics (bags, backing for carpets and rugs) twines and ropes, electrical insulation, fuses
Ramie	Textile, clothing and upholstery, cordage and twine, paper making, fish nets, fire hoses, canvas, industrial packing, filter cloths
Sisal	Agricultural twine, ropes, coarse fabrics for bags, hammocks, shoe soles
Abaca	Cordage, twine and cables, hat braids, woven stiffening materials, padding, paper making
Coir	Marine cordage, clothes, bristles for brushes, door mats, matting, mattress fiber and yarns, fish nets
Animal	Numerous types and grades of textile products, clothing, and other general uses
Mineral	Numerous grades and types of insulating products

Burn tests may be used to characterize a natural fiber. This test involves classifying the fiber according to its behavior when approaching a flame, when it enters the flame, and when it is removed. The ash and odor produced are also characteristic.

Finally, the solubility behavior of fibers in different solvents may also provide additional information when identifying natural fibers. The solubility behavior of fibers is examined using a variety of solvents that vary in polarity and acidity. Here the principle, ‘like dissolved like’ is used. The fibers containing polar groups such as oxygen dissolve more readily in polar solvents. The solubility behavior of most fibers can be found in the literature. However, the experiments are performed using known fibers and the results are compared to the unknown fiber being identified¹.

Natural fibers have many uses. Some of the more common uses are listed in Table 17A-1.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Polarizing light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) focusing ocular with micrometer scale and full wave plate

Fluorescence microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and various excitation and barrier filters

Micro kit

Microscope slides and cover slips

Mounting media

Cargille™ refractive index oils

Vegetable fibers (flax, ramie, jute, hemp, sisal, coir, kapok, cotton, akund, abaca, sunn)

Animal fibers (wool, mohair, alpaca, angora, cashmere, camel, llama, silk)

Mineral fibers (asbestos)

Unbleached cotton, unbleached cotton washed with detergent, washed with detergent and rinsed

Polyethylene or plastic sheets

Hot plate

Pipette tips

Microtome

Thread

Nail polish

Test tubes

95 % ethanol

5 % nitric acid

0.25 N sodium hypochlorite

Bunsen burner

Solvent kit (40 % sodium hydroxide, concentrated sulfuric acid, 75 % sulphuric acid, concentrated hydrochloric acid, concentrated formic acid, dimethylformamide, glacial acetic acid, acetonitrile, chloroform, hexafluoroisopropanol, concentrated nitric acid, cyclohexanone)

¹ Identification of Fibers in Textile Materials; DuPont Technical Information Bulletin X-176, December 1961.

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums and solvents used in the procedures, and use them with appropriate precautions as set by your instructor. The solvent kit contains hazardous materials that should be handled with care. It is important that gloves and eye protection be used. Additionally, proper ventilation is required. Refer to MSDS if necessary. Dispose of glass in an appropriate container.

PART I: NATURAL FIBER EXAMINATION

Procedure

A fiber worksheet is included for Experiment 17, on page 214, and is available at <http://www.wileyeurope.com/college/wheeler>.

1. Obtain the assigned fibers.
2. Examine the first sample with the stereomicroscope. Note the color, length, and longitudinal form of the fiber.
3. Set the compound or polarizing light microscope up for Köhler illumination.
4. Determine the cross-sectional shape. This can be done by making a cross-section (procedures for making cross-sections are outlined in Experiment 15B) or from a liquid mounted fiber.
5. Using the liquid mounted fiber, examine the fiber under plane-polarized light. Determine the diameter and longitudinal shape. Draw what you see. Note any other morphological or microscopic features of the fiber. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Return it to the original position.
6. Examine the fiber under crossed polars. Draw what you see.
7. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Document the extinction and birefringence properties of the fiber.
8. Repeat steps 1–6 for using a higher magnification.
9. Repeat steps 1–8 for all fibers assigned.

PART II: REFRACTIVE INDEX DETERMINATION

Procedure

1. Obtain the assigned fibers.
2. Using the polarizing light microscope, check the lighting to ensure that samples will be viewed using Köhler illumination.
3. Place the unknown fiber on a microscope slide and add a cover slip. Place a drop of Cargille™ oil on the edges of the cover slip and allow it to be drawn up under the cover slip.
4. Place the microscope slide on the stage and focus using high magnification and plane-polarized light.
5. Rotate the fiber so that the length of the fiber is parallel to the plane of polarized light. For most microscopes this would be E–W.
6. Close down the condenser to increase contrast and prevent the formation of a false Becke Line. A false Becke Line may be observed as a second bright line, which moves in the opposite

direction of the Becke Line. This is visible when the difference of the refractive index of a sample and the immersion liquid is low and the true Becke Line is faint.

7. Using the focus knob, adjust the focus while observing the edges of the fiber. When the fiber is slightly out of focus, the Becke Line will appear either inside or outside the fiber edge. The Becke Line will move towards the higher refractive index when the focal distance is increased. Document the medium that has the higher refractive index.
8. Rotate the fiber so it is oriented perpendicular to the plane of polarized light. For most microscopes the fiber would be oriented N–S.
9. Once again focus the edges to determine which medium has the higher refractive index. Document the medium that has the higher refractive index.
10. Repeat with different Cargille™ oils until you are able to determine the refractive indices for your fiber. Determine which liquid to use next, depending on your previous results.
11. Report n-parallel and n-perpendicular for the room temperature.
12. Using the temperature coefficient on the bottle of Cargille™ oils that were a match, report the refractive index at 25°C.
13. Calculate the birefringence of the fiber at 25°C.
14. Repeat steps 1–13 for all assigned fibers.
15. Produce a chart showing your findings.

PART III: OBSERVING FLUORESCENCE

Procedure

1. Obtain a section of unbleached cotton.
2. Turn on the power supply for the specialized illuminator of the fluorescence microscope. Slide the lamp diaphragm into the closed position while the lamp warms up.
3. Prepare a sample of unbleached cotton using non-fluorescent slides, cover slips, and mounting medium.
4. Turn on the power supply for the base light illuminator. Adjust the microscope to obtain Köhler illumination.
5. Using the lowest magnification, place the prepared sample on the microscope stage and bring it into focus. Lower the intensity of the base light and room lighting. Steps 3 and 4 are only necessary if the base light will be used to aid in the visualization of samples.
6. Open the lamp diaphragm on the specialized illuminator. Refocusing of the sample may be necessary.
7. Observe the sample using the various excitation and barrier filters available.
8. Prepare a sample of cotton that has been washed with detergent and rinsed and a sample of cotton that has been washed with detergent and is not rinsed.
9. Observe both samples using the various excitation and barrier filters available.
10. Compare each of the treated samples to the unbleached sample. Produce a chart showing your findings.

PART IV: TWIST TEST

Procedure

1. Obtain the assigned fibers.
2. Soak the first fiber in water.

3. Using tweezers, hold the fiber above a hot plate, with the free end faced toward you.
4. Observe the direction of twist as the fiber is warmed.
5. Repeat steps 1–4 for all fibers assigned.
6. Produce a chart showing your findings.

PART V: BILLINGHAME TEST

Procedure

1. Obtain the assigned fibers.
2. Wash the first fiber sample with 95 % ethanol to remove any oils and allow to dry.
3. Boil the sample in 5 % nitric Acid for 5–10 minutes.
4. Remove the excess acid and immerse the sample in cold 0.25 N sodium hypochlorite solution for 10 minutes.
5. Remove the sample and allow to dry. Note the color of the fiber.
6. Repeat steps 1–5 for all fibers assigned.
7. Produce a chart showing your findings.

PART VI: HERZOG TEST

Procedure

1. Obtain the assigned fibers.
2. Mount the first fiber in a Cargille™ oil.
3. Setup Köhler illumination on a polarizing light microscope.
4. Rotate the stage so the mounted fiber is in an E–W orientation under crossed polars.
5. Insert the first order red compensator.
6. Observe the retardation color change.
7. Repeat steps 1–6 for all fibers assigned.
8. Produce a chart showing your findings.

PART VII: BURN TEST

Procedure

1. Obtain the assigned fibers.
2. Beginning with the first sample, hold the fiber using tweezers. Note the behavior of the fiber as it slowly approaches the flame.
3. Note the behavior of the fiber as it enters the flame.
4. Note the color of the flame.
5. Note the behavior of the fiber after removal of the flame.
6. Note the type of ash.
7. Note the odor produced.
8. Repeat steps 1–7 for all fibers assigned.
9. Produce a chart showing your findings.

PART VIII: SOLUBILITY TEST

Procedure

Additional Safety Notice:

The solvent kits contain organic solvents, concentrated acids, and bases and oxidizers. All solvents must be used with proper ventilation. Precautions should be taken to avoid inhalation and contact with the skin and eyes. Gloves and eye protection must be worn when handling these reagents.

1. Obtain the assigned fibers.
2. Place a single fiber on a microscope and add a small cover slip.
3. Place the microscope slide on the microscope stage.
4. Using a capillary tube, draw up a small amount of the first solvent from the solvent kit. Allow the solvent to be drawn under the cover slip.
5. Observe any changes to the fiber. Document your findings by notes and/or drawings.
6. Repeat with another fiber from the same source using a second solvent. Repeat until the fiber sample has been tested with all solvents.
7. Repeat steps 1–6 for all assigned fibers.
8. Produce a chart showing your findings.

PART IX: UNKNOWN NATURAL FIBER

Procedure

1. Obtain an unknown fiber.
2. Using any or all of the procedures above, examine the unknown fiber to determine its identity.
3. Describe your examination procedure for the unknown sample.
4. Include the unknown fiber number and what you identified the fiber to be. State your reasons to defend your conclusion.

REPORT REQUIREMENTS

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

REPORT QUESTIONS

1. What are the main divisions for natural fibers? Pick a fiber from each division and describe the microscopic characteristics that would be used to identify it.
2. What are the differences between seed, stem, and leaf plant fibers? Pick a fiber from each category and identify its source.

3. Describe each of the following terms as it relates to natural fibers: lumen, fiber bundles, cuticle scales, isotropic. Include drawings and/or examples with your description for each term.
4. How does a cross-section assist in determining the identity of a natural fiber?
5. How does refractive index assist in determining the identity of a natural fiber?
6. How does fluorescence assist in fiber examinations?

Experiment 17B: Man-Made Fiber Examinations

Recommended pre-lab reading assignments:

- Textile Institute. *Identification of Textile Materials*. 7 ed. Manchester, England: The Textile Institute; 1975; 95–133.
- Eyring MB, Gaudette BD. An Introduction to the Forensic Aspects of Textile Fiber Examination. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 245–81.
- Palenik SJ. Microscopical Examination of Fibres. In: Robertson J, Grieve M, eds. *Forensic Examination of Fibres*. London: Taylor and Francis; 1999; 153–76.

OBJECTIVE

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

1. general characteristics of man-made fibers
2. use of the stereomicroscope to identify and visualize the characteristics of man-made fibers
3. use of the polarizing light microscope to identify and visualize the characteristics of man-made fibers
4. determination of the cross-sectional shape of man-made fibers
5. measurement of the refractive indices of man-made fibers
6. determination of the sign of elongation
7. use of the fluorescence microscope to visualize characteristics of man-made fibers
8. use of complementary testing such as burn and solubility tests

INTRODUCTION

The Locard Exchange Principle states that whenever two objects come in contact, an exchange of matter occurs. This theory applies to fiber evidence. Fibers are easily shed and transferred whenever there is contact between two objects, two individuals, or an individual and another object. Therefore, fiber examinations are often used to associate people with items or scenes. This makes fibers an important source of information in forensic investigations.

Various characteristics are used by the forensic scientist to identify fibers. Man-made fibers are created from raw materials, either cellulose (natural) or chemical based, and possess various macroscopic and microscopic characteristics, which make identifications and further association with a particular source sometimes possible. Because of this fact, man-made fibers are routinely examined in forensic casework. Common man-made fibers are discussed below.

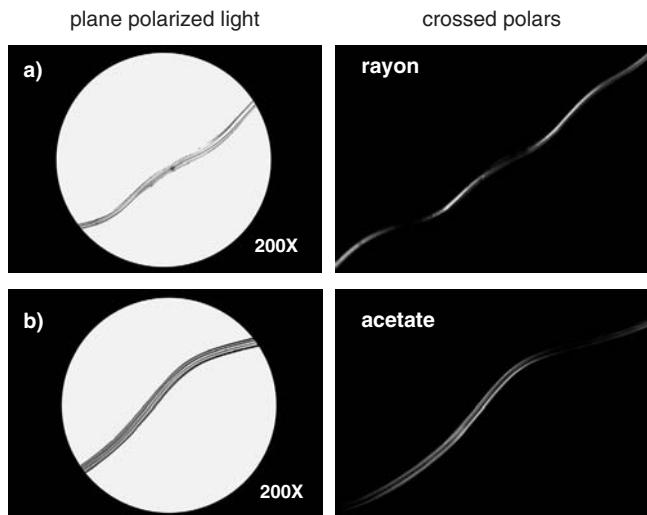


Figure 17B-1 Common cellulose-based polymer fibers under plane polarized and crossed polarized light. A full-color version of this figure can be found in the color plate section of this book.

Cellulose-based Polymers²

- **Acetate:** Acetate is a manufactured fiber in which the fiber-forming substance is cellulose acetate (see Figure 17B-1b).
- **Triacetate:** The production of triacetate is similar to acetate only differing in that the initial reaction product is hydrolyzed to a product in which not less than 92 % of the hydroxyl groups are esterified.
- **Rayon:** Rayon is a manufactured fiber composed of regenerated cellulose, as well as manufactured fiber composed of regenerated cellulose in which substituents have replaced not more than 15 % of the hydrogens in the hydroxyl groups (see Figure 17B-1a).
- **Lyocell:** The production of lyocell is similar to rayon differing only in that the fiber is composed of cellulose precipitated from an organic solution, and in which no substitution of the hydroxyl groups takes place and no chemical intermediates are formed.
- **Regenerated protein:**
 - **Azlon:** Azlon is a manufactured fiber in which the fiber-forming substance is composed of any regenerated, naturally occurring protein.

Chemical-based (Synthetic) Polymers³

- **Polyamide fibers:**
 - **Nylon:** Nylon is a manufactured fiber in which the fiber forming substance is a long chain synthetic polyamide with less than 85 % of the amide linkages being attached directly to the two aromatic rings (see Figure 17B-2b).

² Federal Trade Commission, Textile Products Identification Act, 1954.

³ Federal Trade Commission, Textile Products Identification Act, 1954.

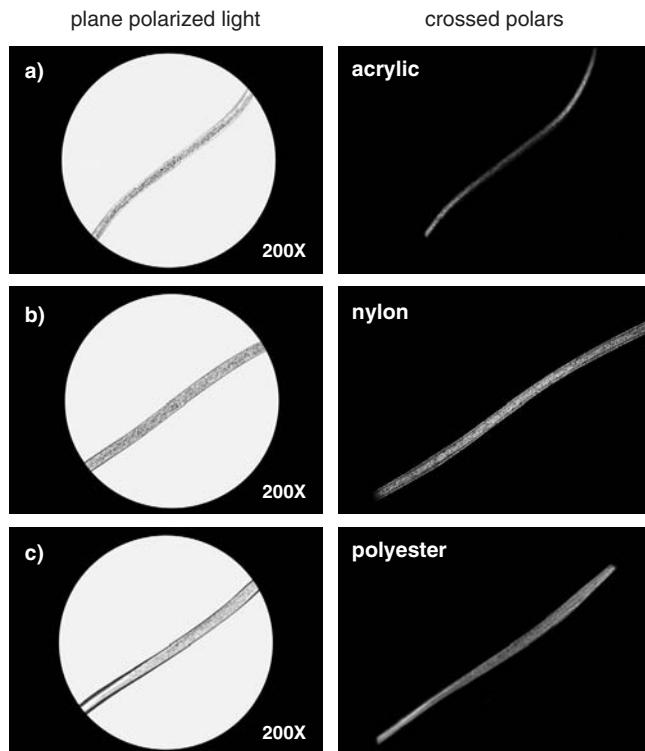


Figure 17B-2 Common chemical-based polymer fibers under plane polarized and crossed polarized light. A full-color version of this figure can be found in the color plate section of this book.

- **Nylon-6,6:** Nylon-6,6 is designed using adipic acid and diamine from which a polymer is synthesized containing 6 carbons. Coal tar intermediates or other derivatives are used as the starting materials.
- **Nylon-6:** Nylon-6 is produced through the use of caprolactam, the internal cyclic amide of aminocaproic acid derived petrochemically. Caprolactam is polymerized by ring opening, followed by repetitive addition, until the desired molecular weight is achieved.
- **Qiana:** Qiana, a nylon fiber, is a condensation polymer containing alicyclic rings.
- **Aramid:** Aramid is a manufactured fiber in which the fiber-forming substance is a long chain synthetic polyamide with at least 85 % of the amide linkages being attached directly to two aromatic rings.
- **Polyester:** Polyester is a manufactured fiber in which the fiber-forming substance is a long chain synthetic polymer composed of at least 85 % by weight of an ester of a substituted aromatic carboxylic acid, including, but not restricted, to substituted terephthalate units and para substituted hydroxyl-benzoate units (see Figure 17B-2c).
- **Acrylic:** Acrylic is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 85 % by weight of acrylonitrile units (see Figure 17B-2a).
- **Modacrylic:** Modacrylic fibers are also based on acrylonitrile; however, lower unit content is used (less than 85 % but at least 35 % by weight of acrylonitrile units).
- **Vynylon:** Vynylon is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 85 % by weight of vinyl chloride units.

- **Saran:** Saran is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 80 % by weight of vinylidene chloride units.
- **Vinal:** Vinal is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 50 % by weight of vinyl alcohol units, and in which the total of the vinyl alcohol units and any one or more of the various acetal units is at least 85 % by weight of the fiber.
- **Spandex:** Spandex is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 85 % segmented polyurethane.
- **Olefin:** Olefin is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 85 % by weight of ethylene, propylene, or other olefin units except amorphous polyolefins qualifying under rubber or vinal.
- **Fluorocarbon:** Fluorocarbon fibers are manufactured containing at least 95 % of a long chain polymer synthesized from aliphatic fluorocarbon monomers.
- **Nytril:** Nytril is a manufactured fiber in which the fiber-forming substance is a long chain polymer of vinylidene dinitrile, with the vinylidene content being no less than every other unit in the polymer chain.
- **Novoloid:** Novoloid is a manufactured fiber in which the fiber contains at least 85 % by weight of a cross-linked nonolac.
- **Anidex:** Anidex is a manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of at least 50 % by weight of one or more esters of a monohydric alcohol and acrylic acid.
- **Metallic:** Metallic fibers are manufactured fibers composed of metal, plastic-coated metal, metal-coated plastic, or a core completely covered by metal.
- **Sulfar:** Sulfar is a manufactured fiber in which the fiber-forming substance is a long chain synthetic polysulfide with at least 85 % of the sulfide linkages being attached directly to two aromatic rings.
- **PBI:** PBI is a manufactured fiber in which the fiber-forming substance is a long chain aromatic polymer containing reoccurring imidazole groups as an integral part of the polymer chain.
- **Elastoester:** Elastoester fibers are manufactured fibers in which the fiber-forming substance is a long chain synthetic polymer composed of at least 50 % by weight of an aliphatic polyether, and at least 35 % by weight of polyester.
- **Melamine:** Melamine fibers are manufactured fibers in which the fiber-forming substance is a synthetic polymer composed of at least 50 % by weight of a cross-linked melamine polymer.
- **Glass:** Glass fibers are a manufactured fiber in which the fiber-forming substance is glass.

With the wide variety of manufactured fibers available, numerous uses for these fibers have also been employed. See further scientific literature for more details on the microscopic characteristics and uses of the various manufactured fibers.

Although size and shape are used in natural fiber identification, the man-made fiber manufacturing process lends itself to an endless variety of size and shape possibilities for most man-made fibers. However, this manufacturing process also produces characteristics that remain constant and uniform. Therefore, the appearance, or lack of, the following characteristics are noted and used for identification during a microscopic examination of manufactured fibers: color, luster, thickness, inclusions, cross-section, variations in characteristics within the fiber, refractive index, birefringence, pleochroism, and sign of elongation. Fluorescence of optical brighteners, dyes, and contaminants such as oils, fats, adhesives, pigments, toothpaste and denture cleaners, food products, cosmetics, paints, and soil may also be observed.

Two of the identifying characteristics of all fibers are their refractive indices and birefringence. Appendix B contains a table of refractive indices and birefringence for many common

man-made fibers. With man-made fibers, these properties can be used to distinguish between fibers and manufacturers. The refractive index of a fiber is a measurement of how much light is slowed as it passes through the fiber. For fibers, there are two refractive indices: parallel and perpendicular. The refractive indices are determined by examining the fiber with orientation parallel and perpendicular to the plane of polarization. The Becke Line method is also used to determine the refractive index of fibers. The Becke Line is a bright halo appearing at the boundary of a particle. This halo will move as the focus is adjusted up and down. As the working distance of a microscope is increased, the Becke Line travels towards the medium of higher refractive index. The halo moves towards the lower refractive index medium as the focal length is decreased. The birefringence of fibers can also be used to aid in the identification of a fiber. Once the birefringence is determined, the thickness of the fiber can be measured. Using these two characteristics and the Michel-Lévy birefringence chart, the fiber identification can be determined.

Another identifying characteristic is the sign of elongation. If the refractive index for light parallel to the fiber exceeds that of the light perpendicular to the fiber, the elongation is said to be positive. If the reverse is true, the fiber is negative. Sign of elongation can be determined by two methods: a) when using immersion oils, the sign can be determined by mathematical subtraction – $n_{\text{parallel}} - n_{\text{perpendicular}}$; or b) by inserting a compensator into the optical path. When inserting a compensator, the retardation will either be ‘added’ or ‘subtracted.’ This can be determined by using the Michel-Lévy birefringence chart as a reference.

Burn tests may also be used to characterize a man-made fiber. This test involves classifying the fiber according to its behavior when approaching a flame, when it enters, and when it is removed. The ash and odor produced are also characteristic. Solubility tests may also provide additional information when identifying man-made fibers. The solubility behavior of fibers is examined using a variety of solvents that vary in polarity and acidity. Here the principle ‘like dissolved like’ is used. The fibers containing polar groups such as oxygen will dissolve more readily in polar solvents. The solubility behavior of most fibers can be found in the literature⁴. However, the experiments are performed using known fibers and the results are compared to the unknown fiber being identified.

An understanding of the macroscopic and microscopic characteristics found in man-made fibers allows the forensic scientist to perform man-made fiber examinations. Fiber examinations involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine optical properties such as refractive index, birefringence, and sign of elongation. Fluorescence may also be determined. Fluorescence of optical brighteners, dyes, and contaminants such as oils, fats, adhesives, pigments, toothpaste and denture cleaners, food products, cosmetics, paints, and soil may also be observed. To do this, a cross-section and either a semi-permanent or permanent mount are used. At times, burn or solubility tests may also be performed during man-made fiber examinations. Further testing can be performed using a variety of techniques and instruments to identify additional characteristics. Fourier Transform Infrared Microspectrophotometry (Experiment 22), UV-VIS-NIR Microspectrophotometry (Experiment 23), Scanning Electron Microscopy (Experiment 25), and Pyrolysis Gas Chromatography are all complementary techniques commonly performed on fibers.

⁴ Identification of Fibers in Textile Materials: DuPont Technical Information Bulletin, December 1961.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Polarizing light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) focusing ocular with micrometer scale and 530-nm gypsum plate

Fluorescence microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and various excitation and barrier filters

Micro kit

Microscope slides and cover slips

Mounting media

Cargille™ refractive index oils

Man-made fibers (acetate, acrylic, aramid, azlon, glass, fluorocarbon, lyocell, modacrylic, novoloid, nylon, nitril, olefin, polyester, rayon, saran, spandex, sulfar, triacetate, vinal, vinylon)

Man-made fabrics that have been stained with a variety of fluorescent contaminants (e.g., oil, fats, toothpaste, creamer, paint)

Polyethylene or plastic sheets

Hot plate

Pipette tips

Microtome

Thread

Nail polish

Bunsen burner

Solvent kit (40 % sodium hydroxide, concentrated sulfuric acid, 75 % sulphuric acid, concentrated hydrochloric acid, concentrated formic acid, dimethylformamide, glacial acetic acid, acetonitrile, chloroform, hexafluoroisopropanol, concentrated nitric acid, cyclohexanone)

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums and solvents used in the procedures, and use them with appropriate precautions as set by your instructor. The solvent kit contains hazardous materials that should be handled with care. It is important that gloves and eye protection be used. Additionally, proper ventilation is required. Refer to MSDS if necessary. Dispose of glass in an appropriate container.

PART I: MAN-MADE FIBER EXAMINATION

Procedure

A fiber worksheet is included for Experiment 17, on page 214 and is available at <http://www.wileyeurope.com/college/wheeler>.

1. Obtain the assigned fibers.
2. Examine the first sample with the stereomicroscope. Note the color, length, and longitudinal form (Figure 17-2) of the fiber.

3. Set the compound or polarizing light microscope up for Köhler illumination.
4. Determine the cross-sectional shape. This can be done by making a cross-section (procedures for making cross-sections are outlined in Experiment 15B in Chapter 15) and comparing to Figure 17-2 or from a liquid mounted fiber described in the next step.
5. Using the liquid mounted fiber, examine the fiber under non-polarized light. Determine the diameter and longitudinal shape using Figure 17-2. Draw what you see. Note any other morphological or microscopic features of the fiber. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Return it to the original position.
6. Examine the fiber under crossed polars. Draw what you see.
7. Rotate the fiber 90°, making note of any changes to the fiber as you rotate it. Document the extinction and birefringence characteristics of the fiber.
8. Repeat steps 1–6 for using a higher magnification.
9. Repeat steps 1–8 for all fibers assigned.

PART II: REFRACTIVE INDEX DETERMINATION

Procedure

1. Obtain the assigned fibers.
2. Using the polarizing light microscope, check the lighting to ensure that samples will be viewed using Köhler illumination.
3. Place the unknown fiber on a microscope slide and add a cover slip. Place a drop of Cargille™ oil on the edges of the cover slip and allow it to be drawn up under the cover slip.
4. Place the microscope slide on the stage and focus using high magnification and plane-polarized light.
5. Rotate the fiber so that the length of the fiber is parallel to the plane of polarized light. For most microscopes this would be E–W.
6. Close down the condenser to increase contrast and prevent the formation of a false Becke Line. A false Becke Line may be observed as a second bright line, which moves, in the opposite direction of the Becke Line. This is visible when the difference of the refractive index of a sample and the immersion liquid is low and the true Becke Line is faint.
7. Using the focus knob, adjust the focus while observing the edges of the fiber. When the fiber is slightly out of focus, the Becke Line will appear either inside or outside the fiber edge. The Becke Line will move towards the higher refractive index when the focal distance is increased. Document the medium that has the higher refractive index.
8. Rotate the fiber so it is oriented perpendicular to the plane of polarized light. For most microscopes the fiber would be oriented N–S.
9. Once again focus the edges to determine which medium has the higher refractive index. Document the medium that has the higher refractive index.
10. Repeat with different Cargille™ oils until you are able to determine the refractive indices for your fiber. Determine which liquid to use next, depending on your previous results.
11. Report n_{parallel} and $n_{\text{perpendicular}}$ for the room temperature.
12. Using the temperature coefficient on the bottle of Cargille™ oils that were a match, report the refractive index at 25°C.
13. Calculate the birefringence of the fiber at 25°C.
14. Repeat steps 1–13 for all assigned fibers.
15. Produce a chart showing your findings.

PART III: SIGN OF ELONGATION

Procedure

Reminder:

A fiber having its slow component (high refractive index) vibration parallel to its length is said to have a positive sign of elongation. Addition of retardation results in a + sign of elongation. Subtraction of retardation results in a negative sign of elongation.

1. Using the polarizing light microscope, check the lighting to ensure that samples will be viewed using Köhler illumination.
2. Obtain a prepared slide of Dynel (modacrylic).
3. Observe the Dynel fiber under cross polars. Rotate this fiber into an upper left, lower right position and draw.
4. Insert the first order red compensator and draw the colors.
5. Rotate the stage 90° and redraw the colors.
6. Consult the Michel-Lévy interference color chart and determine if addition or subtraction of interference colors is occurring.
7. Prepare a slide with acrylic fibers.
8. Observe the fiber under crossed polars. Rotate the fiber into the upper left, lower right position and draw.
9. Insert the first order red compensator plate and draw the colors.
10. Rotate the stage 90° and redraw the colors.
11. Consult the Michel-Lévy interference color chart and determine if addition or subtraction of interference colors is occurring.
12. Report your findings including your handwritten notes, explaining how you reached your conclusion.
13. Repeat this test for your assigned fibers.
14. Produce a chart showing your findings.

PART IV: OBSERVING FLUORESCENCE

Procedure

1. Obtain a section of stained fabric.
2. Turn on the power supply for the specialized illuminator of the fluorescence microscope. Slide lamp diaphragm into the closed position while the lamp warms up.
3. Prepare a sample from the unstained portion of the fabric using non-fluorescent slides, cover slips, and mounting medium.
4. Turn on the power supply for the base light illuminator. Adjust the microscope to obtain Köhler illumination.
5. Using the lowest magnification, place the prepared sample on the microscope stage and bring it into focus. Lower the intensity of the base light and room lighting. Steps 3 and 4 are only necessary if the base light will be used to aid in the visualization of samples.
6. Open the lamp diaphragm on the specialized illuminator. Refocusing of the sample may be necessary.

7. Observe the sample using the various excitation and barrier filters available.
8. Prepare a sample from the stained portion of the fabric.
9. Observe this sample using the various excitation and barrier filters available.
10. Compare your results for the unstained and stained samples.
11. Repeat steps 1–10 for all assigned fibers.
12. Produce a chart showing your findings.

PART V: BURN TEST

Procedure

1. Obtain the assigned fibers.
2. Beginning with the first sample, hold the fiber using tweezers. Note the behavior of the fiber as it slowly approaches the flame.
3. Note the behavior of the fiber as it enters the flame.
4. Note the color of the flame.
5. Note the behavior of the fiber after removal of the flame.
6. Note the type of ash.
7. Note the odor produced.
8. Repeat steps 1–7 for all fibers assigned.
9. Produce a chart showing your findings.

PART VI: SOLUBILITY TEST

Procedure

Additional Safety Note:

The solvent kits contain organic solvents, concentrated acids, and bases and oxidizers. All solvents must be used with proper ventilation. Precautions should be taken to avoid inhalation and contact with the skin and eyes. Gloves and eye protection must be worn when handling these reagents.

1. Obtain the assigned fibers.
2. Place a single fiber on a microscope and add a small cover slip.
3. Place the microscope slide on the microscope stage.
4. Using a capillary tube, draw up a small amount of the first solvent from the solvent kit. Allow the solvent to be drawn under the cover slip.
5. Observe any changes to the fiber. Document your findings by notes and/or drawings.
6. Repeat with another fiber from the same source using a second solvent. Repeat until the fiber sample has been tested with all fiber solvents.
7. Repeat steps 1–6 for all assigned fibers.
8. Produce a chart showing your findings.

PART VII: UNKNOWN MAN-MADE FIBER

Procedure

1. Obtain an unknown fiber.
2. Using any or all of the procedures above, examine the unknown fiber to determine its identity.
3. Describe your examination procedure for the unknown sample.
4. Include the unknown fiber number and what you identified the fiber to be. State your reasons to defend your conclusion.

REPORT REQUIREMENTS

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

REPORT QUESTIONS

1. What are the main divisions for man-made fibers? Pick a fiber from each division and describe the microscopic characteristics that would be used to identify it.
2. What is refractive index? How can it be determined?
3. What is sign of elongation? How can it be determined?
4. Describe each of the following terms as it relates to man-made fibers: n-parallel, n-perpendicular, birefringence. Include examples with your description for each term.
5. How does a cross-section assist in determining the identity of a man-made fiber?
6. How does refractive index assist in determining the identity of a man-made fiber?
7. How does the sign of elongation assist in determining the identity of a man-made fiber?
8. How does fluorescence assist in fiber examinations?
9. What are the advantages and disadvantages of using refractive index and birefringence for identification of fibers?
10. Explain the logic behind the burn test and solubility test. Were these useful in determining the identification of your unknown fiber?

Experiment 17C: Fiber Comparisons

Recommended pre-lab reading assignments:

Eyring MB, Gaudette BD. An Introduction to the Forensic Aspects of Textile Fiber Examination. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 280–4.

OBJECTIVE

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

1. techniques used for comparison of fibers

INTRODUCTION

Fiber comparisons are performed to determine possible associations between people, places, and objects. This association is accomplished through the comparison of macroscopic and microscopic characteristics. Learning to recognize the characteristics and the range found within a sample allows the forensic scientist to identify and compare fibers. Careful examination of known and unknown samples can provide information as to similarities or dissimilarities for an investigation.

Generally, unknown fibers are examined first. Macroscopic and microscopic characteristics are determined so that the fiber can be identified. Comparisons to known samples are then performed. Like hair comparisons, a suitable known fiber sample is extremely important when doing comparison examinations. There is such a variety of macroscopic and microscopic characteristics among fibers that known samples should be collected from each possible fiber source. So, if the unknowns to be compared are both blue cotton and red polyester, a known sample should be collected from all possible blue cotton and red polyester sources. It is also necessary to have a known sample that contains the range of characteristics found within a particular source. For instance, if the known source is a pair of blue jeans, samples should be taken from all sections of the fabric making up the jeans: right leg front, right leg back, left leg front, left leg back, pockets. This should provide samples containing the range of characteristics that might be found from that source. Usually fabric sections for a garment are cut from the same piece of cloth; however, this may not always be the case. Minor dye differences between sections may not be visually identifiable, but under the microscope small differences could be detected preventing an association if suitable knowns had not been collected. Because of this fact, it is usually easier for the analyst to collect his or her own knowns. When handled this way, if more than one section needs to be tested, it is possible for the analyst to obtain another sample, but at the same time if the sample shows similarities, duplicate samples would not have to be received and tested.

An understanding of the macroscopic and microscopic characteristics found in fibers allows the forensic scientist to perform fiber comparisons. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. Further examination is performed using a compound or comparison light microscope and a fluorescence microscope to determine the microscopic



Figure 17C-1 A comparison polarized light microscope for performing fiber comparisons. The optical bridge shown here connects to Leica DM EPT™ polarizing microscopes. (Reproduced with permission of Leica Microsystems, Inc.)

characteristics of the fiber sample. To do this, a cross-section is prepared, and either a semi-permanent or permanent mount is used. Known fiber samples are identified first. Unknown samples are then examined to determine their identity.

If there is the possibility of a match, comparisons are then performed. This is usually performed on a comparison compound, polarizing light (see Figure 17C-1), and/or fluorescence microscope. These comparison microscopes are composed of two similar microscopes, which are connected by an optical bridge. The bridge contains prisms and mirrors that are used to direct the light to a common set of oculars. A set of knobs is used to adjust the field of view, so that items can be viewed from either microscope independently or combined. In the combined view the field of view is split into side-by-side views of portions from each microscope. This allows the examiner to view two items side-by-side on a microscopic scale. These comparison microscopes usually use at least one translational stage instead of a rotating stage, allowing precise alignment of two fibers.

Comparison compound microscopes may be used for comparison of many of the fiber characteristics, however a comparison polarized light microscope should also be used to view some optical properties that are found in fibers. Refractive index, birefringence, sign of elongation, and pleochroism (Experiments 3A, 3B, 3C in Chapter 3) may be compared using the comparison polarized light microscope. It is also necessary to use the comparison fluorescence microscope when determinations are made concerning the similarity of fiber dyes, whiteners, and brighteners. If all the characteristics displayed by the unknown fiber are found within the known sample, an association may be reached. However, when an association is reached as to a possible origin of the fiber, it should be pointed out that a similarity to a known sample does not exclude the possibility of another similar source.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Comparison compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Comparison polarized light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Comparison fluorescence microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and various excitation and barrier filters

Comparison light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) and focusing ocular with micrometer scale

Micro kit

Microscope slides and cover slips

Mounting media

Cargille™ refractive index oils

Polyethylene or plastic sheets

Hot plate

Pipette tips

Microtome

Thread

Nail polish

Test tubes

95 % ethanol

5 % nitric acid

0.25 N sodium hypochlorite

Bunsen burner

Solvent kit (40 % sodium hydroxide, concentrated sulfuric acid, 75% sulphuric acid, concentrated hydrochloric acid, concentrated formic acid, dimethylformamide, glacial acetic acid, acetonitrile, chloroform, hexafluoroisopropanol, concentrated nitric acid, cyclohexanone)

Known fiber samples

Unknown fiber samples

SAFETY

Additional Safety Note:

The solvent kit contains organic solvents, concentrated acids, and bases and oxidizers. The organic solvents must be used with proper ventilation. Precautions should be taken to avoid inhalation and contact with the skin and eyes. Gloves and eye protection must be worn when handling these reagents.

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the mounting mediums and solvents used in the procedures, and use them with appropriate precautions as set by your instructor. The solvent kit contains hazardous materials that should be handled with care. It is important that gloves and eye protection be used. Additionally, proper ventilation is required. Refer to MSDS if necessary. Dispose of glass in an appropriate container.

PART I: FIBER COMPARISONS

Procedure

A fiber worksheet is included for Experiment 17, on page 214.

1. Obtain both a known and an unknown fiber sample.
2. Examine the known sample with the stereomicroscope.
3. If the fiber can be determined to be natural, proceed with testing as outlined in Experiment 17A. If the fiber is determined to be man-made, proceed with testing as outlined in Experiment 17B.
4. Repeat step 3 with the unknown fiber sample.
5. Compare the macroscopic and microscopic characteristics that you have documented for the unknown sample and the known sample.
6. If possible, use the various comparison microscopes to examine both samples simultaneously. If you believe that the samples are similar, find areas in the known sample that have similar characteristics to that in the unknown fiber. Draw what you see.
7. Make sure your documentation includes the appropriate labeling (name, sample numbers, lab).
8. Include the unknown fiber number and state the result of your fiber comparison. State your reasons to defend your conclusion.

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. Why is it possible to perform fiber comparisons?
2. How is a fiber comparison conducted?
3. Can a fiber be associated with only one source? Explain your answer.
4. Is it possible for an unknown fiber from a murder scene to be similar to a known sample, but for that source to have no involvement in the crime? Explain your answer.
5. Is it possible for an unknown hair from a murder scene to be dissimilar to a known sample, but for that source to be involved in the crime? Explain your answer.

RECOMMENDED AND FURTHER READING

- ASTM. *Standard Test Materials for Identification of Fibers in Textiles*. Philadelphia, PA: ASTM; 1996; 276–89.
- Carroll GR, Demers J. Technical Note: A New Method for Determining the Refractive Indices and Birefringence of Textile Fibers. *Canadian Society of Forensic Science Journal*. 1993; 26: 15–17.
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- Paulsson N, Stocklassa B. A Real-Time Color Image Processing System for Forensic Fiber Investigations. *Forensic Science International*. 1999; 103(1): 37–59.
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Soil Examination

Experiment 18: Soil Examinations

Recommended pre-lab reading assignments:

- Murray RC, Solebello LP. Forensic Examination of Soil. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 615–25.
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- McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978; 125–168.

OBJECTIVE

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

1. general characteristics of soil
2. color of soil
3. particle size distributions of soil
4. density of soil

INTRODUCTION

Soil is a common form of physical evidence found at most crime scenes. Soil may be picked up by a vehicle, thus providing a valuable link between the car and a scene. Similarly, soil found adhering to clothing or shoes may provide an association that can link a suspect to a particular crime scene. Since there is the possibility of soil associations, soil examinations have become a valuable portion of investigations. There are two types of soil examinations encountered in the forensic laboratory. The majority of soil examinations involve comparisons between soil samples collected from known locations and unknown soil samples. However, some soil examinations may

be performed to identify and compare additives or contaminants to the soil samples themselves. This evidence is generally used in conjunction with other forensic evidence.

Soil is a mixture of decaying vegetable matter and rock that has weathered and decayed by erosion, oxidation, hydration, and the actions of acids such as carbonic on the rock. As it decays, water, wind, gravity, plants, and animals move it, causing a mixture of the minerals present. Through this constant mixing, soils form in a variety of manners and different locations, yielding characteristics that allow an examiner to look for unique properties in a soil identification and comparison.

Common soil mineral constituents are clay, silica, calcite, numerous silicoaluminates, and smaller quantities of mica, hornblends, and iron oxides. A variety of quartz and feldspars, halite, garnet, tourmaline, rutile, epidote, pyrite, pumice, obsidian, augite, gypsum, talc, limonite, serpentine, magnetite, dolomite, and hematite may also be found in soil samples. In addition to the mineral constituents, the organic and miscellaneous constituents (e.g., coal, cinders, diatoms, pollen, seeds, peat) also contribute to the variety of characteristics found in soils.

Since soil is a complex mixture, the examination is a detailed procedure, using several techniques. The method for collecting appropriate soil samples relies on the reliability of the source. Soil samples can vary greatly within short geographical distances. They can change vertically as well as horizontally. Therefore, soil samples should be taken from the area and to the depth that is believed to be the actual source. It is essential that further samples be taken near the source, so that the variability of the soil may be established. Each should be packaged separately and in a container to allow drying. A handful is usually an appropriate size sample.

Once the size and collection of a soil sample has been determined to be sufficient, analysis can begin. Soil color is one of the most obvious features of a soil sample. However, soil color is very dependent on soil moisture. Since unknown soil samples and soil samples collected from scenes may vary in moisture content, samples must be dried before color comparisons can be performed. The color of the soil is compared to a set of standard colors, usually the Munsell™ soil color chart. In this classification system, colors are expressed on a numerical basis in terms of their hue, value, and chroma. Hue is the base color of the soil. The system uses five primary hues (blue, green, purple, red, yellow) and five intermediate hues for a total of ten hue names. The intermediate hues are green-yellow (GY), yellow-red (YR), blue-green (BG), red-purple (RP), and purple-blue (PB). Each of these ten hues is divided into colors that are equally spaced. These are given numerical values before the hue name. For example, 10Y marks a limit of yellow hue. Four equally spaced steps of the adjacent yellow-red (YR) hue are identified as 2.5YR, 5YR, 7.5YR, and 10YR respectively. The standard chart for soil has separate hue cards from 10R through 5Y. Value is a measure of the lightness or darkness of this color. Neutral gray is used to show levels of light and dark on an achromatic scale, with values extending from pure black (0/) to pure white (10/). The value notation is a measure of the amount of light that reaches the eye under day light conditions. Gray is perceived as about halfway between black and white and has a value notation of 5/.

Chroma is the degree of saturation of the color or the strength of the color. For soils, the scales of chroma extend from /0 for neutral colors to a chroma of /8 as the strongest saturation. Figure 18-1 shows how the color chips are arranged in the Munsell™ color book for soils. Chroma increases horizontally from left to right on the color card, and increases as the color saturation reaches pure color hues (this is the furthest from black). Value increases vertically from bottom to top. Since value is a measure of how much light reaches the eye, the value increases as it gets further from black (total absence of light). Value also increases as each card corresponds to a different hue. For example, a dark reddish brown soil would have Munsell™ color notation of 5YR 3/4 where YR is the hue corresponding to the card containing the matching color, 3 is

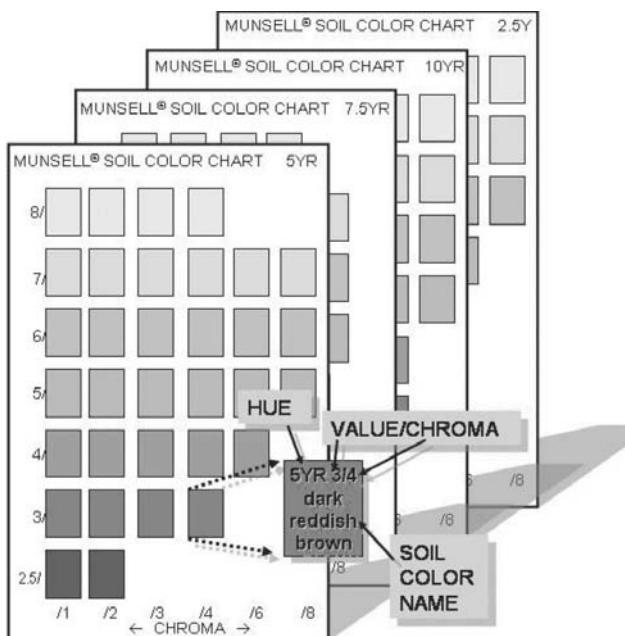


Figure 18-1 Arrangement of color in soil in the Munsell Color Book™. (Reproduced with permission of Weil RR. *Laboratory Manual for Introductory Soils*. 7 ed. Dubuque, IA. Kendall/Hunt Publishing Company, 2005.)

the value corresponding to the matching row, and 4 is the chroma corresponding to the matching column. The Munsell™ system allows analysts to speak the same color language and normalizes color descriptions. Munsell is not the only color system used in forensic science, but it is the most common system used for soils.

Next, a stereomicroscopic examination of the soil sample is performed. Any contaminants, such as paint chips, brick, glass, or cement, are noted. For some contaminants, further analysis and comparison may be possible. The dried soil samples are then sieved into sections and the colors compared again. The soil samples are passed through a set of wire sieves with the size of the openings decreasing from top to bottom. Sieve sizes should range from 40 to 280 mesh. After sieving, the relative amount of each particle size is determined. The particle size distribution in soil can be used to distinguish and compare samples and to determine its texture class. The three primary texture classes are sand (diameters between 0.05 and 2.0 mm), silt (diameters between 0.002 and 0.05 mm), and clay (diameters less than 0.002 mm). Variations in the particle sizes are distinctive and can be used to distinguish different samples. Because some of these particles are smaller than the smallest sieve they can only be determined using the so-called ‘settling methods.’ These methods are based on the idea that larger particles fall more quickly through water than smaller ones. Equal volume aliquots of the soil suspension are taken from the same depth in a measuring vessel at different time intervals and allowed to evaporate. In both the sieving and settling methods, the dried particles from each fraction are weighed and the weight of each is plotted in a graph as a function of sampling interval for comparison purposes.

Usually the 40 and 60 mesh fractions are used for density comparisons and identification of mineral content. Density comparisons are usually performed through the use of density gradient

tubes. Each gradient tube is composed of numerous layers starting with an undiluted heavy density liquid, followed by various dilutions on the heavy density liquid and water to provide a range of densities with the final layer being unmixed water. For instance, if using Poly-Gee™, which is a saturated solution of sodium polytungstate and water, ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10 might be used. The density of the liquid for each layer may be determined by using the following equation:

$$D_T = \frac{D_{PG,T}V_{PG} + D_{DW,T}V_{DW}}{V_{PG} + V_{DW}} \quad (18-1)$$

where $D_{PG,T}$ is the density of Poly-Gee™ in g/mL at temperature T, V_{PG} is the volume of Poly-Gee™ in mL, $D_{DW,T}$ is density of distilled water at temperature T, and V_{DW} is the volume of distilled water. Since density is temperature dependent it is important to record density at the specific temperature, T. (*Note:* Temperature does not have to be as carefully controlled when comparisons are made using the density gradient method. However, if it is necessary to report a numerical value for density the temperature should be kept constant using a circulating water bath or other method.)

Color, particle size, and texture class are used to compare different soils. However, they have limited discriminating value in forensic cases. If it is necessary to further differentiate soil samples the analyst would identify the mineral content of the soil by analyzing the optical properties of the sieved portions as discussed in Experiment 18A.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Compound light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) focusing ocular with micrometer scale and 530 nm gypsum plate

Micro kit

Microscope slides and cover slips

Known soil samples (or mineral combinations)

Unknown soil samples (or mineral combinations)

Munsell™ soil color chart

Sieve set (20, 40, 60, 80, 100 mesh sizes and trap)

Density tubes

Poly-Gee™

1–250 mL or 400 mL beaker

Plastic weigh boats

20 % hydrogen peroxide solution

Hot plate

Stirring rod

Mortar and pestle

Balance for weighing at least +/- 0.1g

Disposable glass or plastic pipets and bulbs

Para-film™

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. It is important to wear safety glasses and gloves when handling hydrogen peroxide solutions. Contact with the eyes can cause serious long-term damage. The hydrogen peroxide solution is corrosive and can cause skin burns. Slow decomposition of the solution in storage may lead to a build-up of pressure in sealed containers. Hydrogen peroxide can form potentially explosive compounds with a wide variety of materials. Refer to the MSDS if necessary.

PART I: COLOR AND OVERALL COMPOSITION

Procedure¹

Students will work in pairs for Part I. One student will examine the known sample and one will examine the unknown.

1. Weigh a clean evaporation dish to at least 0.1 g.
2. Weigh the evaporation dish with the soil sample. Use the same balance for all weighing in this procedure.
3. Dry the soil samples in an oven overnight at about 65°C in the evaporation dish.
4. Reweigh the evaporation dish with the soil sample. Subtracting the weight of the evaporation dish determine the mass of the soil sample. Calculate the percent weight of the moisture in the original sample using the following equation:

$$\text{Percent Moisture Content} = \frac{\text{mass of dried sample (g)}}{\text{mass of wet sample (g)}} \times 100 \quad (18-2)$$

5. Break up any large clumps and determine the color using the Munsell™ soil color chart. For comparison to the color charts use daylight if possible because the type of illumination will affect your results.
6. Examine each sample with the stereomicroscope to identify any foreign contaminants that might be present in the samples.
7. Sieve the soil samples into the designated portions.
8. Weigh the portion that cannot pass through the largest sieve. This is generally referred to as the gravel portion of the sample. Calculate the percent weight of the gravel portion using the following equation:

$$\text{Percent Weight} = \frac{\text{weight of fraction (g)}}{\text{weight of moisture free sample (g)}} \times 100 \quad (18-3)$$

This formula can also be used to calculate the percent weight for the other fractions.

9. Classify the color of this portion using the Munsell™ soil color chart.

¹ Procedure adapted from Quarino L. Soil Identification – Particle Size Distribution. Trace Evidence and Microscopy. Allentown, PA: Cedar Crest College; 2006.

10. Organic matter in the remaining portions needs to be oxidized before an accurate weight can be determined. Weigh a 200 or 400 mL beaker. Transfer the unsieved fraction to the beaker and reweigh. Calculate the mass of this unsieved fraction.

Important Safety Reminder:

ALWAYS wear safety glasses and gloves when handling hydrogen peroxide solutions. Hydrogen peroxide solutions should be diluted with lots of water before they are poured into the sink.

11. Add 100 ml of 20 % hydrogen peroxide. Add in increments, stirring well between increments. Allow to stand overnight in the hood with a watch glass covering the beaker. Make sure your name and a description of the contents of the beaker are written on the beaker.
12. Place the beaker in an oven for two hours to destroy the remaining organics and to decompose the remaining peroxide. Look at the beaker periodically to make sure the liquid does not froth. If frothing occurs, wash the sides of the beaker with distilled water using a wash bottle.
13. Add enough distilled water to bring the volume to 150 ml and stir well. Cool and allow to stand overnight – any remaining large organic particles will float to the surface.
14. Carefully decant off as much of the clear fluid as possible with a disposable glass or plastic pipet without disturbing the solid matter. Boil the remaining liquid on a hot plate to remove as much water as possible. Place the remainder in an oven for as long as it takes to dry. Cool in a desiccator for 30 minutes and then weigh, subtracting the weight of the beaker to give the weight of the mineral soil free of organic matter. Calculate the percent organic matter using the mass of the unsieved fraction determined in step 10, according to the following equation:

$$\text{Percent organic matter} = \frac{\text{mass after digestion (g)}}{\text{mass before digestion (g)}} \times 100 \quad (18-4)$$

15. Transfer the completely dry material to a mortar and pestle and break apart any clumps in the sample. Minerals should not adhere to each other.
16. Pour the sample into the set of remaining sieves, retaining the fine particles beneath the sieve in a collecting tray. As best you can, make sure that all the soil is transferred from the mortar and pestle. Shake the sieve set making sure that particles get trapped on the proper sieve.
17. Transfer the particles from each sieve to a plastic weigh boat. Record the weight of each fraction by subtracting the weight of each weigh boat.
18. Determine the color of each fraction using the Munsell™ color charts.
19. Calculate the percent weight of each fraction using the original dry weight of the soil sample and Equation 18-3.
20. For both the known and the unknown soil samples, prepare a histogram of percent weight of each fraction (including the gravel fraction) versus fraction name by mesh size. Sum the cumulative weight of each fraction and compare to the original weight of the soil sample. Account for any differences in these masses in your report. Tabulate the color of each fraction and any additional observations about the fraction. Report the percent moisture content and organic matter for your sample.
21. Pooling data with your partner, compare the known and unknown samples using all the data collected. Are the two samples from a probable common source? Why or why not?
22. Keep either the 40 or 60 mesh fraction for density testing in Part II.

PART II: DENSITY DISTRIBUTION OF MINERAL CONTENT

Procedure

Additional Note:

This procedure uses Poly-Gee™ (sodium polytungstate solution) and water.

The instructor may choose to substitute bromoform and bromobenzene for this procedure to have a wider range of density available.

Both bromoform and bromobenzene are extremely toxic and suspected carcinogens and should be used in a hood, with protective eyewear.

1. Obtain two dry tubes. Position the tubes on a stand so that you will be able to add liquids evenly to create two comparable density gradient tubes.
2. Using water (s.g. of 1.00) and Poly-Gee™ (s.g. of 2.89), you need to prepare five mixtures to get a density distribution that could be used to separate minerals ranging in densities from 1.68–2.89. (100 % water and 100 % Poly-Gee™ will be your sixth and seventh portions.) Select five density values and calculate the amounts of water and Poly-Gee™ needed to prepare about 10 ml of each density mixture. You can prepare this in small beakers. Be sure the beakers are CLEAN and DRY before you begin.
3. When preparing the mixtures, add water to the beaker FIRST, and then add the Poly-Gee™. This will make it easier to form the solutions. Stir very well.
4. Starting with the most dense mixture, 100 % Poly-Gee™, slowly add about 5 ml to each tube. Remember, you will be comparing the mineral samples in the two tubes, so you want the liquid levels to be identical.
5. Slowly add about 5 ml of the next densest mixture into each tube. Add the liquids carefully so that minimal mixing occurs.
6. Proceed with each additional liquid mixture (in the order of steps 3–5) slowly adding portions until the final mixture is added. Add 5 ml of 100 % water to each tube.
7. Cover each density gradient tube with a small section of Para-film™.
8. Allow the density gradient tubes to equalize at least 24 hours prior to use.
9. Obtain your known mineral mixture and unknown sample. If continuing from Part I, use the 40 or 60 mesh portion of the sieved samples.
10. Examine a small portion of the known sample under a stereoscope. Make sure you do not contaminate this sample, because you will return it to the known vial.
11. Examine your unknown sample under the stereomicroscope. Make sure you do not contaminate this sample, because you will return it to the unknown vial.
12. Slowly add 0.1 g of the sample for comparison into each density gradient tube (known sample into one tube and unknown sample into the other).
13. Allow the samples to separate and equilibrate for approximately 48 hours.
14. Compare levels of separation for the samples.
15. If possible take a photograph of the two tubes. Count the number of different types of mineral grains found in each sample. If possible count the number of grains within each mineral type and tabulate the data. Note color differences between different mineral types. Write a concise statement about the two samples. Are they from a probable common source? Why or why

- not? Explain your choice. Comment on the accuracy and precision of the method and give sources of error with the procedure.
16. Record room temperature. Are the results here accurate without temperature control?

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. How are soil samples collected?
2. What information can be gained in an investigation with a soil comparison?
3. How is color determined for soil samples? Name three things that affect the color of soil samples.
4. Why were the Munsell™ color determinations done using daylight?
5. Estimate the color of an object that has a Munsell™ color notation of: a) 5Y 6/1; and b) 7.5G 2/6.
6. Describe how the density distribution of a mineral portion is determined.
7. If two samples are being compared for possible differences, which do you think would be the most discriminating: a) density distribution of mineral content; b) percent organic matter; c) particle distribution; or d) color? Fully explain your choice.
8. What are three texture classes for soil? How would the texture class be determined?
9. What determined the amount of organic matter in soil? Would the amount of organic matter affect the color of soil?
10. What was the purpose of the hydrogen peroxide?
11. A trace examiner compares two soils samples by determining the color and percent weight of the sieved fractions. They were found to be similar in all characteristics. If this is all that was done, could the examiner state that the two were of a probable common source? Why or why not?
12. A trace examiner compares two soils samples by determining the density distribution of the minerals found in the soil. They were found to be similar in all characteristics. If this is all that was done, could the examiner state that the two samples were of a probable common source? Why or why not?
13. Could the density determinations of mineral content be determined for the known sample on one day and compared to the density determination of minerals for the unknown sample on the next day? Could different laboratories work on the known and unknown samples and compare the results? Why or why not?

Experiment 18A: Identification of Minerals in Soil

Recommended pre-lab reading assignments:

- Murray RC, Solebello LP. Forensic Examination of Soil. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 615–25.
- Petraco N, Kubic T. *Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators*. Boca Raton, FL: CRC Press; 2004; 135–49.
- McCrone WC, McCrone LB, Delly JG. *Polarized Light Microscopy*. Ann Arbor, MI: Ann Arbor Science, 1978.

OBJECTIVE

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

1. conoscopic observations of minerals
2. determining the orientation of a uniaxial mineral to determine which refractive index is being observed
3. use of conoscopic illumination to determine if a mineral is + or –
4. distinction of uniaxial and biaxial minerals using interference figures
5. use of the compound or comparison light microscope to visualize soil characteristics

INTRODUCTION

It has been said that ‘soil is merely a rock on its way to the sea.’² Because soil is a product of rock decay it is possible to discriminate soils samples by identification of the many microscopic mineral particles found in the soil. Minerals are a naturally occurring inorganic material with a definite chemical composition. Minerals are composed of atoms that are arranged in a regular pattern to produce a crystalline form. The ordered nature of minerals makes them anisotropic with the notable exception of minerals belonging to the cubic crystal system. Optical properties covered in earlier chapters such as birefringence, refractive index, and relief can be used in mineral identifications. In addition to these properties mineral identification is aided by the use of color, optic sign, optic angle, and interference figures. Crystal form and cleavage also offer insights into mineral identification.

Since crystals possess an orderly arrangement, if a beam of white light enters a crystal, several things can happen depending on the crystal structure. The reaction to a beam of white light is shown by the use of an optical indicatrix. An indicatrix is the geometric figure that shows the refractive index and vibrational direction for light passing through a material in any direction. Each vector of the figure is drawn proportional in length to the material’s refractive index for light

² Weil RR. *Laboratory Manual for Introductory Soils*. 6 ed. Dubuque, IA: Kendall/Hunt Publishing Company; 1998; 1.

Table 18A-1 Refractive index for common isotropic crystals.

Mineral	n
Opal	1.43
Fluorite	1.433
KCl	1.490
Pumice	1.450
Halite	1.544
NH ₄ Cl	1.640
Sphalerite	2.37
Magnetite	2.42
Diamond	2.42
Cuprite	2.85

Barthelmy D. Minerals by Physical and Optical Properties Tables. 2005 [updated 2005; cited 2007 November 20]; Available from: <http://www.webmineral.com/>.

vibrating parallel to that vector direction. The geometric figure is a surface that connects all the tips of these vectors.

Minerals, like other crystals and particles, can be isotropic and have one refractive index (Table 18A-1) or anisotropic and have more than one refractive indices. In isotropic materials, the chemical bonding is the same in all directions, so the velocity of light passing through is the same in all directions. The refractive index remains constant regardless of the direction in which the light is vibrating, therefore, the indicatrix shape is a sphere (see Figure 18A-1). The same can be said for all isometric (cubic) crystals. Anisotropic minerals, however, react differently. Anisotropic minerals are those in the remaining six crystal systems: hexagonal, rhombohedral, tetragonal, orthorhombic, monoclinic, and triclinic. In these anisotropic materials, the chemical bonding is different in parallel and perpendicular directions, so the velocity of light passing through is different in parallel and perpendicular directions. The refractive index is different depending on the direction in which the light is vibrating. Therefore, the indicatrix shape is an ellipse (see Figure 18A-1).

Anisotropic minerals may be further divided into uniaxial (having a single unique optic axis and two refractive indices) or biaxial (having two optic axes and three refractive indices).

Uniaxial minerals belong to the hexagonal, rhombohedral, and tetragonal crystal systems and have directions that are alike (multiple a-axes) and one distinct (c-axis) (see Figure 18A-1). The name uniaxial is used because only one axis is unique to the other directions and is commonly referred to as the optic axis. A uniaxial mineral under crossed polars produces two rays of light, known as the e-ray (with refractive index, n_e or epsilon) and the o-ray (with refractive index, n_o or omega), which vibrate perpendicular to each other. Depending on the orientation of the crystal when viewed under the microscope, n_e and n_o are visible, or some combination of the two known as $n_{e'}$. For a review of determining refractive indices of anisotropic materials see Experiment 3B on page 48.

Table 18A-2 lists a number of common uniaxial minerals and their different refractive indices. In order to know which refractive index one is observing the analyst must determine the orientation

Crystal Systems	Indicatrices	Conoscopic Figures	
		No conoscopic figures	+
	Isotropic		
Cubic: $a = b = c$ $\alpha = \beta = \gamma = 90^\circ$			
Hexagonal: $a_1 = a_2 = a_3 = c$ $\alpha = \beta = 90^\circ; \gamma = 120^\circ$			
Rhombohedral: $a_1 = a_2 = a_3 = a$ $\alpha = \beta = \gamma \neq 90^\circ$			
Tetragonal: $a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$			
Rhombic: $a \neq b \neq c$ $\alpha = \beta = \gamma = 90^\circ$			
Monoclinic: $a \neq b \neq c$ $a = \gamma = 90^\circ$ $\beta \neq 90^\circ$			
Triclinic: $a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90^\circ$			

Figure 18A-1 The seven crystal systems. Cubic crystals have one refractive index and are isotropic. Hexagonal, rhombohedral, and tetragonal crystals have two refractive indices and one unique optic axis and are uniaxial. Rhombic, monoclinic, and triclinic crystals have three refractive indices and are biaxial. (Reproduced with permission of Leica Microsystems, Inc.)

Table 18A-2 Refractive index for common uniaxial crystals.

Mineral	n_{ω}	n_{ϵ}
Quartz	1.544	1.553
Beryl	1.581	1.564
Apatite	1.630	1.632
Tourmaline	1.650	1.621
Calcite	1.658	1.486
Dolomite	1.682	1.503
Corundum	1.7681	1.759
Zircon	1.91	1.968
Rutile	2.616	2.903
Carborundum	2.654	2.697
Hematite	3.15	2.87

Barthelmy D. Minerals by Physical and Optical Properties Tables. 2005 [updated 2005; cited 2007 November 20]; Available from: <http://www.webmineral.com/>.

of the uniaxial mineral. When minerals are first mounted they will be in random orientations. It is possible to perform a random search of each mineral in the preparation to find one with the proper orientation. Another technique used to align minerals is to roll the mineral on a spindle stage or after mounting it in a very viscous medium. In both the random search and rolling methods, interference figures are used to confirm orientation of the c-axis.

Depending on the orientation of the mineral particle with respect to the optic axis, three possible views can be seen. 1) When the mineral particle is oriented so that one is looking ‘down the c-axis,’ only the ω refractive index will be seen. This orientation aligns the c-axis parallel to the optic axis of the microscope. 2) A mineral particle perpendicular to the optical axis of the microscope shows two refractive indices, ω and ϵ_{\max} . 3) Orientations in between parallel and perpendicular will show ω and some ϵ value ranging from zero to ϵ_{\max} . Therefore, ω is common to every view of the uniaxial crystal and ϵ will range from zero to ϵ_{\max} .

During this experiment we will illustrate these orientations by mounting the mineral in a refractive index equal to ω and viewing the mineral grain with polarized light and the analyzer removed. What is actually seen varies depending upon the three orientations previously described. 1) The first orientation describes a situation where only ω is visible. Therefore, the mineral will appear isotropic upon stage rotation and mineral particles with this orientation will exhibit minimum birefringence under cross polars. Mounting the mineral in a refractive index equal to ω , if known, will cause the mineral to remain invisible at all locations of stage rotation when the analyzer is removed. 2) Observations in polarized light (analyzer removed) with the mineral oriented with its c-axis perpendicular to the optic axis of the microscope will result in disappearance when only ω is observed. Only ω is seen when the e-ray’s vibration direction is N–S and blocking the E–W polarized light allowed by the polarizer. Because the mineral is mounted in a liquid matching the o-ray and the e-ray is blocked the mineral appears to disappear at two locations 180° apart during a 360° stage rotation. At two other locations, maximum relief is observed when ϵ_{\max} is the only visible refractive index. At these locations the o-ray is vibrating N–S and blocking the E–W polarized light allowing only ϵ_{\max} to be seen. 3) At locations in between parallel and perpendicular, the mineral will always show ω and some ϵ in between zero and ϵ_{\max} . The relief of the particle will then vary between a minimum and a maximum value upon stage rotation.

Table 18A-3 Refractive index for common biaxial crystals.

Mineral	n_α	n_β	n_γ
Gypsum	1.521	1.523	1.530
Orthoclase	1.518	1.524	1.526
Albite	1.528	1.529	1.536
Biotite	1.565	1.605	1.605
Talc	1.539	1.589	1.589
Microcline	1.520	1.523	1.530
Vermiculite	1.525	1.545	1.545
Muscovite	1.552	1.582	1.584
Topaz	1.619	1.620	1.627
Olivine	1.635	1.665	1.670
Barite	1.636	1.637	1.648
Hornblende	1.646	1.656	1.661
Olivine	1.662	1.680	1.699
Malachite	1.655	1.875	1.909
Augite	1.680	1.684	1.706
Epidote	1.723	1.730	1.736
Azurite	1.73	1.758	1.838
Carnotite	1.75	1.90	1.95
Monazite	1.79	1.795	1.842

Barthelmy D. Minerals by Physical and Optical Properties Tables. 2005 [updated 2005; cited 2007 November 20]; Available from: <http://www.webmineral.com/>.

A biaxial mineral under crossed polars produces three rays of light – X, Y, and Z – which vibrate in different directions and are always mutually perpendicular. In contrast the three crystallographic directions, (a, b, and c) are not perpendicular in monoclinic and triclinic crystals (see Figure 18A-1). The refractive indices corresponding to the three orientations are referred to as n_α (alpha), n_β (beta), and n_γ (gamma). Table 18A-3 contains a list of common biaxial minerals and their refractive indices. The slowest vibration direction is Z, which is the n_γ refractive index and n_α , which is always the highest refractive index and is the X vibrational direction. The intermediate refractive index is n_β , corresponding to the Y direction. The X and Z directions are always in the optical axis plane, making Y the optical normal.

It is possible to classify minerals using a microscope by viewing their interaction with polarized light. Isotropic minerals, which belong to the isotropic cubic system, can immediately be identified because they remain dark under crossed polars. Uniaxial and biaxial minerals require the use of conoscopic illumination for identification.

Conoscopic illumination refers to the observation of the back focal plane of the objective. It is obtained by removal of the ocular or insertion of the Bertrand lens to obtain a magnified image of this area (see Figure 18A-2.) With conoscopic illumination, the light is now focused at five locations along the optical path:

- the lamp filament
- the substage aperture diaphragm
- the objective back focal plane
- the intermediate image
- the retina of the eye.

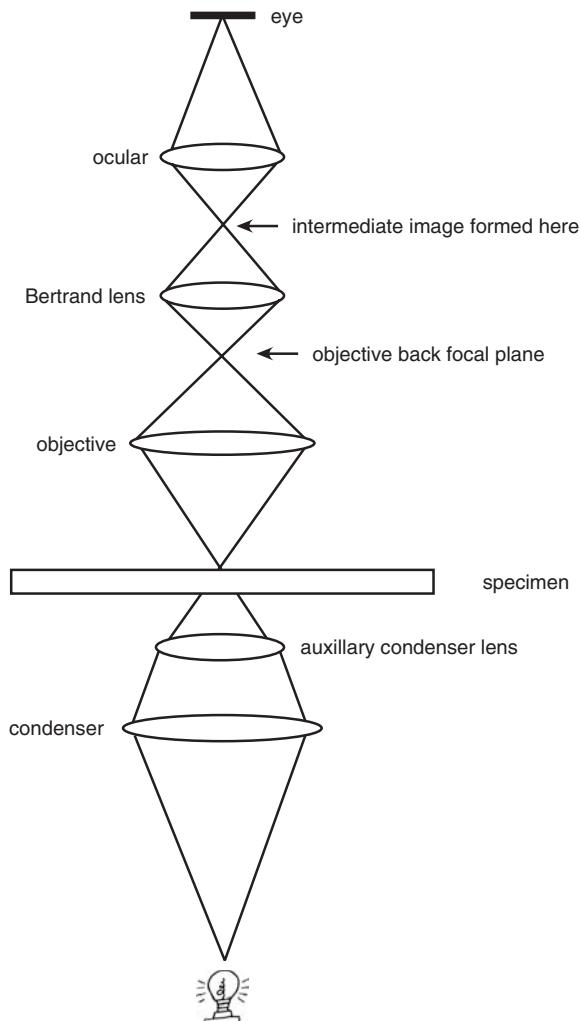


Figure 18A-2 Conoscopic illumination is used to focus on the objective back focal plane for observation of interference figures.

At the objective back focal plane, an anisotropic mineral gives an ‘interference figure.’ Interference figures are light patterns that emerge from a crystal when viewed under crossed polars with conoscopic illumination. This light pattern provides information about the mineral’s orientation and can also be used to determine if the mineral is uniaxial or biaxial. It is also possible to determine the optic sign for a mineral; whether or not the mineral is positive or negative. A more advanced topic, which is beyond the scope of this book, is determination of the optic axial angle, ‘optic angle,’ for biaxial minerals. The optic angle is the acute angle between the two optic axes (this is the $2V$ angle shown in the biaxial indicatrices of Figure 18A-1).

Interference figures result when the many rays passing through the mineral under crossed polarized light converge as a cone of light. The light rays always converge evenly with orthoscopic illumination; however, they are considered less convergent and the term orthoscopic suggests an

almost parallel illumination. For conoscopic illumination it is sometimes necessary to increase the numerical aperture (NA) of the condenser by insertion of an auxiliary condensing lens in the light path. This causes the illumination rays to be highly convergent so that they focus within a mineral on the stage, which produces conoscopic illumination where a cone of light emerges from the mineral diverging rapidly. Removal of the ocular or insertion of the Bertrand lens allows an analyst to interpret the emergent cone of light.

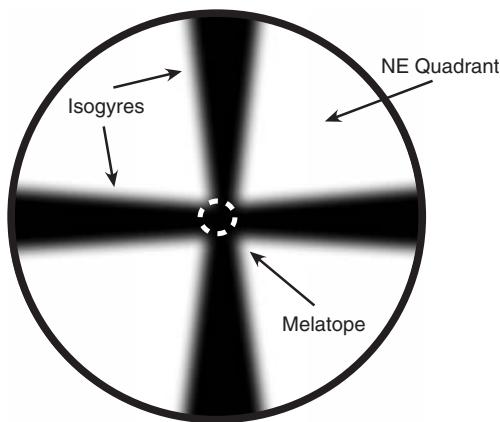


Figure 18A-3 A uniaxial interference figure.

Figure 18A-3 shows a typical uniaxial interference figure observed when the optic axis (c-axis) is vertical. The figure shows a cross whose arms intersect at the center, or melatope. The arms or isogyres remain N–S and E–W during 360° stage rotation as long as the mineral is oriented with its c-axis parallel to the optical axis of the microscope. To obtain a vertical c-axis a mineral is selected that exhibits minimum birefringence under crossed polars with orthoscopic illumination. The optic sign of the mineral is determined by insertion of a first order red plate, which adds or subtracts 530 nm of retardation (see Figure 18A-4). In the northeast quadrant of the interference figure, upon insertion of the first order red plate, the overall retardation will become blue (addition) for a + mineral and yellow (subtraction) for a - mineral. This classification refers to $n_e > n_\omega$ for a + mineral and $n_\omega > n_e$ for a - mineral. If there is any inclination of the optic axis then an off-centered figure is obtained. Rotation of the stage and isogyres allows the analyst to identify the northeast quadrant and repeat the test for retardation color.

As with uniaxial minerals, interference figures can be used to determine the optic sign of biaxial minerals as well as distinguish uniaxial minerals from biaxial minerals. The biaxial mineral has two optic axes due to the three vibrational directions, creating an interference figure, called a centered acute bisectrix figure (see Figure 18A-5a, b). The isogyres appear as a black cross that changes into a pair of hyperbolic isogyres with interference colors four times with each complete rotation of the stage. Distinction of positive and negative optic sign for biaxial crystals is determined by orienting the optic plane NE–SW as in Figure 18A-4 and observing addition or subtraction of light upon insertion of the first order red at the center of the cross-hairs. The optic sign of a biaxial mineral is positive when all vibration components parallel to the optic plane are fast. Since the Z direction (slow ray or γ) is along the acute bisectrix, and $n_\gamma - n_\beta > n_\beta - n_\alpha$, retardation will always subtract between the isogyres for positive crystals, which will yield yellow at the center

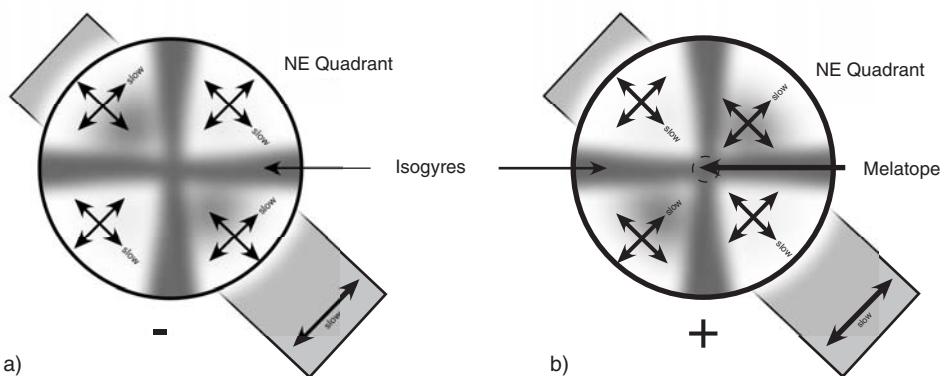


Figure 18A-4 A typical interference figure for a uniaxial mineral. The isogyes appear first order red upon insertion of the first order red plate. a) A negative mineral will appear yellow in the NE quadrant because subtraction has occurred (slow rays of mineral and accessory plate are perpendicular). b) A positive mineral will appear blue in the NE quadrant because addition has occurred (slow rays of the mineral and accessory plate are parallel). A full-color version of this figure can be found in the color plate section of this book.

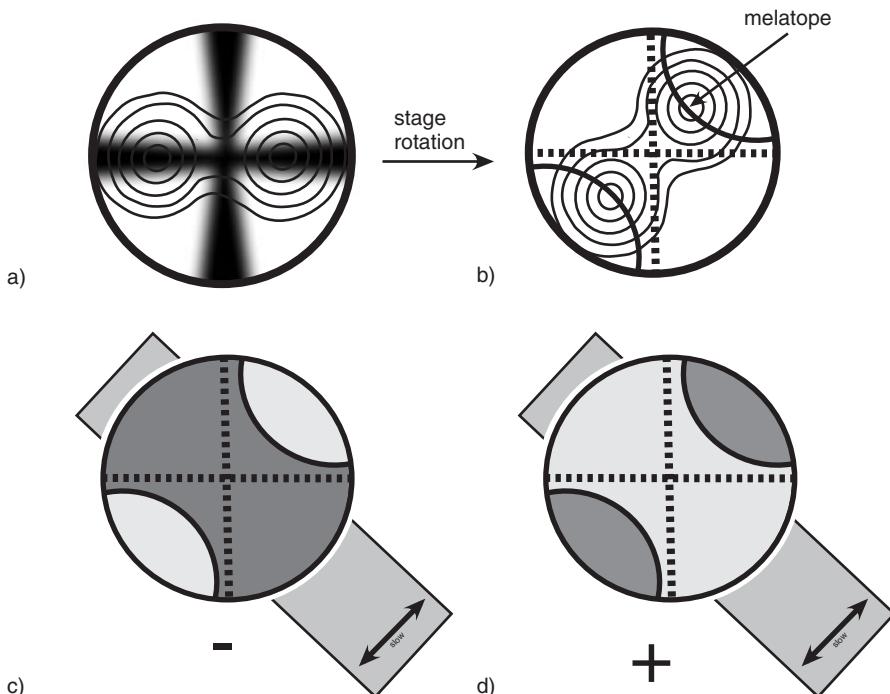


Figure 18A-5 A biaxial interference figure. With stage rotation, the interference figure changes from a) a black cross above interference rings with melatopes to b) two hyperbolic isogyes above each melatope. Upon insertion of the first order red plate the center of the crosshairs in b) turn c) blue for a negative mineral and d) yellow for a positive mineral. A full-color version of this figure can be found in the color plate section of this book.

upon insertion of the first order red plate (Figure 18A-5c). Likewise, the optic sign of a biaxial mineral is negative if $n_\gamma - n_\beta > n_\beta - n_\alpha$ and retardation adds at the center position, yielding blue upon insertion of the first order red plate (Figure 18A-5d).

EQUIPMENT AND SUPPLIES

Stereomicroscope

Polarized light microscope with objectives of various magnifications (e.g., 4X, 10X, 20X, 40X) focusing ocular with micrometer scale and first order red compensator (530–550 nm)

Micro kit

Microscope slides and cover slips

Cargille™ refractive index liquids

Flo-texx™

Calcite (from 100–200 mesh sieve)

Sodium nitrate (ACS grade)

Sodium sulfite (from 100–200 mesh sieve)

Quartz or apatite

Muscovite plates

Unknown minerals

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. Refer to MSDS if necessary.

PART I: OBSERVING THE TWO REFRACTIVE INDICES IN A UNIAXIAL CRYSTAL

Procedure

1. Examine a prepared slide of calcite. Using the circle template located in Appendix F, draw a few representative particles in both uncrossed and crossed polars.
2. View the particles while rotating the stage under both uncrossed and crossed polars. What do you see? (Include these comments under each of the previous drawings.)
3. Mount a few particles of crushed calcite in an immersion oil with a refractive index of 1.658 (this matches the refractive index of ω). Using the lowest magnification, observe the particles with both uncrossed and crossed polars. Slowly rotate the stage 360° and observe the relief of some of the particles. What happens to the particles as the stage is rotated? (Remember that the particles are in random orientations). With the analyzer out find a grain that disappears or

shows minimum relief twice at two positions 180° apart during each complete rotation of the stage. Which refractive index are you observing?

PART II: DETERMINING THE ω REFRACTIVE INDEX FOR A UNIAXIAL CRYSTAL

1. Using the Becke Line immersion method discussed in Experiment 2D, determine the ω refractive index for sodium sulfite.
2. Find an immersion oil where the minerals disappear twice upon 360° stage rotation at locations 180° apart.
3. Record the room temperature for this measurement and correct the temperature according to Equation 2C-1.
4. Report the ω refractive index of sodium sulfite at 25°C .

PART III: OBSERVING A TYPICAL UNIAXIAL INTERFERENCE FIGURE. DETERMINING THE OPTIC SIGN OF A UNIAXIAL CRYSTAL

Procedure

1. Place several particles (totaling about the size of a pencil eraser) of sodium nitrate on a microscope slide. Since sodium nitrate has a high melting point ($>300^\circ\text{C}$) place the particles near one end of the slide to decrease the chance of breaking the slide by uneven heating.
2. Cover the particles with a cover slip. Make a melt mount by fusing the particles of sodium nitrate between the slide and cover slip (this is done by heating the microscope slide on a hot plate). When the sodium nitrate melts, push the cover slip down using a pencil eraser to obtain a thin, even crystal. Remove the microscope slide from the hot plate and allow it to cool. Remember, the slide will be very hot!
3. Place the microscope slide containing the recrystallized sample on the microscope. Sodium nitrate typically recrystallizes so that the c-axis is parallel to the optic axis of the microscope, making an interference figure easily visible.
4. Focus on the sample using low magnification. Search for a portion that shows the lowest retardation throughout stage rotation under crossed polars. Switch to the 40X objective and check your focus. Now observe the sample with the Bertrand lens inserted. A good interference figure will have a black cross (isogyres) with concentric circles of interference colors. Remember, you may need to refocus slightly since you have switched objectives. If you cannot obtain a black cross, rotate the stage to see if the isogyres move across the field of view. If this happens you have selected a mineral whose c-axis orientation is off-centered. To proceed you can either chose another mineral or try to locate the NE quadrant as discussed in the next step.
5. Observe the colors in each quadrant. Draw what you see. To determine the optic sign for the crystal, insert the first order red plate. Observe and draw the color change in the NE (upper right) quadrant. If you are using an off-centered interference figure determine the NE quadrant by rotating the stage until the east isogyre just passes by. The NE quadrant is after the east isogyre but before the north isogyres passes by.
6. Determine if sodium nitrate is a + or - uniaxial crystal by observing either addition or subtraction in the NE quadrant. Compare the colors seen upon insertion of the first order accessory

plate to the Michel-Lévy chart using Figure 18-1 as a guide. If the colors move up the chart (blue) they are said to add and the crystal is positive. If they move down the chart (yellow) the colors are said to subtract and the mineral is negative.

PART IV: DETERMINING CRYSTAL ORIENTATION

Procedure

1. Observe a typical uniaxial crystal by mounting a few grains of quartz or apatite on a slide using Flo-texx™.
2. Find a grain showing the lowest retardation throughout stage rotation and observe its interference figure. Leave that grain in the center of the field of view, switch to the 40X objective, and insert the Bertrand lens.
3. If necessary focus the interference figure to the largest circle by adjusting the focus very slightly using the fine focus knob. You should see a black cross with concentric circles of interference colors, a typical uniaxial interference figure. Remember it may be off-center so you may only see the arms of the cross. Rotating the stage is necessary to determine if you are indeed seeing a cross.
4. Repeat this process until at least a centered interference figure is obtained:
 - (a) Draw the crystal under conoscopic illumination.
 - (b) Draw the crystal under orthoscopic illumination.
5. Explain the orientation of the c-axis for the crystal grain that gave you the centered interference figure on the circle template. On the orthoscopic drawing made in the previous step indicate the location and direction of the c-axis.

PART V: OBSERVING A TYPICAL BIAXIAL INTERFERENCE FIGURE. DETERMINING THE OPTIC SIGN OF A BIAXIAL CRYSTAL

Procedure

1. To observe a biaxial crystal interference figure, place a thin section of muscovite on a slide without a cover slip. This mineral has the acute bisectrix parallel to the optical axis of the microscope.
2. Focus on the muscovite using low magnification. Switch to the 40X objective and refocus if necessary, cross the polars, and insert the Bertrand Lens to obtain an interference figure. With biaxial minerals the black cross will change into a pair of hyperbolic isogynes with interference colors upon rotation of the stage. This should happen four times during one complete rotation of the stage. Remember, you may need to refocus slightly because you have switched objectives. If you cannot find an interference figure initially, move the slide around to obtain the minerals preferred orientation. Draw what you see.
3. To determine the optic sign for the mineral, rotate the stage until the interference figure resembles Figure 18-2. Insert the first order red plate. Compare the colors seen upon insertion of the first order accessory plate to the Michel-Lévy chart to determine whether muscovite is a + or - biaxial crystal. Check for the color change at the center of the two melatopes. If the

color changes to yellow, the optic sign is positive. If the color changes to blue, the mineral is negative.

4. Draw the interference figure with the first order red plate inserted. Make sure that the stage is rotated to show the two hyperbolic isogyres above each melatope. Record the optic sign under the drawing

PART VI: UNKNOWN CRYSTAL TYPE AND OPTIC SIGN

Procedure

As you have observed from Parts III and V, biaxial crystals have a different interference pattern than uniaxial crystals. Obtain an unknown sample. Mount it on a microscope slide using Flo-texx™. Using conoscopic illumination determine whether the sample is biaxial or uniaxial by its interference figure. Also, report if the crystals are positive or negative.

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 foci obtained with conoscopic illumination? How does this differ with orthoscopic illumination?
2. What is a uniaxial crystal? Define e-ray, o-ray, n_e , and n_o .
3. Why is the mineral called uniaxial given that it has three axes?
4. Which refractive index are you observing for the calcite particles in Part I when they disappear at the two locations 180° apart? Explain your answer.
5. Which refractive index are you observing 90° from the two locations in the previous problem positions? *Note:* these particles have the most contrast or highest relief. Explain your answer.
6. Which refractive index are you observing in the other grains showing intermediate relief? Explain your answer.
7. What would you expect to see if the mineral was isotropic? Explain your answer.
8. What is a biaxial mineral? Define acute bisectrix. Define + biaxial mineral and how the optic sign is determined using an interference figure.
9. Why is a mineral called biaxial when it has three axes?
10. Draw an off-centered interference figure for a uniaxial mineral and label the isogyres, melatope, and NE quadrant.
11. Explain why the black isogyres turns into red isogyres upon insertion of the first order red plate.
12. Explain how you could find a mineral grain whose c-axis was oriented parallel to the optical axis of the microscope using an interference figure. Which refractive index is being observed with this orientation? Explain.

RECOMMENDED AND FURTHER READING

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- Rawlins BG, Kemp SJ, Hodgkinson EH, Riding JB, Vane CH, Poulton C, et al. Potential and Pitfalls in Establishing the Provenance of Earth-Related Samples in Forensic Investigations. *Journal of Forensic Sciences*. 2006; 51(4): 832–45.
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- Stoiber RE. *Crystal Identification with the Petrographic Microscope*. Hanover, NH, 1962.
- Wanogho S, Gettinby G, Caddy B, Robertson J. Determination of Particle-Size Distribution of Soils in Forensic-Science Using Classical and Modern Instrumental Methods. *Journal of Forensic Sciences*. 1989; 34(4): 823–35.
- Weil RR. *Laboratory Manual for Introductory Soils*. 7 ed. Dubuque, IA: Kendall/Hunt Publishing Company, 2005.

Experiment 19: Microchemical Testing – Inorganic Ions

Recommended pre-lab reading assignment:

Chamot EM, Mason CW. *Handbook of Chemical Microscopy*. New York, NY: John Wiley & Sons, Inc.; 1938.

OBJECTIVE

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

1. microchemical testing
2. techniques for performing microchemical tests
3. several tests for determination of ions/anions
4. use of the compound microscope to visualize test results

INTRODUCTION

With current analytical instrumental methods for the identification of unknown materials one might think that microchemical testing is outdated. However, the techniques used are widely accepted and easily obtainable. Because microchemical testing is simple, very inexpensive, sensitive, reliable, and quick, its popularity in forensic science remains. It is commonly used as a screening technique with other instrumental confirmatory tests to follow. In a few forensic disciplines, such as drug analysis and trace evidence, microchemical testing is widely used (Experiment 20). The principles behind inorganic microchemical testing follow the qualitative analysis scheme commonly used to separate and detect cations and anions in a sample substance. The ‘semi-micro’ level of qualitative analysis employs methods used to detect 1–2 mg of an ion in 5 mL of solution and detection is visually observed. Using a microscope allows analysts to identify single grains of the sample substance.

Almost any chemical test performed in a qualitative analysis scheme can be carried out on a microscope slide. There are six main procedures used in microchemical testing: reagent mixing, solubility, evaporation, decantation, sublimation, and fusion. Reagent mixing can be achieved using one of the three methods described in Figure 19-1.

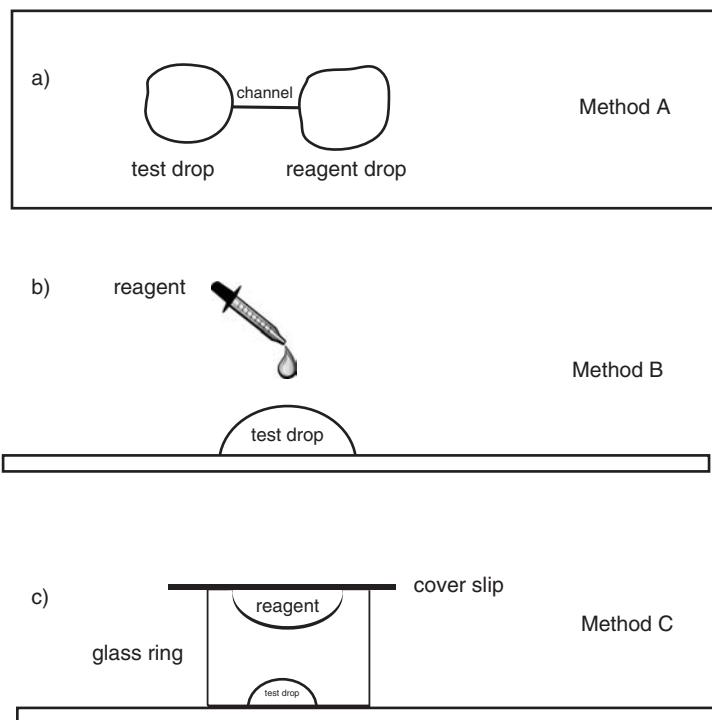


Figure 19-1 Inorganic ion microcrystalline test methods. a) The reagent and test sample are dissolved. The reaction occurs when connected by a channel formed with a toothpick; b) reagent is dissolved then dropped onto the dissolved or solid test sample; and c) reagent is suspended over the test sample that is dissolved in a glass ring.

Solubility testing involves placing a single crystal or grain in a drop of water or other solvent. For many of the reactions performed on the microscopic level, water turns out to be the best solvent, however, certain reactions work better when the solution is slightly acidic or basic. The analyst should note if the test sample is soluble, slightly soluble, or insoluble in the solvent. The initial test of solubility tells the analysts something about the polarity of the sample substance and illustrates what is meant by the comment ‘like dissolves like.’ Polar compounds dissolve in polar solvents and nonpolar compounds dissolve in nonpolar solvents.

Evaporation entails heat being applied to a solvent that contains the test sample. Sources of heat used for evaporation include an alcohol flame, a warm lamp bulb, or a hot plate if spark free heating is needed. The microscope slide is held over the heat. When it is warm it is common to blow gently across the drop so that a uniform film of the substance is produced. After evaporation has occurred, characteristic crystals often remain that can be used in identification.

Decantation involves separating a precipitate from the solvent. The solvent is drawn away from the precipitant with a micro spatula that is then allowed to dry. Back flow can be avoided by

making a channel between the excess solvent and precipitant using a small piece of filter paper and slight warming of the channel area.

Sublimation, the transformation of the solid phase to a gaseous phase without passing through the liquid phase, can occur on the microscopic scale. Crystal formation after sublimation can be used in identification. A reagent drop is suspended over the solid test sample by use of a hanging drop on a cover slip over a glass ring as shown in Figure 19-1c (on page 266). Upon heating sublimation occurs and crystals form on the underside of a cover glass.

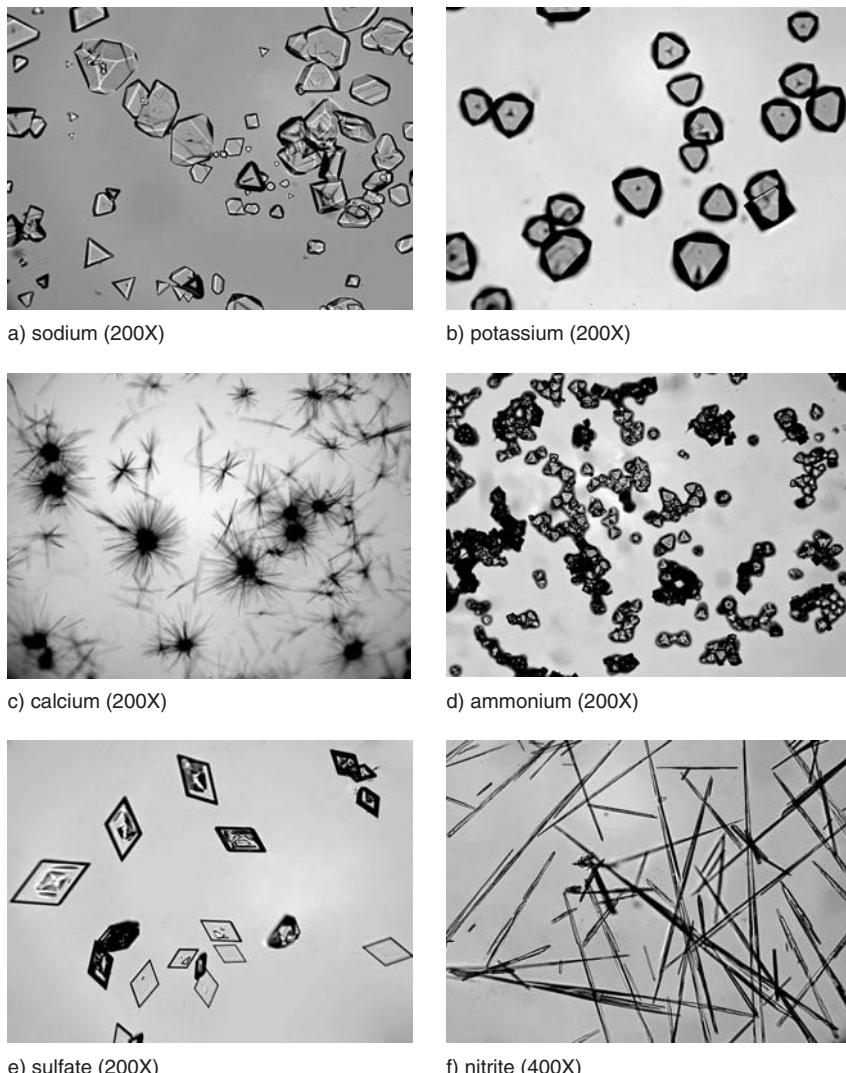


Figure 19-2 Characteristic crystals for some common microchemical tests for inorganic ions: a) sodium with zinc uranyl acetate dissolve in acetic acid; b) potassium with chloroplatinic acid; c) calcium with sulfuric acid; d) ammonium with chloroplatinic acid forms crystals with exactly the same habit as potassium in b); e) sulfate with silver nitrate; f) nitrite with silver nitrate. Not shown: chloride with silver nitrate (white precipitate) and carbonate with dilute hydrochloric acid (bubble formation).

In fusion methods the test sample is mixed with a flux agent such as borax and heated on a platinum wire. A glassy bead is formed that may then be studied for its unique interference colors, crystal habit, or other unusual properties. Fusion methods are particularly useful for identifying insoluble compounds, although tabulated information about the fusion properties are not readily available.

This is just a short summary of the many variations of these types of procedures, resulting in a wide variety of microchemical tests.

In this experiment we will focus on microchemical crystal testing, which involves the application of known reagents to small amounts of unknown material to form identifiable crystalline products. These reactions are carried out on a microscope slide using a test drop (drop containing substance) and a reagent drop (reagent dissolved in appropriate solvent). In general, the reagent drop is placed in contact with the unknown. This is usually accomplished by drawing the two drops together using a glass rod or a toothpick as shown in Figure 19-1a. A reaction between the two substances occurs. The result of the reaction can vary from a color change, gas bubble formation, or characteristic crystals of the resulting compound. These can be observed with a compound microscope and polarized light if crystalline product is expected using 100X–400X magnification. Sample concentration, impurities, and reagent problems may cause difficulties when performing microchemical testing. Because of these problems, a control test must be performed at the same time that an unknown is tested. It is important to note that a positive result must be determined by a comparison to a control test and not to published literature that uses different reagents and techniques. Students should run positive controls of all ions in question to become familiar with the microchemical technique, procedures used, and the appearance of crystals prior to attempting work with unknown samples. It is also important to run negative controls using all reagents without analyte to ensure that the reagent itself does not cause formation of the observed crystals. Although the positive and negative controls are often run as preliminary procedures, it is important that they be run at the same time as the unknown in a side-by-side manner.

The sensitivity of these tests means that special precautions should be taken to guard against contamination. All equipment (slides, micropipettes, microspatulas) that comes in contact with the unknown or reagents should be scrupulously clean. Microscope slides must be cleaned prior to use. Flat wooden toothpicks serve nicely as disposable microspatulas. It is very important to label all slides and keep your work area well organized and neat to minimize confusion and the opportunity for contamination.

EQUIPMENT AND SUPPLIES

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

Micro kit

Microscope slides (specialized spot well slides may be used)

Cover slips

Toothpicks (or closed end capillary tubes)

Chemicals as listed with each test

Known samples containing anions and cations to be tested

Unknown samples

SAFETY

Use standard laboratory safety procedures as described in rules set by your instructor. Be cautious of microscope light levels to avoid eye damage. Students should know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. Refer to MSDS as needed. Students should wear personal protective equipment such as goggles and nitrile gloves for all parts of this lab. Several of the reagents contain concentrated acid solutions and should be handled with caution.

STANDARD METHODS FOR MICROCRYSTALLINE TESTS

Since the crystal formation occurs when the test sample and reagents are at a specific ratio, crystals may not be immediately obtained. In this case, set the test slide aside and check for crystal formation after a few minutes. This allows for the solutions to evaporate, encouraging crystal formation.

Method A

Place a small amount of the reagent and test substance in separate drops of water. After they are dissolved, join the two drops by a thin ‘channel’ using a toothpick. Characteristic crystals will form as the reaction occurs between the two sample drops. See Figure 19-1a.

Method B

Dissolve the test substance in a drop of water. Add a small amount of reagent directly to the test drop. Characteristic crystals will form as the reaction occurs between the samples. At times, higher concentrations of the test sample quicken the crystal formation. In these situations, the test sample can be added directly into the reagent. See Figure 19-1b.

Method C

A hanging drop is subjected to the gases of a reaction between the sample test drop and a reagent. A spacer (made of cut 1/4 inch (6 mm) glass tubing) is used to hold the cover slip holding the reagent over the dissolved sample. Once crystals have formed, invert the cover slip and place it on a microscope slide for viewing. See Figure 19-1c.

Cations

- Na^+ The best reaction for sodium is obtained with zinc uranyl acetate using method A. Start by dissolving the sodium containing solid in distilled water. Next dissolve the zinc uranyl acetate in acetic acid. Excess acetate ions are necessary for crystal formation. Characteristic crystals will appear as monoclinic polysynthetic twins possessing octahedral aspects. Photos of crystals are shown in Figure 19-2a.

- **K⁺** Dissolve potassium salts in dilute hydrochloric acid. React this solution with chloroplatinic acid using method A. Characteristic well-formed colorless highly birefringent octahedral and octahedron and cubes will appear. Elongated and distorted shapes may also appear. Elongated octahedral crystals appear as orthorhombic crystals. Because of their birefringence the crystals are best observed using crossed polars. It is helpful if the test drop is cold and relatively dilute. Photos of crystals are shown in Figure 19-2b.
- **Ca²⁺** Using method A, add a drop of dilute sulfuric acid to the dissolved calcium sample. Colorless birefringent needles will form, appearing as starbursts. These thin crystals are hard to view, however; because of their birefringence the crystals are best observed using crossed polars. Photos of crystals are shown in Figure 19-2c. An alternate procedure is to add a drop of sulfuric acid to the solid calcium salt. This produces larger needle crystals.
- **NH₄⁺** Using the hanging drop method (method C), add dilute sodium hydroxide to the test sample. Quickly cover this sample with a cover slip that holds a hanging drop of chloroplatinic acid. Highly refractive octahedra are produced (Figure 19-2a), which have exactly the same habit as the potassium crystals obtained with chloroplatinic acid (shown in Figure 19-2b).

Anions

- **Cl⁻** Characteristic finely grained octahedra are formed when a chloride containing compound has been exposed to silver nitrate using method A. This appears as an amorphous white precipitate unless viewed at high magnification (>500X). Because many anions form a white precipitate with silver nitrate, further testing is required.
- **SO₄²⁻** Place a drop of sulfuric acid on a microscope slide. Neutralize this with dilute sodium hydroxide. Place a few crystals drops of silver nitrate on the slide next to the drop. Add a drop of distilled water to dissolve the solid. Allow the two to react using method A. Characteristic colorless, highly refractive rhomb shaped tablets and prisms with angular ends are formed (see Figure 19-2e).
- **NO₂⁻** A mass of fine needles with long acicular prisms form (see Figure 19-2f) when nitrites are exposed to solutions of silver nitrate using method A. Upon standing, short stout prisms with imperfect ends are formed.
- **CO₃²⁻** When dilute hydrochloric acid is added to a carbonate containing compound using method A or B, effervescence occurs as carbon dioxide forms.

PART I: MICROCHEMICAL TESTING OF KNOWN SAMPLES

Procedure

Worksheets are included for this procedure at the end of this experiment and at <http://www.wileyeurope.com/college/wheeler>.

1. Samples containing known cations and anions will be provided.
2. Perform positive controls. Using the procedures listed above perform microchemical testing of the known samples.
3. Place the slide on a polarizing compound microscope and observe the test under 100X–200X magnification using plane polarized light. Be careful not to get any reagent on the objective. If this does occur ask the instructor how to clean the objective. Use the search and find technique

to locate any crystals that may have formed. It is especially important to look at the junction of the two drops and near the edges of the liquid.

4. Fully describe your observations in Table 19-1 for each of the known samples. Draw the characteristic crystals obtained and note any special distinguishing features observed.
5. Perform negative controls. For each test mix the reagents with a drop of water side-by-side to the positive control. Observe the negative control to ensure the reagent itself does not result in the observed reaction. Report the results in Table 19-2.
6. Once you have learned the methods used to perform the reactions and observe the results move to Part II.

PART II: MICROCHEMICAL UNKNOWNNS

1. Samples containing unknown cations and anions will be provided. Repeat the tests performed for the positive controls and report the results in Table 19-3.
2. In identifying the ions in your unknown sample, only one positive crystal test for each ion need be obtained.
3. As part of your lab report write a paragraph that justifies your answer.

MICROCHEMICAL TESTS WORKSHEETS

Table 19-1 Positive control using knowns.

Table 19-2 Negative control.

Table 19-3 Tests on unknown samples.

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 microchemical tests? Give the purpose and value of the tests.
2. List three reactions that are used for microchemical testing. Give an example of each.
3. If you were given a sample that was thought to be gypsum, how would you use microchemical testing to prove/disprove this?
4. If you were given an acid, how would you determine which acid it was?
5. What are positive and negative controls? Why are they required?
6. You have a white sample particle that is about $40 \mu\text{m} \times 50 \mu\text{m} \times 3 \mu\text{m}$ that you believe to be lead paint. Find a microchemical test for lead. Write the procedure and properly reference the literature source. A website is not an appropriate source for this question.
7. Write the chemical reaction responsible for carbon dioxide formation from acids reacting with carbonate ion.

RECOMMENDED AND FURTHER READING

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- Schaeffer HF. *Microscopy for Chemists*. New York: Dover Publications, 1966.

Experiment 20: Microscopic Analysis of Controlled Substances

Recommended pre-lab reading assignment:

- Moffat AC. *Clarke's Isolation and Identification of Drugs*. London, England: The Pharmaceutical Press; 1986; 128–47.
- Fiegl F. *Spot Test in Organic Analysis*. Amsterdam, Netherlands: Elsevier Scientific, 1966.
- Johns SH, Wist AA, Najam AR. Spot Tests: A Color Chart Reference for Forensic Chemists. *Journal of Forensic Sciences*. 1979; 24(3): 631–41.
- Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006; 270–294.

OBJECTIVE

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

1. difference in presumptive and confirmatory tests
2. color tests for drug testing
3. microcrystalline test for drug testing

INTRODUCTION

The microscope can be used to perform presumptive tests for forensic drug analysis. Presumptive tests are used to tentatively identify possible components of a sample. They are similar to field screening tests performed by police officers. These tests provide the analyst with an initial idea of when a drug might be present so that other tests can be performed to identify the substance conclusively. These additional tests are called confirmatory tests. Confirmatory tests identify the presence of a specific drug or drug metabolite (i.e., IR, GC-MS). The two main types of presumptive tests used for drug analysis are color tests and microcrystalline tests, both of which are sometimes called spot testing.

Color tests are the most common form of spot testing. They use reagents that react with a particular compound or a functional group. Forensic drug analysis targets three primary functional groups: phenols, aromatic rings, and amines. Since many drugs have more than one active group, understanding the mechanism for color can be quite complicated. In general, the most commonly used color tests are Duquenois-Levine for marijuana, cobalt thiocyanate for cocaine, Marquis for opium and amphetamines, and p-DMAB (p-dimethylaminobenzaldehyde) for LSD¹. The expected results for the color test to be performed in this experiment are given in Table 20-1 for some common substances.

Table 20-1 Expected results for common color tests.

Reagent	Classes of controlled substance	Expected positive result for some common drugs ¹
Duquenois-Levine	marijuana	marijuana (blue-violet color in the organic layer)
Cobalt Thiocyanate	cocaine or related substance	cocaine HCl (blue precipitate) cocaine (blue) quinine sulfate (blue) chlordiazepoxide HCl (no change)
Mandeline	steroids, alkaloids, aspirin	amphetamine sulfate (green) acetaminophen (olive green) aspirin (green) quinine sulfate (light green)
Marquis	opium derivatives, amphetamines, other alkaloids	amphetamine sulfate (green) methamphetamine HCl (light green) aspirin (green) d-propoxyphene HCl (brown/purple) benzphetamine HCl (effervescence, developing green) morphine HCl (olive) codeine phosphate (olive)
Mecke	alkaloids, opiates	oxycodone HCl (yellow → olive green) opium (green) morphine base (blue green) morphine HCl (green) heroin HCl (green-blue)

¹ Johns SH, Wist AA, Najam AR. Spot tests: A color chart reference for forensic chemists. *Journal of Forensic Sciences*. 1979; 24(3): 631–41.

Color tests are performed using a spot plate, which can be glass, ceramic, or a test tube. To perform the test a small amount of the unknown sample is placed in a well of the spot plate. A drop or two of the reagent(s) is added to the sample and the change in color is observed under a stereomicroscope. A known sample should be analyzed at the same time to ensure that the reagents are working properly. An important component of color testing is the use of control samples. A color change can occur even when the drug is not present due to contamination, side reactions, or

¹ Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006; 270.

oxidation. Therefore a blank or negative control is run using only reagent in the neighboring well of the spot plate. If a color change is observed with a negative control the test is said to give a false positive. If there is no color change when the reagent is tested on a known sample with a target analyte, the test is said to give a false negative. If a questioned sample contains only a small amount of the target analyte it is possible to obtain a negative result because the concentration of the analyte is below the detection limit of the test. Generally, presumptive tests have a detection limit of 1 to 50 µg². When a test gives a positive result with very small amounts of analyte it is said to be a sensitive test. However, this does not mean it can differentiate different drugs. In general, color tests react with certain classes of drugs and are not necessarily selective for an individual analyte.

Color tests are used extensively for screening purposes because of their ease of use and robustness. Microcrystalline tests on the other hand have the reputation of being difficult presumptive tests to perform. One reason for this is that crystal growth in general is tricky and has often been referred to as more of an art than a science. However, some microcrystalline tests are robust enough to have practical use in forensic science. In contrast to color testing microcrystalline testing can be much more selective if the analyst can learn to distinguish different crystal forms. For example, microcrystalline tests are useful for differentiation of optical isomers such as *d*-amphetamine from *d,l*-amphetamine and *d*-cocaine from *l*-cocaine³. Crystal formation requires a supersaturated solution, which is a solution whose concentration exceeds the solubility concentration. The solubility concentration is the concentration of the analyte in equilibrium with solid. Very high super-saturation will lead to precipitation or the formation of amorphous (disordered) solid. Concentrations below the solubility concentration will not produce any solid. Crystals form in the metastable region of the solubility diagram. During microcrystalline testing it is important to realize that the concentration of the reagents varies throughout the drops on the slide. A search and find technique is used to locate crystals that often form near the edges of the drop, where concentrations are higher due to evaporation or near the junction where the sample and the reagent are mixed. Crossed polars can be used in the search for crystals because it enhances our ability to see small crystals, as long as the crystals formed are not cubic.

EQUIPMENT AND SUPPLIES

Steromicroscope with magnifications of 10X–40X

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

Micro kit

Spot plate

Microscope slides

Toothpicks

Test tubes

Reagents and samples as listed with each test

² O'Neal CL, Crouch DJ, Fatah AA. Validation of Twelve Chemical Spot Tests for the Detection of Drugs of Abuse. *Forensic Science International*. 2000; 109: 189–201.

³ Allen AC, Cooper DA, Kiser WO, Cottrell RC. The Cocaine Diastereoisomers. *Journal of Forensic Sciences*. 1981; 26(1): 12–26.

SAFETY

Use standard laboratory safety procedures as described in rules set by your instructor. Be cautious of microscope light levels to avoid eye damage. Students should know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as set by your instructor. Refer to MSDS as needed. Students should wear personal protective equipment such as goggles and nitrile gloves for all parts of this lab. All work done with chloroform and organic solvents should be completed in a hood or with adequate ventilation. Several of the reagents contain concentrated acid solutions and should be handled with caution.

PART I: COLOR TEST

Duquenois-Levine Color Test for Marijuana

This spot test will identify the presence or absence of a 1,3-resorcinol structure in a suspected marijuana sample. A color change from gray to green through blue to violet-blue suggests the presence of cannabis. Roasted coffee and patchouli oil also produce a positive result. Fresh marijuana leaves give the best results.

Reagents needed

- Duquenois reagent: Mix 0.63 mL acetaldehyde, 0.5 g vanillin, and 25 mL of 95 % ethanol. Place into a dropper bottle and label
- Concentrated hydrochloric acid
- Chloroform

Known samples

Known samples include coffee beans and plant samples including marijuana.

Unknown sample

Obtain an unknown sample extract from your instructor and record its number in your notes.

Procedure

1. Observe the plant sample under the stereomicroscope. Pick a few leaves that have some obvious resin glands and place in a labeled test tube.
2. Grind a coffee bean in a mortar and pestle. Place a few coffee grinds in another labeled test tube.
3. Add 20 drops of Duquenois reagent and then 20 drops of concentrated HCl. Cover the test tube with a cork or Para-film™ and shake. A purple color should form.
4. Next add approximately 20 drops of chloroform to the vial. Cover and shake. The appearance of a blue-violet color in the organic layer (bottom layer) indicates a positive test for marijuana.
5. Repeat this test using an empty test tube for a control.
6. Repeat this test using your unknown sample.

7. Fully describe your observations in your notes of each of the known samples and the control. Assign Munsell™ color notation for each final color using the Munsell Color Book⁴. The Munsell color system is explained in Experiment 18.

Cobalt Thiocyanate Color Test for Cocaine and Related Drugs

In this test the pink solution of cobalt thiocyanate turns blue if cocaine or a related substance such as procaine is present through an ion-pairing mechanism. Procaine can be distinguished from cocaine because the blue product dissolves upon addition of stannous chloride.

Reagents needed

- Cobalt thiocyanate reagent: Mix 0.5 g of Co(SCN)₂ in 25 mL of water. Place into a dropper bottle and label.
- Stannous chloride reagent: 5.0 g of stannous chloride is added to 10 mL of concentrated HCl. Enough distilled water is added to produce 100 mL. Place in a dropper bottle and label.

Known samples

Known samples include solid forms of cocaine HCl, procaine HCl, quinine sulfate, and chlor diazepoxide HCl. *Note:* Substitution of 5–10 mg/mL standard solutions can be made in methanol. These solutions can be used for color testing by placing a few drops of the drug standard in each well. Due to the sensitivity of this test the 1 mg/mL standards solutions available commercially do not give a color change.

Unknown samples

Obtain an unknown sample from your instructor and record its number in your notes. A single unknown will be used for all color tests.

Procedure

1. Place a clean spot plate under the stereomicroscope. Place a 25 µL drop of the appropriate known in separate wells of the spot plate using the pipet and tips provided. For solid samples use only a few grains.
2. Add 1 drop of Co(SCN)₂ reagent dissolved to each well. Add 1 drop of Co(SCN)₂ to an empty well as a control. Note the presence or absence of blue color.
3. Add 2 drops of stannous chloride reagent. Note the persistence or disappearance of blue color. Also note any solid gelatinous precipitate that may form.
4. Fully describe your observations in your notes of each of the known samples and the control. Assign Munsell™ color notation for each final color using the Munsell Color Book. The Munsell color system is explained in Experiment 18.
5. Test your unknown sample using the same procedure and report your observations including Munsell color notation.

⁴ *Munsell Book of Color*, Matte Edition, may be purchased from the X-Rite Master Product Catalog. Available from: <http://www.xrite.com>.

Mandeline Color Test for Opium Derivatives, Amphetamines, and Other Alkaloids

A colored vanadium complex is formed with steroids, alkaloids, or aspirin. Many compounds give a color change with this reagent so it is important to interpret this test by comparing to a positive control. For example, hydrochlorides give a red color, and compounds containing ring bound sulfur produce a color as long as the ring does not contain more than one nitrogen atom, as do aromatic rings with saturated 5-, 6-, or 7- membered rings containing only one nitrogen atoms.

Reagent needed

- Mandelin reagent: Mix 0.25 g of ammonium vanadate in 25 mL of concentrated sulfuric acid. Place into a dropper bottle and label.

Known samples

Known samples include solid forms of amphetamine sulfate, acetaminophen, Excedrin, and quinine sulfate. *Note:* Substitution of 1 mg/mL standard solutions can be made. These solutions can be used for color testing by placing a few drops of the drug standard in each well. *This test works best when the color reagent is added directly to the 1 mg/mL solution.*

Unknown samples

Obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. Place a clean spot plate under the stereomicroscope. Place a 25 μ L drop of the appropriate known in separate wells of the spot plate using the pipet and tips provided. For solid samples use only a few grains.
2. Add 2 drops of the Mandelin reagent to each well and observe the color change. Add 2 drops of the Mandelin reagent to an empty well as a control.
3. Fully describe your observations in your notes of each of the known samples and the control. Assign Munsell™ color notation for each final color using the Munsell Color Book. The Munsell color system is explained in Experiment 18.
4. Test your unknown sample using the same procedure and report your observations including Munsell color notation.

Marquis Color Test for Opium Derivatives, Amphetamines, and Other Alkaloids

All colors of the visible spectrum can be obtained with this test so it is important to conduct positive controls. However, compounds that tend to produce color in decreasing order of efficacy are: ring sulfur, aromatic ring oxygen, and aromatic compounds consisting only of C, H, and N.

Reagent needed

- Marquis reagent: Mix 1 mL of 40 % formaldehyde mixed 20 mL of concentrated sulfuric acid. Place into a dropper bottle and label.

Known samples

Known samples include solid amphetamine sulfate, methamphetamine, aspirin, propoxyphene, benzphetamine, morphine HCl, and codeine. *Note:* Substitution of 1 mg/mL standard solutions for controlled substances can be made with the exception of methamphetamine, which needs a higher concentration. These solutions can be used for color testing by placing a few drops of the drug standard in each well. *This test works best when the color reagent is added directly to the 1 mg/mL solution.*

Unknown samples

Obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. Place a clean spot plate under the stereomicroscope. Place a 25 µL drop of the appropriate known in separate wells of the spot plate using the pipet and tips provided. For solid samples use only a few grains.
2. Add 2 drops of the Marquis reagent to each well and observe the color change. Add 2 drops of the Marquis reagent to an empty well as a control.
3. Fully describe your observations in your notes of each of the known samples and the negative control. Assign Munsell™ color notation for each final color using the Munsell Color Book. The Munsell color system is explained in Experiment 18.
4. Test your unknown sample using the same procedure and report your observations including Munsell color notation.

Mecke's Color Test for Opiates

The Mecke's reagent produces a green color when in contact with opiates.

Reagent needed

- Mecke's reagent: Mix 0.25 g selenious acid added to 25 mL concentrated sulfuric acid. Place in a dropper bottle and label.

Known samples

Known samples include solid samples of oxycodone HCl, nutmeg, and sugar. *Note:* Substitution of 1 mg/mL standard solutions for controlled substances can be made. These solutions can be used for color testing by placing a few drops of the drug standard in each well. *This test works best when the color reagent is added directly to the 1 mg/mL solution.*

Unknown samples

Obtain an unknown sample from your instructor and record its number in your notes.

Procedure

1. Place a clean spot plate under the stereomicroscope. Place a $25\ \mu\text{L}$ drop of the appropriate known in separate wells of the spot plate using the pipet and tips provided. For solid samples use only a few grains.
2. Add 2 drops of Mecke's reagent. To an empty well add 2 drops of Mecke's reagent as a control.
3. Fully describe your observations in your notes of each of the known samples and the negative control. Assign Munsell™ color notation for each final color using the Munsell Color Book. The Munsell color system is explained in Experiment 18.
4. Test your unknown sample using the same procedure and report your observations including Munsell color notation.

PART II: MICROCRYSTALLINE TESTS

Reagents needed

- Gold chloride reagent (5 % HAuCl_4) in a dropper bottle
- 3 M HCl in a dropper bottle

Known samples

Known samples include solid samples of aspirin, caffeine, and procaine, and standard solutions (1 mg/mL) of heroin and cocaine in methanol.

Unknown samples

Obtain an unknown sample from your instructor and record its number in your notes.

Procedure for Solid Samples

1. Obtain a mortar and pestle and grind the tablets to obtain a fine grain sample.
2. Obtain a clean microscope slide and sprinkle just a few grains of one of the known samples onto the surface. Dissolve the sample in a tiny drop of 3M HCl.
3. Next to this drop add a tiny drop of gold chloride reagent. Mix these two drops using method A of Figure 19-1. Using a toothpick draw the reagent drop to the sample drop. (If a precipitate immediately forms your analyte concentration was too high leading to high super saturation and you will need to repeat step 1 with less sample.)

Procedure for 1 mg/mL Standard Solutions

1. Add a drop of the standard solution to the inside of a glass ring that has been placed on a microscope slide (see method C of Figure 19-1 for arrangement). The glass ring prevents the standard solution from spreading out on the microscope slide. Allow all of the solvent to evaporate.

2. Remove the glass ring. Add a drop of crystallizing reagent to the region that has most of the dried drug solid (method B of Figure 19-1). A second crystallizing reagent drop can be added to another region of the same slide.

Procedure for Observing Crystals

1. Place the slide on a polarizing compound microscope and observe the reaction mixture under 200X magnification using crossed and uncrossed polars. Be careful not to get any reagent on the objective. If this does occur ask the instructor how to clean the objective. Use the search and find technique to locate any crystals that may have formed. It is especially important to look at the junction of the two drops and near the edges of the liquid where solvent evaporation may have lead to crystal formation. Use Figure 20-1 as a guide to crystal forms obtained for gold chloride. However, it is important to note that positive identification requires comparison to known standards and not to published photographs, because reagents and conditions may alter the crystal form.

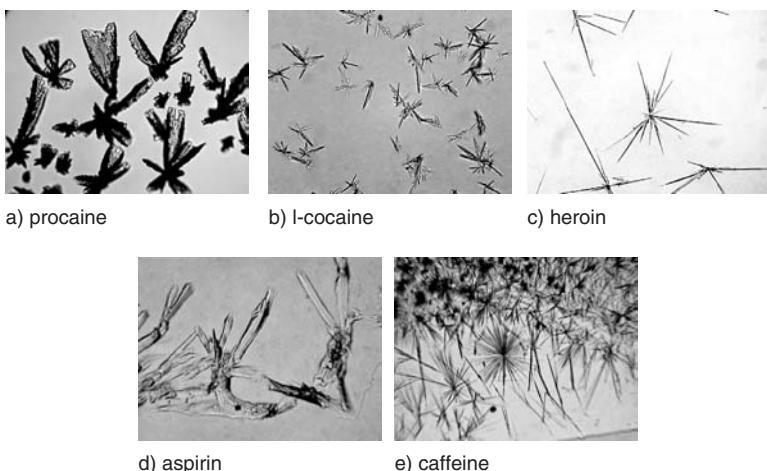


Figure 20-1 Crystal forms obtained using gold chloride (200X) from a) procaine(s); b) l-cocaine (1 mg/mL standard solution in methanol); c) heroin (1 mg/mL standard solution in methanol); d) aspirin(s); and caffeine(s).

2. If no crystals are found, set the microscope slide to the side and work with another drug sample. With time crystals may form so it is important to recheck the slide under crossed and uncrossed polars.
3. Fully describe your observations in your notes for each of the known samples.
4. Test your unknown sample using the same procedure and report your observations.

REPORT REQUIREMENTS

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

REPORT QUESTIONS

Part I: Color Tests

1. Write a paragraph describing how a color test is performed including the value and purpose of the test.
2. Define 'false positive.' Give three examples of a false positive color test.
3. Define 'false negative.' Give three examples of a false negative color test.
4. What test can be used to detect thiamine.
5. Describe the use of blanks and controls in spot testing.
6. What effect would mixtures have on spot test results?
7. What effect does time have on color test reagents?
8. Describe the difference between 'sensitivity' and 'selectivity' as they relate to presumptive tests. Are color tests or microcrystalline tests more selective?
9. Describe the Munsell Color System.
10. Estimate the color of an object that has a Munsell color notation of
 - a) 5YR 7/10
 - b) 2.5P 6/4.
11. What are some substances that will give a false positive for the Duquenois-Levine color test? Did you observe this with any of the samples you tested?

Part II: Microcrystalline Tests

1. Write a paragraph describing what microcrystalline tests are and how they are performed. Give the value and purpose of the test.
2. Define the following terms: precipitate, solubility, super saturation, and crystallization.
3. Why was the search for crystals carried out under crossed polars?
4. Explain why precipitate formed in some of the microcrystalline tests.
5. What are optical isomers?
6. Describe the difference between 'sensitivity' and 'selectivity' as they relate to presumptive tests. Are color tests or microcrystalline tests more selective?

RECOMMENDED AND FURTHER READING

- Cassista AR, Sandercock PML. Comparison and Identification of Automotive Topcoats: Microchemical Spot Tests, Microspectrophotometry, Pyrolysis-Gas Chromatography, and Diamond Anvil Cell FTIR. *Canadian Society of Forensic Science Journal*. 1994; 27: 209–23.
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- Fulton CC. *Modern Microcrystalline Tests for Drugs*. New York: John Wiley & Sons, Inc., 1969.
- Hauber DJ. Marijuana Analysis with Recording Botanical Features Present and without the Environmental Pollutants of the Duquenois-Levine Test. *Journal of Forensic Sciences*. 1992; 37(6): 1656–61.
- Jeffery W. Colour Tests. In: Moffat AC, Osselton MD, Widdop B, editors. *Clarke's Analysis of Drugs and Poisons*. London, UK: Pharmaceutical Press; 2004; 279–300.
- Johns SH, Wist AA, Najam AR. Spot Tests: A Color Chart Reference for Forensic Chemists. *Journal of Forensic Sciences*. 1979; 24(3): 631–41.
- Kebabj DE. The Differentiation of D and L Isomers of Propoxyphene by Mixed Crystal Test. *Microgram*. 1979; 12(11) – access restricted to direct application to the US Drug Enforcement Agency.

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- Novoseisky Y, Glattstein B, Volvok N, Zeichner A. Microchemical Spot Tests in Toolmark Examinations. *Journal of Forensic Sciences*. 1995; 40(5).
- O'Neal CL, Crouch DJ, Fatah AA. Validation of Twelve Chemical Spot Tests for the Detection of Drugs of Abuse. *Forensic Science International*. 2000; 109: 189–201.
- Schaeffer HF. *Microscopy for Chemists*. New York: Dover Publications, 1966.
- Swiatko J, De Forest PR, Zedeck MS. Further Studies on Spot Tests and Microcrystal Tests for Identification of Cocaine. *Journal of Forensic Sciences*. 2003; 48(3): 581–5.
- Wielbo D, Tebbett IR. The Use of Microcrystal Tests in Conjunction with Fourier-Transform Infrared-Spectroscopy for the Rapid Identification of Street Drugs. *Journal of Forensic Sciences*. 1992; 37(4): 1134–48.

Experiment 21: Semen Examinations

Recommended Pre-lab Reading:

Shaler R. Modern Forensic Biology. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2002; 525–613.

OBJECTIVE

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

1. microscopic identification of semen

INTRODUCTION

Seminal fluid is an organic liquid that is secreted by the sexual glands and organs of males. It is discharged through the process of ejaculation. Each human ejaculate is approximately 2–6 mL in volume. This fluid usually contains spermatozoa (sperm), which is the cellular structure that may be found within the seminal fluid. Some disease stages, genetic disorders, excessive drug or alcohol abuse, and elective surgical procedures can result in low or complete absence of sperm. It is the characterization of the sperm cell that is important to the forensic scientist.

A sperm cell is usually 55–70 μm in length. It is composed of a head, midsection, and a thin flagellate tail. The presence of sperm in a biological sample or stain is a conclusive test for semen. This usually indicates some type of sexual contact has occurred. Motile or intact sperm may be encountered by the forensic scientist. Motile sperm are active, living cells. Motile sperm may be present in samples collected from a vagina for 1–8 hours and present in samples collected from the cervix for several days. Intact sperm are cellular structures that are complete, but no longer living. Intact sperm may be present in samples collected from the vagina for up to 26 hours, present in post coitus samples from the rectum for 6–65 hours, and present in samples collected from the

mouth for rarely more than 6 hours. Because forensic scientists generally receive samples that have been previously collected, it is usually intact sperm that are identified when sperm are present.

Once the presumptive test for acid phosphatase has been performed, it is necessary to confirm the presence of semen through microscopic identification of the sperm cell. Sperm identification is done by differential staining. This process involves exposing the sample to various chemical solutions. Using multiple stains can distinguish between different structures or cellular components of a single organism. Depending on the sample, portions of the cellular structure may absorb stain. Once the cellular structure is visible, human and animal sperm can be easily differentiated. Most species have a similar sperm length as human sperm, however, the head shape and head size will be different. When viewing human sperm from the front, the head has an oval shape. It is approximately 4–5 μm in length and 2–3 μm in width. If the sperm cell is oriented on its side, the head will appear pear-shaped. Although the overall length of animal sperm is similar to human sperm, the attachment of the head and the size and shape of the head is completely different for animal sperm. Sperm from pets (dog and cat) and farm animals (bull, horse, sheep, and pig) can be differentiated from human sperm by the evident truncation where the head neck meets the midsection. Many animal species have sperm heads that are an elongated oval (i.e., horse, cow, goat, pig) and other animal sperm heads have completely different shapes. The sperm head of rat, turkey, and chicken are very thin and elongated, whereas moose, deer, and ram sperm head have very distinctive shapes and sizes. Depending on the sample origin and stain used to visualize the sperm, the head, midsection, and tail may also stain various colors. Two stains are commonly used in forensic laboratories: Christmas Tree stain and Hematoxylin-Eosin stain.

Complete forensic biology examinations involve the use of a variety of microscopes, techniques, and instruments. Presumptive and confirmatory tests are performed to identify semen in cases where sexual contact is suspected. DNA testing can be performed for additional information when necessary.

EQUIPMENT AND SUPPLIES

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

Biological samples

Micro kit

Reagents for staining (see specific stains below)

Immersion oil for use with oil objective

Centrifuge

Pipet tips

Test tubes

Pipettor needed for extraction

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Students should know the hazards associated with the solvents used in the procedures, and use them with appropriate precautions as

set by your instructor. Refer to MSDS as needed. Personal protective equipment (gloves, masks, safety glasses, etc.) should be used when working with biological materials.

PART I: SAMPLE SLIDE PREPARATION

Procedure

If a sample slide has not been provided (as in some sexual assault kits), it is necessary to extract the biological stain and make one.

1. Extract a portion of the stain by placing a small cutting of fabric in 1 mL of distilled water. Allow it to extract for approximately 20 minutes.
2. Place the cutting and liquid in a pipet tip that is over a test tube. This allows the liquid to collect in a test tube. Centrifuge for 3 minutes at approximately 3000 rpm.
3. Draw off the majority of the extract, being careful not to disturb the sample pellet at the bottom. Leave a few drops of extract with the pellet.
4. Mix the remaining drops and the pellet by pipetting up and down. This should re-dissolve the sample pellet.
5. Pipet the sample onto a microscope slide and allow the sample to thoroughly dry.

PART II: SPERM SLIDE STAINING

Christmas Tree Stain

The anterior portion of human sperm heads will stain clear to pink. The posterior portion of the head will stain dark pink to red. The midsection will stain blue and the tail will stain yellowish-green. Nucleated epithelial cells will also be visible as green rhomboid-like structures with a red/pink nucleus.

Reagents needed

- Stain A: Nuclear Fast Red: Mix 2.5 g aluminum sulfate in 100 ml warm water. Then add in 50 mg nuclear fast red. Filter when cool.
- Stain B: Picro Indigo Carmine: Mix 1.3 g picric acid in 100 ml warm water. Add 0.33 g indigo carmine and stir overnight.

Procedure

1. Using a dried sample slide, place a sufficient quantity of Christmas Tree stain A to cover the sample. Allow it to sit for approximately 5–10 seconds.
2. Gently rinse the slide with distilled water to remove the Christmas Tree stain A.
3. Apply Christmas Tree Stain B (green stain) to the slide. Allow it to sit for 5–10 seconds.
4. Gently rinse the slide with 95 % ethanol to remove the Christmas Tree stain B.
5. Allow the slide to air dry.

6. Apply 1–2 drops of immersion oil to the sample slide. View the slide with the compound light microscope using a 100X oil immersion objective. Identify any sperm cells present.

Hematoxylin-Eosin

Human sperm heads will stain purple, while the tail portion will stain pink.

Reagents needed

- Solution A: Formal alcohol: Mix 260 ml 95 % ethanol, 90 ml distilled water, and 150 ml 30 % formaldehyde.
- Solution B: Hematoxylin: Mix 1 g Hematoxylin, 0.2 g sodium iodate, 50 g aluminum potassium sulfate, and 1 l distilled water. Filter before use.
- Solution C: Acid alcohol: Mix 350 ml of 95 % ethanol, 125 ml distilled water, and 25 ml concentrated hydrochloric acid.
- Solution D: 0.5 % Disodium phosphate: Mix 2.5 g disodium phosphate with 500 ml distilled water.
- Solution E: 2 % Eosin: Mix 10 g eosin Y with 500 ml distilled water.

Procedure

1. Using a dried sample slide, place a sufficient quantity of solution A to cover the sample. Allow it to sit for approximately 30 seconds.
2. Gently rinse the slide with distilled water to remove solution A.
3. Apply solution B. Allow it to sit for approximately 10 minutes.
4. Gently rinse the slide with distilled water to remove solution B.
5. *Omit this step if using modified Mayer's Hematoxylin.* Apply solution C. Allow it to sit for approximately 30 seconds. Gently rinse the slide with distilled water.
6. Apply solution D. Allow it to sit for approximately 30 seconds.
7. Gently rinse the slide with distilled water to remove solution D.
8. Apply solution E. Allow it to sit for approximately 15–20 seconds.
9. Gently rinse the slide with distilled water to remove solution E.
10. Allow the slide to air dry.
11. Apply 1–2 drops of immersion oil to the sample slide. View the slide with the compound light microscope using a 100X oil immersion objective. Identify any sperm cells present.

REPORT REQUIREMENTS

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

REPORT QUESTIONS

1. What is the difference between a presumptive and confirmatory test?

2. Explain differential staining as it relates to forensics.
3. Define motile and intact sperm cells.
4. How long after a sexual assault would you expect to find identifiable sperm?
5. How is human sperm differentiated from dog sperm?
6. Will human sperm always be identified in cases of sexual contact?

RECOMMENDED AND FURTHER READING

- Aiken MM, Muram D, Keene PR, Mamelli J. Evidence Collection in Cases of Child-Abuse – the Detection of Seminal Fluid. *Adolescent and Pediatric Gynecology*. 1993; 6(2): 86–90.
- Albrecht K, Schultheiss D. Seminal Stains in Legal Medicine. An Historical Review of Forensic Proof. *Urologe A*. 2005; 44(5): 530.
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- Rand S, Wiegand P, Brinkmann B. Problems Associated with the DNA Analysis of Stains. *International Journal of Legal Medicine*. 1991; 104(5): 293–7.
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- Shaler R. Modern Forensic Biology. In: Saferstein R, ed. *Forensic Science Handbook*. Upper Saddle River, NJ: Pearson Education; 2005; 525–613.

Instrumental Microscopy

Experiment 22: Fourier Transform Infrared Microspectrometry

Recommended pre-lab reading assignments:

- Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006; 149–169.
- Ryland SG. Infrared Microspectroscopy of Forensic Paint Evidence. In: Humecki H, ed. *Practical Guide to Infrared Microspectroscopy*. New York, NY: Marcel Dekker; 1995; 163–243.
- Kirkbridge KP, Tungol MW. Infrared Microspectroscopy of Fibres. In: Robertson J, Grieve M, eds. *Forensic Examination of Fibres*. London: Taylor and Francis; 1999; 179–222.
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OBJECTIVE

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

1. general infrared spectrometry principles
2. general micro-FTIR principles
3. sample preparation for micro-FTIR analysis
4. use of the FTIR microspectrometer to identify paint characteristics
5. use of the FTIR microspectrometer to identify fiber characteristics

INTRODUCTION

Another microscopic application adapted to a conventional instrument is the Fourier transform infrared (FTIR) microspectrometer. Since this spectrometer measures the interaction of infrared light with matter we must first discuss basic principles of light and matter.

Light is found all around us in the form of the electromagnetic radiation. When an electric field joins perpendicularly and in phase with a magnetic field, electromagnetic waves are formed.

The electromagnetic spectrum defines the array of radiation as we know it into specific ranges. The various electromagnetic ranges are determined by the wavelength. The distance between one wave crest to the next is called the wavelength. Waves vary from very long radio waves to very short gamma-rays. The area of the electromagnetic spectrum that interests us in this experiment – infrared light – falls within a narrow range.

Infrared light is found within the electromagnetic spectrum from the rear end of the visible spectrum to the microwaves, including radiation at wavelengths from approximately 14,000 nm to 20 nm. The spectral range of greatest use is the mid-infrared region, which covers the frequency range from 200 cm^{-1} to 4000 cm^{-1} . Infrared spectroscopy involves the interaction of infrared light with matter. Twisting, bending, rotating, and vibration motions of the atoms occur within a molecule when subjected to infrared light. Upon interaction, portions of the incident radiation are absorbed at particular wavelengths. The uniqueness of the infrared spectrum arises from the multiplicity of vibrations occurring simultaneously. This is displayed by absorption, which is characteristic of the functional groups comprising the molecule and the overall configuration of the atoms as well. The absorption results in a peak in a spectrum that is a graph of wavelength versus percent transmittance (%T), which is related to absorption by $A = 2 - \log \%T$.

The FTIR spectrometer (see Figure 22-1) is composed of an interferometer, which is an instrument that divides a beam of light into two beams and then recombines the light after introducing a phase difference in one of the beams. The light beam from the source is split with a beam splitter and reflected off two mirrors to be recombined at the beam splitter. One of the mirrors is fixed and the other movable. It is the moving mirror that introduces the phase difference. Combining

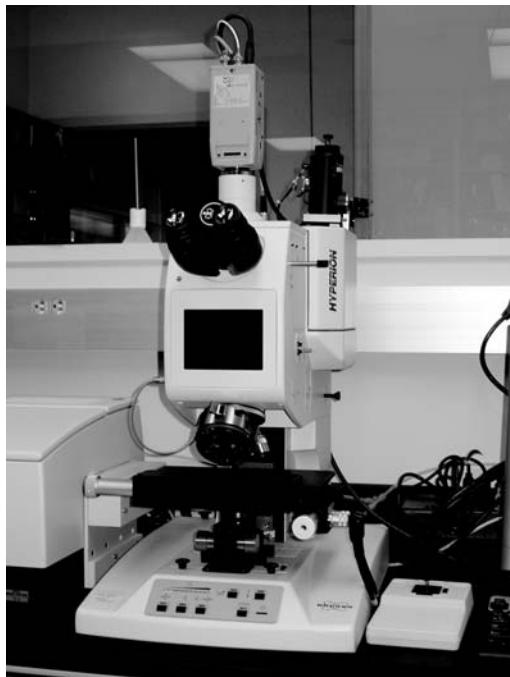


Figure 22-1 A Bruker™ FTIR microspectrometer. This instrument allows forensic scientists to acquire FTIR data from microscopic size forensic samples. While the light beam is generated from the FTIR bench, a separate detector is used for the microscope.

two beams of light that are ‘in phase’ results in constructive interference and a resulting intensity enhancement. The combining of light that is ‘out of phase,’ results in destructive interference and an intensity decrease. If the intensity of the two beams of light remain the same and the phase difference is introduced incrementally then the intensity difference will manifest itself as a cosine wave, which has a frequency dependent upon the speed of the moving mirror. This resultant waveform is called an interferogram. If a sample is placed in the beam the cosine wave will be altered according to whatever interaction the sample will have with the light itself.

In an infrared spectrometer, all wavelengths are measured at the same time. The resultant waveform is quite complex, but mathematically can be solved by use of the Fourier Transform. The moving mirror increments the phase differences in small fractions of a wavelength (frequency). This means that the position of the mirror is critical to any meaningful measurement. Using mechanical measuring devices for mirror positions proved inadequate for frequency measurements. This brought about the advent of the double-beam interferometer. The double-beam interferometer uses a second mirror and laser light source. Laser light is monochromatic and the interferogram is fairly simple and easy to track. By counting the highs and lows in the laser interferogram it is possible to track the mirror position very precisely. Being able to track the mirror produces a reproducible spectrum and allows for the use of multiple scan averaging. Multiple scan averaging improves signal to noise ratios and results in cleaner spectra. The most common detectors for FTIR are the triglycine sulfate (TGS) and the mercury-cadmium-telluride (MCT). The TGS operates at room temperature but the MCT must be cooled with liquid nitrogen. These detectors respond only to the modified signal coming from the interferometer and therefore are not subject to stray light or emission from the sample. Usually the bench operates with a TGS detector and the microscope attachment has a MCT detector.

The power of FTIR microspectroscopy is that it provides information about the chemical structure of the evidence being examined. Fibers and paint are composed of bonded atoms, which rotate, bend, and vibrate in different modes when placed in infrared light. To determine the type of bonds and atoms in the molecule the peaks in the spectrum must be interpreted. Table 22-1 lists the common bond types for the functional group regions.

Table 22-1 Common bond types for the functional group region.

Region (cm^{-1})	Bond type	Frequency (cm^{-1}), intensity, shape
4000–2700	OH	3600–3500 s, broad
4000–2700	NH ₂ or NH	3500–3300 w–m, broad
4000–2700	=C–H	3100–3000 w
4000–2700	–C–H	3000–2800 s
2700–1850	C=N	2300–2200 w
1850–1500	C=O	1780–1650 s
1850–1500	C=C	1670–1640 w–m

Two regions are assigned to the infrared spectrum. The first region is below 2000 cm^{-1} and is the so called ‘fingerprint region’, which is named after the numerous peaks that can be used to distinguish two samples. This region can be difficult to interpret due to the large number of peaks, mainly due to bending type vibrations. Therefore, it is best to start interpretation at the high wave numbers and identify functional groups first. The functional group region has fewer peaks that remain fairly consistent. In addition to the location of the peak it is useful to interpret

the intensity of the peak, which is related to the path length of the sample and the magnitude of the dipole moment change incurred during vibration. In addition to manual interpretation there are numerous databases and computer library searches available that can be used to identify the exact composition of the evidence.

The shape and thickness of samples becomes very important when using the microscope on the FTIR spectrometer. The ideal sample is approximately 5–15 µm thick. However, even thick samples can be sufficiently prepared by cutting and flattening them prior to acquiring a spectrum.

Examinations of paint are usually performed to identify a paint and then to compare a paint back to a possible paint source. Initially a stereomicroscope is used to examine the paint for layer structure and their solubilities in different solvents (Experiment 14). FTIR microspectrometry is an additional technique that may be used to identify and compare the many layers found in a paint sample. Original finish automotive paints will have absorption peaks at 1550 and 815. Automotive repaints will not have these bands. Using functional group interpretation of paint samples, many binders, resins, pigments, and extenders can often be identified. Common binders and resins are listed in Table 22-2. Common pigments and extenders, which may be identified by micro-FTIR, are listed in Table 22-3.

Table 22-2 Absorption bands for some common paint binders and resins.

Binder/resin	Identifying absorption bands (cm ⁻¹)									
Acrylic	1450	1380	1270/1240	1150*	1070	840	750	705		
Acrylic-melamine	1550*	1480	1370	1170*	1090	815*	(may also have 1270/1240)			
Acrylic-alkyd	1260*	1180*	1130	1070						
Acrylic-urethane	1530*	1240*	1170	1070						
Orthophthalic alkyd	1450	1380	1270*	1130*	1070*	740	700			
Isophthalic alkyd	1475	1373	1305	1237*	1135	1074	730*			
Terephthalic alkyd	1270	1250*	1120	1105	1020	730*				
Alkyd-melamine	1550*	1270*	1120	1070	815*	740	700			
Alkyd-urea	1650*	1540*	1270	1120	1070	770*	740	705		
Polyester-melamine	1550*	1330*	1240*	815*	750	730	705			
Benzoguanamine	1590	1540	825	780	710					
Epoxy	1510*	1240	1180	830*						
Melamine	1550	815								
Nitrocellulose	1650*	1280*	840*	750						
Polybutadiene	970	915								
Polyurethane	1690	1530*	1470	1250	1070					
epoxy modified	1730	1510								
water based	1690	770								
Styrene	1490	1450	760	700						
Styrene-butadiene	1600	1495	1450	760*	700*					
Urea	1655									
PVA	1735	1370	1240*	1135–1020	945*	605*				

*main absorption bands

Table 22-3 Absorption bands for some common paint pigments and extenders.

Pigment/extender	Identifying absorption bands (cm^{-1})									
Calcium carbonate										
aragonite	1445	870	857	712	317					
calcite	1445	870	712	317						
Barium chromate	935	896	860							
Potassium zinc chromate	950	880	805							
Strontium chromate	911	887	875	844						
Chromium oxide	680	634	582	446	417	400				
Iron oxide										
red	560–530	480–440	350–310							
yellow	899	797	606	405	278					
Silicon dioxide										
cristobalite	1090	795	621	485	387	300				
diatomaceous silica	1100	800	480							
quartz	1081	798	779	512	460	397	373			
Titanium dioxide										
rutile	600	410	340							
anatase	600	340								
Zinc phosphate	1120	1080	1020	950	630					
Zinc oxide	1096	888	520	501	401					
Silicate										
talc	1030	1015	670	460	450	420	390	345		
clay	1120	1030	1010	940	910	540	470	430	350	280
mica	1065	1032	936	834	756	699	535	478	411	
Barium sulfate	1175	1080	980	640	610					
Lead carbonate	1412	1047	848	695	683	404				
Lead sulfate	1410	1172	1078	969	687	632	600	428	363	
Cobalt aluminate	1102	1035	1012	905	735	652	558	508	239	
Cuprous oxide	626	425								

Fiber examinations were discussed in Experiments 17, 17A, 17B, and 17C. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine optical properties such as refractive index and extinction. At times, burn or solubility tests may also be performed during man-made fiber examinations. FTIR microspectrometry is an additional technique that is frequently used in fiber examinations because of the characterization potential of FTIR.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Paint samples

Fiber samples

- Micro kit
- Microscope slides
- FTIR microspectrometer (salt plate and salt plate holder)
- Liquid nitrogen, thermos, and cryo-gloves
- Small metal roller

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Use of safety glasses and cryo-gloves when handling liquid nitrogen is recommended. Refer to MSDS if necessary.

PART I: FAMILIARIZATION WITH THE FTIR

Since each instrument may be operated differently and use different software, please refer to the operational manual of your instrument to familiarize yourself with operating the instrument.

PART II: PAINT

Procedure

1. Obtain a paint sample.
2. Cut a thin slice from the top layer. This may be either a clear or colored layer.
3. Place the slice on a hard surface (microscope slide) and flatten it using the roller.
4. Obtain a FTIR spectrum using the microscope attachment. Remember, the sensitivity of the microscope detector cuts off around 650 nm.
5. Identify any binders, pigments, extenders, or resins present in the top layer of the paint sample.
6. Cut a thin slice from the primer layer.
7. Place the slice on a hard surface (microscope slide) and flatten it using the roller.
8. Obtain a FTIR spectrum using the microscope attachment. Remember, the sensitivity of the microscope detector cuts off around 650 nm.
9. Identify any binders, pigments, extenders, or resins present in the primer layer of the paint sample.
10. Repeat steps 2–9 for any assigned paints.
11. Include a copy of the FTIR spectra obtained from the paint samples. Determine any possible pigments, binders, extenders, or resins present in the paint sample. Give the chemical structures of these ingredients. Relate at least two peaks in the spectra to chemical structures present in the paint.

PART III: FIBERS

Procedure

1. Obtain a fiber sample.

2. Cut a small section of fiber.
3. Place it on a hard surface (microscope slide) and flatten it using the roller.
4. Obtain a FTIR spectrum using the microscope attachment. Remember, the sensitivity of the microscope detector cuts off around 650 nm.
5. Identify the fiber.
6. Repeat steps 2–5 for any assigned fibers.
7. Include a copy of the FTIR spectra obtained from the fiber samples. Draw the chemical structure of the fiber and its chemical formula. Relate at least two peaks in the spectra to the chemical structure of the fiber.

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. Explain how a FTIR microspectrometer operates. Include a drawing of the mechanics of the bench of an FTIR.
2. Why is this a useful instrument to forensic laboratories?
3. Include a copy of the FTIR spectra obtained from the fiber samples. Determine the identity of the fiber. Give the chemical structure of the fiber and relate at least two peaks to the chemical structure.
4. The Scientific Working Group for Materials Examinations (SWGMA) Forensic Paint Analysis and Comparison Guidelines state that ‘Certain types of coatings, including automotive undercoats and many types of architectural coatings..., usually contain significant amounts of inorganic pigments.’ Since these pigments tend to have most of their significant infrared absorptions in the lower frequency spectral regions, some well below 700 nm, how can these be identified with a FTIR?
5. The American Society for Testing and Materials (ASTM) guidelines for fiber examinations state that microscopic examination is indispensable for identification of cellulose and animal fibers, while infrared and solubility relationships are essential for identifying man-made fibers. Explain why.

RECOMMENDED AND FURTHER READING

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Experiment 23: UV-Visible-NIR Microspectrophotometry

Recommended pre-lab reading assignments:

Bell S. *Forensic Chemistry*. Upper Saddle River, NJ: Pearson Education, 2006; 149–161.

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OBJECTIVE

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

1. general microspectroscopy principles
2. sample preparation for analysis with the microspectrophotometer
3. use of the microspectrophotometer to analyze paint characteristics
4. use of the microspectrophotometer to analyze fiber characteristics
5. use of the microspectrophotometer to analyze ink characteristics

INTRODUCTION

Spectroscopy is the branch of science dealing with the theory and interpretation of spectra. In Experiment 22, infrared light was used to produce an infrared spectrum in order to gain structural information about the molecule. The UV-visible-NIR microspectrophotometer on the other hand is used to gain information about the ultra-violet (UV) region (190–400 nm), visible region (400–800 nm), and near-infrared (NIR) region (800–2500 nm) of the electromagnetic spectrum.

The visible region provides information about the color of the sample under study. The ability to determine color accurately is an important skill for forensic scientists. The analyst must rely on color changes determined in presumptive tests, and also in the comparison of colors of fibers, paints, and inks frequently encountered in case work. There are many disadvantages in determining

color by visual inspection. First, each person perceives colors differently. Second, color can be difficult to describe without a common color language. Finally, the color observed depends on the source of illumination, which is often not considered in visual exams. Use of an instrument to obtain a color spectrum avoids these problems and provides additional information for regions of the spectrum outside those seen by the human eye.

In addition to color measurements within the visible spectral range, a microspectrophotometer provides measurements in the UV and NIR range (see Figure 23-1). In the UV region only two types of electronic transitions can occur. Therefore, only compounds containing π electrons will contribute peaks in this region. This will provide information about the presence or absence of double bonds and conjugation in the sample. The NIR range yields information about the vibrational and rotational transitions for molecules such as titanium oxide found in paint evidence and carbon dioxide.



Figure 23-1 A SEET™ UV-Visible-NIR microspectrophotometer. This instrument allows forensic scientists to acquire data from microscopic size forensic samples. Several settings and light sources are used to view microscopic samples by transmittance, absorbance, reflectance, and fluorescence.

Four types of spectra can be obtained by a microspectrophotometer:

- transmittance
- absorbance
- reflectance
- fluorescence

These will each be described briefly. Transmission spectra can be obtained by measuring the amount of light transmitted by the sample at each wavelength. Transmitted light (T) is determined by the following equation:

$$T = \frac{I}{I_0} \quad (23-1)$$

where I_0 is the intensity of radiant energy striking the sample and I is the intensity of radiant energy emerging from the sample.

These types of spectra are useful for comparing transparent evidence. A more common spectrum is the absorbance spectra, which is obtained by measuring the amount of light absorbed (A) at each wavelength. A is measured by

$$A = -\log T = \log \frac{I_0}{I} \quad (23-2)$$

The Beer-Lambert Law relates transmittance, sample thickness, and concentration according to

$$A = \varepsilon bc \quad (23-3)$$

where ε is the molar absorptivity, b is the path length, and c is the concentration of the absorbing species.

It is the relationship between absorbance and path length that at times requires samples to be flattened with a metal roller. This reduces the path length, b , and the absorbance, A , allowing consistent spectra to be obtained.

If the sample is opaque, such as paint samples, it is not possible to measure the amount of light that can pass through the sample. To determine the visible spectrum of these materials the light reflected off the samples is measured with wavelength. There are two ways to obtain a reflectance spectrum. The first is known as the relative reflectance. Here the final spectrum of the sample contains some information about a reference material. Equation 23-4 determines the reflectivity, R :

$$R = \frac{I}{I_0} \quad (23-4)$$

where I_0 is the intensity of energy reflected from the reference sample and I is the intensity of energy reflected from the sample.

Comparing to a reference material corrects for variations in source intensity and detector sensitivity with wavelength. However, there are times when it is not possible to obtain a suitable reference location. In this situation an absolute reflectance spectra can be measured where the final spectrum of the sample is independent of a reference material.

Finally, some samples contain chromophores, which fluoresce when excited with light of particular wavelength. Fluorescence is the emission of light as molecules return to their ground state configuration. Fluorescence (F) is measured in counts and not corrected to any standard because it is measured according to the following equation:

$$F = I \quad (23-5)$$

where I is the intensity of radiant energy measured by the detector.

In most situations, the forensic scientist performs examinations in the reflectance, transmittance, and fluorescence modes.

In a typical spectrophotometer white light containing all wavelengths passes through the sample. The sample absorbs certain wavelengths of light depending on the electronic structure of the

molecules in the sample. Light that is not absorbed enters a monochromator, which separates the wavelengths of light, which are in turn detected by an array detector. The detector array measures the intensity of light at each wavelength. A microspectrophotometer differs from a typical spectrophotometer only in that the amount of light transmitted or reflected by the sample is collected by the objective and imaged onto the mirrored aperture of the attached spectrophotometer, the ocular, or a digital video camera (see Figure 23-2). The spectrophotometer separates the light into its component wavelengths using a holographic grating. The separated rays of light are imaged onto a charged-coupled device (CCD) detector array where their intensity is measured. The intensity and wavelength are then converted to a spectrum.

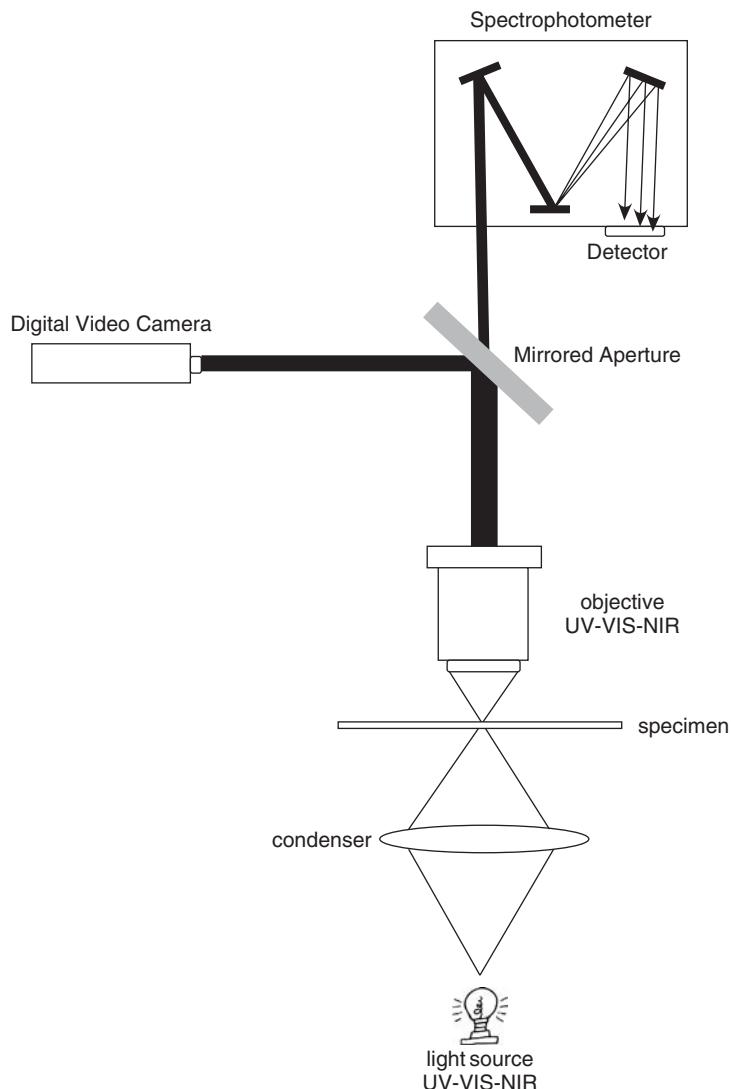


Figure 23-2 Optical path of a UV-VIS-NIR microspectrophotometer.

Microspectrophotometers are single-beam instruments, so a standard or background spectrum is acquired prior to analyzing the sample. The resulting sample data is obtained by comparing the light reflected, transmitted, or fluoresced from the sample to the light reflected, transmitted, or fluoresced from the reference standard. This is visualized by a spectrum, which is plotted with wavelength on the X-axis while the Y-axis can be transmittance, absorbance, reflectance, or fluorescent intensity. The many different types of spectra that can be acquired mean that it is important for the forensic scientist to pay special attention to the axes (i.e., reflectance, transmittance, fluorescence) when interpreting spectra. As with any spectral interpretation it is the location of peaks, as well as their intensities, which relates to the electronic structure of the molecules in the samples.

The microspectrophotometer is a sensitive instrument that can detect small changes in wavelength and light intensity variations, which the human eye cannot. It is also able to distinguish metamer differences. Metamer differences are when two samples may appear to be the same color under one set of lighting conditions, but exhibit different colors when exposed to a different set of lighting conditions. Because of these facts, use of the microspectrophotometer is one of the first examinations done on paint, fiber, and ink evidence.

Examinations of paint are usually performed to identify a paint and then to compare a paint back to a possible paint source. Initially a stereomicroscope is used to examine the paint for layer structure and their solubilities in different solvents (Experiment 14). Paints may also be examined with the FTIR microspectrophotometer (Experiment 22). Further examination of paint by the microspectrophotometer provides additional information. Paint samples are usually examined as thin sections by transmitted light. This provides information concerning the UV absorbers contained in the clear coat, and classifications of pigments. In some cases, such as metallic layers, reflectance may also be necessary.

Fiber examinations were discussed in Experiments 17, 17A, 17B and 17C. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. Further testing can be performed using a compound or comparison light microscope to determine optical properties such as refractive index and extinction. At times, burn or solubility tests may also be performed during man-made fiber examinations. Fibers may also be examined with the FTIR microspectrometer (Experiment 22). Further examination of fibers by the microspectrophotometer provides additional information. Both natural and manufactured fibers may be examined with the microspectrophotometer. Because of the nature of the manufacturing process, most manufactured fibers are dyed fairly consistently. Natural fibers, however, are generally not dyed uniformly. Therefore, the heterogeneity of the dye must be taken into consideration for fiber examinations. Multiple examinations should be obtained for each fiber, whether it is natural or manufactured. It is also very important to note the orientation of the fiber while examinations are being conducted because some fibers exhibit pleochroism. Information concerning fiber treatments such as whiteners and brighteners may be obtained by UV examination.

Ink compositions may also be examined with the microspectrophotometer, usually in reflectance mode using a clean portion of the paper as the reference. As ink is absorbed by the paper fibers, the heterogeneity of the ink should also be taken into consideration when performing examinations. It is possible to compare ink spectra on a single sheet of paper. However, it is difficult to compare ink spectra from different sheets of paper because the resulting spectra are made up of several components including the ink and the paper.

EQUIPMENT AND SUPPLIES

Stereomicroscope

Paint samples

Fiber samples
Ink samples
Micro kit
Quartz microscope slides
Quartz cover slips
Mounting media (XAMTM, glycerol, PhytohistolTM, FluoroMountTM, PermountTM, Norland 65TM)
Microspectrophotometer with transmittance, reflectance and fluorescence lamps, reference filter, Holmium Oxide filter, Didymium filter
Small metal roller

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Refer to MSDS if necessary.

PART I: FAMILIARIZATION WITH THE MICROSPECTROPHOTOMETER

Since each instrument may be operated differently and use different software, please refer to the operational manual of your instrument to familiarize yourself with operating the instrument.

PART II: PAINT

Procedure

1. Obtain a paint sample.
2. Using the stereomicroscope, cut a thin slice from the top layer. This may be either a clear or colored layer.
3. Place the slice on a clean hard surface such as a microscope slide and flatten it using the roller. Mount the sample using a quartz microscope slide and cover slip and a non-fluorescing mounting media.
4. Obtain a UV and/or visible NIR spectrum using the microspectrophotometer with transmitted light.
5. Cut a thin slice from the second layer. This may be a color coat or a primer layer.
6. Place the slice on a clean hard surface such as a microscope slide and flatten it using the roller. Mount the sample using a quartz microscope slide and cover slip and a non-fluorescing mounting media.
7. Obtain a UV and/or visible NIR spectrum using the microspectrophotometer with transmitted light.
8. Repeat steps 5–7 for each additional layer.
9. Obtain an unknown paint sample.
10. Using steps 2–8, analyze each layer of the sample.
11. Compare your results from each sample for each layer. Include a copy of the spectra obtained from the paint samples.

PART III: FIBERS

Procedure

1. Obtain a fiber sample.
2. Place it on a clean hard surface such as a microscope slide and flatten it using the roller. Mount the sample using a quartz microscope slide and cover slip and a non-fluorescing mounting media.
3. Obtain a UV and/or visible NIR spectrum using the microspectrophotometer with transmitted light. Make sure to test several sections of the fiber to determine the degree of dye heterogeneity.
4. Obtain a visible NIR spectrum using the microspectrophotometer with transmitted light with the light source to obtain sample fluorescence.
5. Obtain an unknown fiber sample.
6. Using steps 2–4, analyze the sample.
7. Compare your results from each sample. Include a copy of the spectra obtained from the fiber samples.

PART IV: INK

Procedure

1. Obtain an ink sample.
2. Using the stereomicroscope, separate several paper fibers with ink samples. Place one on a clean hard surface such as a microscope slide and flatten it using the roller. Mount the sample using a quartz microscope slide and cover slip and a non-fluorescing mounting media.
3. Obtain a UV and/or visible NIR spectrum using the microspectrophotometer with transmitted light. Make sure to test several sections of the ink sample to determine the degree of dye heterogeneity.
4. Obtain a visible NIR spectrum using the microspectrophotometer with transmitted light with the light source to obtain sample fluorescence.
5. Obtain a UV and/or visible NIR spectrum using the microspectrophotometer with reflected light. Make sure to test several sections of the ink sample to determine the degree of dye heterogeneity.
6. Obtain a visible NIR spectrum using the microspectrophotometer with reflected light with the light source to obtain sample fluorescence.
7. Compare your results using transmitted light and reflected light.
8. Obtain an unknown ink sample.
9. Analyze the unknown sample using the lighting (transmitted or reflected) with which you obtained the better results for your previous sample.
10. Compare your results from the unknown sample to the previously examined sample. Include a copy of the spectra obtained from the ink samples.

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. Define transmittance, absorbance, reflectance, and fluorescence.
2. Explain how the microspectrophotometer works.
3. Can you think of an example of two samples that would have different UV visible NIR spectra but similar colors? Explain your answer.
4. Name three samples that would require you to run the microspectrophotometer in reflectance mode.
5. Give two examples of evidence that would use the fluorescence mode of a microspectrophotometer.
6. Why are fiber samples rolled before their spectra are obtained?

RECOMMENDED AND FURTHER READING

- Adolf FP, Dunlop J. *Microspectrophotometry/Colour Measurement*. 2nd ed. London: Francis & Taylor, 1999.
- Cassista AR, Sandercock PML. Comparison and Identification of Automotive Topcoats: Microchemical Spot Tests, Microspectrophotometry, Pyrolysis-Gas Chromatography, and Diamond Anvil Cell FTIR. *Canadian Society of Forensic Science Journal*. 1994; 27: 209–23.
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- Enoch J, Tobey F. Microspectrophotometry and Optical Phenomena: Birefringence, Dichroism and Anomalous Dispersion. *Springer Series in Optical Sciences*. 1981; 23: 337–99.
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- Hartshorne AW, Laing DK. Microspectrofluorimetry of Fluorescent Dyes and Brighteners on Single Textile Fibres: Part 1 – Fluorescence Emission Spectra. *Forensic Science International*. 1991; 51: 203–20.
- Hartshorne AW, Laing DK. Microspectrofluorimetry of Fluorescent Dyes and Brighteners on Single Textile Fibres: Part 2 – Colour Measurements. *Forensic Science International*. 1991; 51: 221–37.
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- Kopchick KA, Bommarito CR. Color Analysis of Apparently Achromatic Automotive Paints by Visible Microspectrophotometry. *Journal of Forensic Sciences*. 2006; 51(2): 340–3.
- Saitoh N, Akiba N. Ultraviolet Fluorescence Imaging of Fingerprints. *Scientific World Journal*. 2006; 6: 691–9.
- Wiggins K, Holness JA. A Further Study of Dye Batch Variation in Textile and Carpet Fibres. *Science & Justice*. 2005; 45(2): 93–6.
- Wiggins KG, Holness JA, March BM. The Importance of Thin Layer Chromatography and UV Microspectrophotometry in the Analysis of Reactive Dyes Released from Wool and Cotton Fibers. *Journal of Forensic Sciences*. 2005; 50(2): 364–8.

Experiment 24: Thermal Microscopy

Recommended pre-lab reading assignments:

- Grieve MC. The Use of Melting Point and Refractive Index Determination to Compare Colorless Polyester Fibres. *Forensic Science International*. 1983; 22: 31–48.
- Hartshoene AW, Wild FM. The Discrimination of Cellulose di- and tri- Acetate Fibers by Solvent Tests and Melting Point Determination. *Journal of Forensic Science Society*. 1991; 31(4): 457–61.
- Cassista AR, Sandercock P. Precision of Glass Refractive Index Measurements: Temperature Variation and Double Variation Methods and the Value of Dispersion. *Canadian Society of Forensic Science Journal*. 1994; 27(3): 203–8.

OBJECTIVE

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

1. set up, operation, and use of a hot stage
2. determination of fiber melting points with the hot stage
3. determination of refractive index with the hot stage

INTRODUCTION

The use of controlled heat (hot stage attachments) has been applied to various analytical techniques currently used in forensics. Hot stage attachments can be used with a compound light microscope to determine fiber melting points, and for glass samples, a phase contrast microscope can be equipped with a hot stage to determine the refractive index of the sample. Both of these analyses involve thermal microscopy. These techniques can also be applied to other forensic samples where it might be necessary to determine the melting point or the refractive index of a substance.

A light microscope equipped with a hot stage (see Figure 24-1) is recommended for observing the effects of heat on man-made fibers (fibers derived from chemical substances of either natural or synthetic origin). Most natural based man-made fibers are composed primarily of cellulose.



Figure 24-1 A Mettler Toledo™ hot stage. When used on a microscope, forensic samples can be viewed under controlled heated settings.

Synthetic fibers are comprised of organic polymers that have been formed by chemical synthesis. Usually, this is a mixture of base polymers that have additives, and also sometimes finishes applied. Since man-made fibers are composed of several chemicals instead of a single compound, they usually exhibit a range of melting point temperatures. Chemical compounds, molecular weights, and the ratio of the compounds affect the melting point. The hot stage and how it is used may also affect results. Reproducible results can be obtained by using a slow heating program when near the actual melting point. Observations of various types may be seen: drop formulation, contraction, softening, charring, bubbling, and melting. Melting points for common fibers are summarized in Table 24-1.

Some fibers do not melt below 300°C: acrylic, aramid, azlon, fluorocarbon, glass, some modacrylics, novoloid, vinal, vinyon, rayon, and natural fibers.

Table 24-1 Melting points for common fibers.

Fiber type	Melting point observations
Saran	Shrinks at 90°C, softens at 115°C, melts at 168°C
Polyethylene	Melts at 119–135°C
Modacrylic	Contracts at 135–155°C, melts at 160°C, Dynel melts at 190°
Polypropylene	Softens at 145–150°C, melts at 167–179°C
Nylon 11	Melts at 182–186°
Rayon	Chars at 185–200°
Nylon 6	Melts at 210–216°C
Spandex	Melts at 230°C
Triacetate	Darkens at 230°C, melts at 290–300°C
Acrylic	Softens at 240°, does not melt
Acetate	Melts at 250–255°C
Nylon 6,6	Melts at 252–260°C
Polyester	PBT melts at 221–222°C PET melts at 256–268°C Kodel melts at 290°C

Complete fiber examinations involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the fiber for macroscopic characteristics. A compound or comparison light microscope is then used to determine optical properties such as birefringence and sign of elongation (Experiment 17). Sometimes, burn or solubility tests may also be performed during man-made fiber examinations (Experiment 17B). Additional examinations may be performed using microspectrometry and micro-FTIR to identify additional characteristics (Experiments 22 and 23). Although thermal microscopy does not lead to identification, it is considered a complementary technique that provides additional information.

A phase contrast light microscope equipped with a hot stage is recommended for observing the effects of heat on the refractive index of glass samples (see Figure 24-2).



Figure 24-2 A Foster + Freeman GRIM™ system. This instrument allows forensic scientists to acquire data from microscopic size forensic samples. The oil immersion method can be used on a phase contrast microscope that has been adapted with a hot stage. By varying the temperature to change the refractive index of a calibrated oil, the RI of an immersed fragment of glass can be determined.

This technique uses varying temperature and wavelength and is called the Emmons double variation method of refractive index determination. The Emmons double variation method relies on the relationship between refractive index, temperature, and wavelength of light. With this method match points, which are matching refractive indices showing minimum relief of the glass particles, are determined at various temperatures by changing the wavelength of light. Variation in the refractive index with the wavelength of light used is called the dispersion of refractive index. All solids demonstrate this dispersion and measurement of this property can be used to assist in identification. A graph of refractive index versus wavelength is a dispersion graph. A Hartmann net is a graph of refractive index versus wavelength that also combines a series of parallel lines, and which represents the refractive index versus wavelength relationship at fixed temperatures for an immersion oil. By using the Hartmann net, the examiner can then plot data obtained from his or her sample to identify the sample's refractive indices at: n_C (656 nm), n_D (589 nm), and n_F (486 nm) (see Figure 24-3).

Automated instruments such as the glass refractive index measurement system (GRIM) also use a variation of this method. With GRIM, measurements are taken at different temperatures with a constant wavelength until a match point is reached. Dispersion curves, which have been calibrated

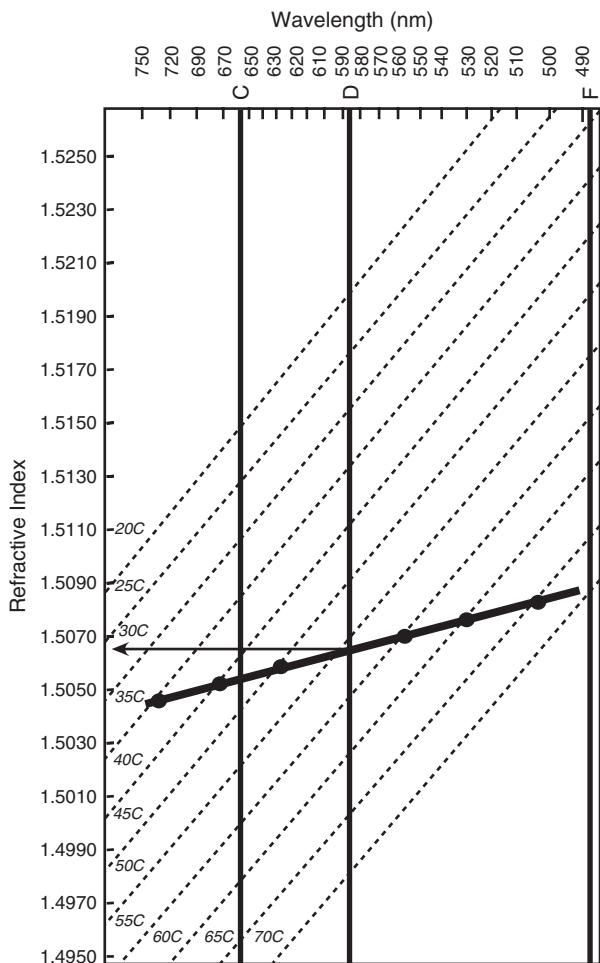


Figure 24-3 This Hartmann net produced for immersion oil 1 covers temperatures from 20°C to 70°C. Data have been plotted for a sample with matching points of 735 at 35°C, 675 at 40°C, 628 at 45°C, 559 at 55°C, 530 at 60°C, and 504 at 65°C. By following the intersecting line at the sodium D line, the refractive index, 1.5066, can be determined for this sample.

for the oils and saved in the computer, are used to determine the refractive index at n_C (656 nm), n_D (589 nm), and n_F (486 nm).

Complete glass examinations involve the use of a variety of microscopes, techniques, and instruments. Initially a stereomicroscope is used to examine the glass for macroscopic characteristics. Further testing can be performed using a compound to determine optical properties. Sometimes, the density of the glass sample (Experiment 16) may also be determined or compared. Further testing can be performed using a scanning electron microscope (SEM) to identify additional characteristics such as elemental composition (Experiment 25). For glass, thermal microscopy allows the analyst to determine the refractive index of a single particle with a single immersion oil. For the Emmons double variation method, samples whose refractive indices differ less than ± 0.0001 can be differentiated. With GRIM, differences of ± 0.00003 can be determined.

EQUIPMENT AND SUPPLIES

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

Hot stage and apparatus

Fiber samples (nylon 6, nylon 6,6)

Unknown fiber

Micro kit

Microscope slides and cover slips

High temperature stable silicone oil (for melting point)

Phase contrast light microscope equipped with a monochrometer

Known refractive index glass sample with dispersion data

Hartmann net calibrated for the oil to be used

High temperature stable silicon oil: LockeTM oils and Dow Corning 710TM silicone oil are recommended for soda-lime-silica glass (e.g., flat glass; Dow Corning 550TM silicone oil is recommended for borosilicate glass (e.g., headlamps); and Dow Corning F/6/7024TM is recommended for glasses with a refractive index above that of the common soda-lime glasses¹.

Unknown glass fragments

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. The hot stage can reach high temperatures so the glass microscope slides will be extremely hot. Use caution when working with these slides. Dispose of glass in an appropriate container.

PART I: MELTING POINT DETERMINATION FOR FIBERS

Procedure

Melting point determinations can be made by using either dry mounts (air) or liquid mounts (heat stable silicon oil).

1. Using the high temperature stable silicon oil, make a liquid mount of nylon 6.
2. Insert the slide into the hot stage that has been positioned on the compound light microscope. Focus on the fiber under 40X magnification. Cross the polars.
3. Quickly raise the temperature to 190°C.
4. Now that you are close to the expected melting point for nylon 6, slowly raise the rate by 2°C per minute.
5. Carefully observe the fiber for changes in optical properties. The melting point range is determined at the temperature when the fiber begins to melt and at the temperature when no more change occurs. Sometimes, viewing the fiber under crossed polars may give clearer indications of the melting point.
6. Repeat the test, using nylon 6,6. Begin at 240°C and then slowly raise the temperature rate until the melting range is determined.

¹ Forensic Science Communications, SWGMAT Standards and Guidelines; Glass Refractive Index Determinations, Jan. 2005.

7. Obtain your unknown fiber. Since you don't know a suspected melting point, begin at the lowest temperature in Table 24-1 (page 316). If there is sufficient sample, a quick temperature ramp can be run to determine an approximate range.
8. Once a suspected range has been determined, make another slide and begin the test at approximately 15°C below the suspected melting point range.
9. Slowly raise the temperature rate until the melting point range is determined.
10. Compare your findings to Table 24-1 to identify the fiber.

PART II: EMMONS DOUBLE VARIATION METHOD OF REFRACTIVE INDEX DETERMINATION FOR GLASS

Procedure

1. Using the high temperature stable oil for refractive index determinations, make a liquid mount of the glass sample.
2. Place the slide containing the glass fragments in the hot stage that has been positioned on the phase contrast light microscope.
3. Focus and align the phase contrast microscope. Adjust the phase plate and phase annulus until the rings are superimposed.
4. Set the hot stage temperature to a low temperature within the liquid's stable range. Using the monochromator, scan the visible range to see if a match point is possible. A match point is determined when there is little or no relief between the glass fragment and oil.
5. If a match point is not found, raise the temperature 5°C and once again scan the visible range. Make sure your temperature equilibrates each time before you begin scanning.
6. Continue raising the temperature and scanning wavelengths until 3–4 match points have been determined.
7. Once several match points have been determined, plot the points on the calibrated Hartmann net to determine the refractive index.

REPORT REQUIREMENTS

Make sure that you include any drawings, calculations, or other information obtained during the laboratory procedure.

REPORT QUESTIONS

1. What is a hot stage? How can it be used in forensic analysis?
2. What is melting point?
3. Why are melting points an identifying characteristic for manufactured fibers, but not natural fibers?
4. Why do nylon 6 and nylon 6,6 have different melting points?
5. Describe the procedure you used to identify your unknown fiber. What is the fiber? Justify your results.
6. Explain the Emmons double variation method.
7. Why is a phase contrast microscope used for this glass examination?

8. What is the refractive index of your unknown glass sample?
9. Why is refractive index reported at n_C (656 nm), n_D (589 nm), and n_F (486 nm)?
10. Research on the Internet to find an explanation of how the automated glass refractive index measurement system (GRIM) works.
11. What are the advantages of using GRIM over the phase contrast microscope/Emmons double variation method for glass examinations?

RECOMMENDED AND FURTHER READING

- Bennett RL, Kim ND, Curran JM, Coulson SA, Newton AWN. Spatial Variation of Refractive Index in a Pane of Float Glass. *Science & Justice*. 2003; 43(2): 71–6.
- Cassista AR, Sandercock PML. Precision of Glass Refractive Index Measurements: Temperature Variation and Double Variation Methods and the Value of Dispersion. *Canadian Society of Forensic Science Journal*. 1994; 27(3): 203–8.
- Causin V, Marega C, Schiavone S, Marigo A. Employing Glass Refractive Index Measurement (GRIM) in Fiber Analysis: A Simple Method for Evaluating the Crystallinity of Acrylics. *Forensic Science International*. 2005; 149(2–3): 193–200.
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- Grieve MC. The Use of Melting Point and Refractive Index Determination to Compare Colorless Polyester Fibres. *Forensic Science International*. 1983; 22: 31–48.
- Hartshoene AW, Wild FM. The Discrimination of Cellulose Di- and Tri- Acetate Fibers by Solvent Tests and Melting Point Determination. *Journal of Forensic Sciences Society*. 1991; 31(4): 457–61.
- Hartshorne AW, Laing DK. The Identification of Polyolefin Fibres by Infrared Spectroscopy and Melting Point Determination. *Forensic Science International*. 1984; 26: 45–52.
- Koons RD, Buscaglia J, Bottrell M, Miller E. Forensic Glass Comparisons. In: Saferstein R, editor. *Forensic Science Handbook*. 2nd ed. Upper Saddle River, New Jersey: Pearson Education; 2002; 161–213.
- Locke J. GRIM: A Semi-Automatic Device for Measuring the Refractive Index of Glass Particles. *Microscope*. 1985: 169–78.
- Locke J, Underhill M. Automatic Refractive Index Measurements of Glass Particles. *Forensic Science International*. 1985; 27: 247–60.
- McCrone WC. Calibration of the Mettler Hotstage. *Journal of the Forensic Science Society*. 1987; 27(3): 207.
- Ojena SM, De Forest PR. Precise Refractive Index Determination by the Immersion Method Using Phase Contrast Microscopy and the Mettler Hot Stage. *Journal of the Forensic Science Society*. 1972; 12: 315–29.
- Was-Gubala J, Krauss W. Damage Caused to Fibres by the Action of Two Types of Heat. *Forensic Science International*. 2006; 159(2–3): 119–26.

Experiment 25: Scanning Electron Microscopy

Recommended pre-lab reading assignments:

Goldstein JI, Newbury DE, Echlin P, Joy DC, Romig AD, Lyman CE, et al. *Scanning Electron Microscopy and X-ray Microanalysis: A Text for Biologists, Materials Scientists, and Geologists*. 2 ed. New York, NY: Plenum Press, 1992.

Almirall J. Elemental analysis of glass fragments. In: Caddy B, ed. *Trace Evidence Analysis and Interpretation*. London, England: Taylor and Francis; 2001; 65–83.

OBJECTIVE

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

1. general scanning electron microscopy principles
2. sample preparation for scanning electron microscopy analysis
3. use of the scanning electron microscope to examine paint samples
4. use of the scanning electron microscope to examine glass samples
5. use of the scanning electron microscope to examine GSR samples

INTRODUCTION

The scanning electron microscope (SEM) is a microscope that uses electrons instead of light to form a magnified image. These electron emissions are viewed on a monitor. When used in combination with other instrumental detectors, the usefulness of the analysis increases.

An SEM is composed of several basic systems, all of which work together to form the image (see Figure 25-1). Basically, the electron column can be thought of as a large vacuum tube with the cathode (electron gun) at the top supplying electrons and the sample at the bottom acting as an anode or target for the electrons. The path of the electron gun is controlled by a series of electromagnetic lenses within the body of the column. These lenses shape and position the electron



Figure 25-1 A Topcon SM-510™ Scanning Electron Microscope, equipped with an Evex™ Energy Dispersive Spectrometer. This instrument allows forensic scientists to acquire data from microscopic size forensic samples. In addition to viewing samples at extremely high magnifications, elemental data can also be acquired from the sample.

beam. The intensity of the electron beam is controlled by the voltage and diameter of the beam (spot size), which are determined by the examiner. The electron beam causes a number of interactions near the surface of the sample. Since it is these interactions that are important to the examiner, the sample chamber contains the sample holder and any additional detectors as well.

Most forensic laboratories use an SEM with two detectors. The first and main detector is a photomultiplier tube for the purpose of imaging. Image formation is accomplished by dividing the sample into an x/y grid and by scanning each division of the grid sequentially. The photomultiplier tube collects electrons, which have been deflected off the sample for each division of the grid, and assigns a light intensity to each division. At the end of a complete scan of the entire grid an image is obtained based on the topical features of the sample. This is viewed on a monitor. The second detector is a semi-conductor, usually an Energy Dispersive detector (EDX). Light is composed of photons and discrete energy levels. When the electron beam strikes the sample X-rays are emitted. Since each element emits X-rays at characteristic energy values, the analyst can identify the elements present in a sample. By changing the location of the electron beam across the sample it is possible to determine each element in the sample. Elements of low atomic number below Na are difficult to detect because of adsorption of the X-rays by the Be window used by most detectors. The EDX detector is essentially a large single crystal semi-conductor. Liquid nitrogen is typically used to cool the detector so that there is little thermionic creation of charge carriers.

Acquiring data on the SEM/EDX unit is fairly straightforward. Samples can be maneuvered by an automated stage to obtain the best image. Once the sample is centered and tilted appropriately, the imaging is automated. However, at times, it may be necessary to make slight adjustments

to the instrument parameters to improve image quality. For the best imaging it is better to set the electron gun voltage as low as possible, but high enough so that you can still see an image on the screen. The spot size should be as low as possible to avoid charging. Charging is an accumulation of electrons on the surface of the sample ‘bleaching’ out the features. Sometimes, in very non-conducting samples, the sample needs to be coated with a conducting material in order to avoid charging. This can be done by sputter coating a sample. Sputter coating deposits an ultra-thin coating of electrically-conducting material such as gold, gold/palladium, platinum, tungsten, or graphite on the sample. Sputter coating will also improve contrast. Adjustments to the contrast and brightness will highlight the morphological features of the sample. The SEM has a relatively large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces high-resolution images, which means that closely spaced features can be examined at a high magnification. Once the image is acceptable on the monitor, further analysis can be performed with the EDX detector.

By using an SEM/EDX system, the analyst combines higher magnification (10 to 100,000X), larger depth of focus, greater resolution, and ease of sample viewing with the opportunity to determine elemental composition of samples. Because of this varied examination, several types of forensic evidence can be analyzed on the SEM. Paint, glass, and gunshot residue (GSR) are a few of the common applications.

Examinations of paint are usually performed to identify a paint and then compare it back to a possible paint source. Initially a stereomicroscope is used to examine the paint for layer structure and its solubilities in different solvents (Experiment 14). Paints may also be examined with the FTIR microspectrometer to examine the binders of a sample (Experiment 22), and with the microspectrophotometer (Experiment 23). Further examination of paint by the SEM/EDX may provide additional information about its composition. Pigments, additives, and fillers can sometimes be identified through their elemental composition. Common paint pigments are listed in Table 25-1.

Table 25-1 Common paint pigments.

Color	Pigments
White	aluminum oxide, antimony oxide, barium carbonate, barium sulfate, lead silicate, lead sulfate, bismuth chloride, calcium phosphate, calcium silicate, calcium carbonate, magnesium carbonate, silicon nitride, silicon oxide, talc, tin oxide, titanated barytes, barium tungsten oxide, iron phosphate, zinc sulfide, zinc oxide, zirconium silicate, zirconium oxide, silicate fillers, clays, micas
Yellow	barium potassium chromate, barium chromate, cadmium sulfite, lead chromate, lead cyanamide, lead titanate, lead oxide, mercuric arsenate, strontium chromate, zinc chromate, nickel phosphate
Orange	lead oxide, cadmium sulfide, lead chromate
Red	antimony sulfide, cadmium sulfide, cadmium selenide, copper oxide, iron oxide, lead oxide
Green	chromium oxide, copper carbonate, barium manganese oxide, iron oxide, zinc chromate
Blue	iron oxide, copper carbonate, cuprous sulfide, barium manganese oxide, nickel-iron, lead sulfate
Purple	iron oxide
Brown	calcium lead oxide, iron oxide, manganous chromate, magnesium ferrite
Black	antimony sulfide, iron oxide, cuprous sulfide, iron titanate
Metallic	aluminum, copper, gold, lead, tin, zinc, iron

Paint samples can be analyzed several different ways with the SEM. Layers can be separated and analyzed independently, or if the chip is fairly thin the entire chip can be analyzed as a whole. For some samples, a cross-section can be prepared and each layer analyzed separately.

Examinations of glass are usually performed to identify a glass and then compare a sample back to a possible source. Initially a stereomicroscope is used to examine the glass for physical properties such as overall color, surface features, flatness/curvature, and thickness. The optical properties of glass such as refractive index (Experiment 16) are also determined to further distinguish glass samples. SEM/EDX is an additional technique that may be used to identify and compare the elements found in a glass sample. Many elements that can be identified come from the glass components themselves and not from surface contaminants. Knowing the elemental composition may assist in the identification of the type and manufacturing process used for the glass sample. The various types of glass and the common compounds and elements found in these types are listed in Table 25-2.

Table 25-2 Common compounds found in various glass types.

Type of glass	Glass components
Silica glass	silicon oxide, commonly combined with barium oxide, strontium oxide, and calcium oxide
Alkali silicate glass	silicon oxide, with additions of lithium oxide, sodium oxide, and potassium oxide
Soda-lime glass	mixtures of alkaline and alkali silicates; generally sodium oxide, calcium oxide, and silicon oxide, in addition to magnesium oxide, aluminum oxide, barium oxide, and potassium oxide
Borosilicate glass	boric oxide
Aluminosilicate glass	aluminum oxides, silicon oxide
Lead glass	lead oxide
Borate glass	various borates, commonly boric oxide
Phosphate glass	phosphoric oxide and phosphates
Chalcogenide glass	based on sulfur, selenium, or tellurium
Halide glass	zinc chloride, beryllium fluoride
Metallic glass	commonly have nickel, zirconium, palladium, iron, phosphorus, or carbon

Elements identified in glass samples may also come from colorants. Common elements associated with various glass colors are listed in Table 25-3. Single glass fragments are analyzed with the SEM.

GSR samples may also be examined using the SEM/EDX. When a gun is fired the firing pin strikes the back of the cartridge and sparks the primer that ignites the ‘gunpowder’ and causes it to burn. This results in the formation of a gaseous vapor, which forces the bullet down the barrel of the gun and out the muzzle. The majority of the vapor proceeds out the muzzle but the vapor can also escape from the cylinder gap in a revolver or the ejection port in a pistol. The vapor that escapes from these areas is called GSR. This analytical procedure for GSR testing starts with sampling the individual’s hands with a sticky aluminum stub. Any possible GSR particles would be transferred to the stub, which is then submitted for SEM testing. GSR contains the three heavy metals: barium, lead, and antimony. With SEM/EDX, the analyst can view the particle morphology and also determine its elemental composition. GSR particles have a characteristic spherical shape, which is roughly spheroidal, globular, or boule-shaped. This morphology is typical for materials

Table 25-3 Common glass colorants.

Color	Contributing elements
Colorless, UV absorbing	titanium, iron
Blue	cobalt, copper, sulfur
Purple	manganese, nickel
Green	chromium, iron, copper, molybdenum
Brown	manganese, iron, titanium, nickel, cesium
Amber	sodium, sulfur
Yellow	cadmium, sulfur, cesium, titanium, silver
Orange	cadmium, sulfur, selenium
Red	cadmium, sulfur, selenium, copper, antimony
Black	copper, manganese, nickel, iron, copper, chromium

that condense rapidly from a vapor. However, with the additional elemental identifications, particles may be attributed to GSR.

EQUIPMENT AND SUPPLIES

Stereomicroscope
Paint samples
Glass samples
Prepared GSR samples
Micro kit
10x15 mm cylindrical aluminum stubs
Sticky tabs
SEM/EDX with sputter coater
Liquid nitrogen, thermos, and cryo-gloves

SAFETY

Use standard laboratory safety procedures as described in guidelines set by your instructor. Be cautious of microscope light levels to avoid eye damage. Use of safety glasses and cryo-gloves when handling liquid nitrogen is recommended. Refer to MSDS if necessary.

PART I: FAMILIARIZATION WITH THE SEM/EDX

Since each instrument may be operated differently and use different software, please refer to the operational manual of your instrument to familiarize yourself with operating the instrument.

PART II: PAINT

Procedure

1. Obtain a paint sample.

2. While viewing the sample under the stereomicroscope, cut a thin slice from the top layer. This may be either a clear or colored layer.
3. Obtain an aluminum stub and place a sticky tab onto one side.
4. Place the prepared paint sample onto the sticky tab using the forceps.
5. Following instrument specifications, obtain an image of the paint sample on the SEM/EDX. If excessive charging is observed, sputter coat your sample. *Please refer to the operational manual of your instrument.*
6. Acquire the elemental data for the paint sample.
7. Identify any elements present and when appropriate the pigment contained within the sample.
8. Repeat steps 2–6 for each additional layer and assigned samples.
9. Include a copy of the SEM/EDX spectra obtained from the paint samples in your report.

PART III: GLASS

Procedure

1. Obtain a glass sample.
2. Obtain an aluminum stub and place a sticky tab onto one side.
3. Place the glass sample onto the sticky tab using the forceps.
4. Following instrument specifications, obtain an image of the glass sample on the SEM/EDX. If excessive charging is observed, sputter coat your sample. *Please refer to the operational manual of your instrument.*
5. Acquire the elemental data for the glass sample.
6. Identify any elements present and when appropriate the elemental components contained within the sample.
7. Repeat steps 2–6 for each assigned sample.
8. Include a copy of the SEM/EDX spectra obtained from the glass samples in your report.

PART III: GSR

Procedure

1. Obtain an aluminum stub that contains a GSR sample.
2. Following instrument specifications, obtain an image of possible GSR particles on the SEM/EDX. If excessive charging is observed, sputter coat your sample. *Please refer to the operational manual of your instrument.*
3. Acquire the elemental data for the particle.
4. Identify any elements present.
5. Repeat steps 2–4 in an attempt to identify barium, lead, and antimony particles.
6. Include a copy of the SEM/EDX spectra obtained from the glass samples in your report.

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. Explain how an SEM operates, discussing each portion of the instrument. Include a drawing of the mechanics of the SEM.
2. Explain how the EDX detector works. What are the advantages of this detector?
3. Which elements cannot be detected with the EDX?
4. What additional detectors are available for SEM use? What are the advantages/disadvantages of each?
5. What is the preferred method for SEM/EDX paint examinations?
6. Can elemental data vary for different locations on the same paint chip/layer?
7. What weight should be placed on elemental data obtained from a glass sample?
8. Barium, lead, and antimony are common elements associated with GSR. To what can these elements be attributed?
9. If an analyst obtains morphology and elemental data identifying barium, lead, and antimony, what result can be reached? What can be inferred from that result?
10. Why is an SEM/EDX a useful instrument to forensic laboratories?

RECOMMENDED AND FURTHER READING

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Appendix A

Optical Properties of Natural Fibers^{1,2}

Plant/Animal fiber	η -parallel	η -perpendicular	Birefringence	Sign of elongation
Abaca	1.571	1.524	0.047	+
Cotton	1.578	1.532	0.046	+
mercerized	1.544	1.524	0.020	+
Flax	1.594	1.532	0.062	+
Hemp	1.585	1.526	0.059	+
Jute	1.577	1.536	0.041	+
Kapok	1.550	1.534	0.016	+
Kenaf	1.567	1.523	0.044	+
Ramie	1.596	1.528	0.068	+
Silk	1.591	1.540	0.051	+
degummed	1.590	1.538	0.052	+
Sisal	1.566	1.528	0.038	+
Wool	1.560	1.547	0.013	+

Mineral fiber	α	β	γ	Sign of elongation
Actinolite	1.6126	1.6288	1.6393	+
Amosite	1.700	*	1.695	+
Anthophyllite	1.6148	1.6273	1.6362	+
Chrysotile	1.529–1.559	1.530–1.564	1.537–1.567	+
Crocidolite	1.680–1.698	1.683–1.700	1.685–1.706	–
Tremolite	1.6063	1.6230	1.6343	+

*This orientation is not reported in literature.

¹ McCrone WC, Delly JG. *The Particle Atlas*, Ann Arbor Michigan; Ann Arbor Science, 1973.

² Textile Institute. *Identification of Textile Materials*. 7 ed. Manchester, England: The Textile Institute, 1975

Optical Properties of Man-Made Fibers^{1,2,3}

Fiber	η -parallel	η -perpendicular	Birefringence	Sign of elongation
Acetate	1.474–1.479	1.473–1.477		
Dicel	1.476	1.473	0.003	+
Acrylic	1.511–1.521	1.512–1.525		
Acrlan	1.52	1.525	–0.005	–
Acrlan 36	1.511	1.514	–0.003	–
Orlon	1.51	1.512	–0.002	–
Aramid	2.050–2.350	1.641–1.646		
Kevlar	2.35	1.641	0.709	+
Nomex	1.80	1.664	0.136	+
Azlon	1.532–1.538	1.526–1.536		
Vicara	1.538	1.536	0.002	+
Calcium alginate	1.524	1.520	0.004	+
Darvan				
Nytril	1.464	1.464	0	
Fluorocarbon	1.385–1.389	1.345–1.350		
Teflon	1.385	1.345	0.040	+
Glass	1.523–1.552	1.523–1.552	0	
Lyocell	1.562–1.564	1.520–1.522	0.050	+
Tencel	1.57	1.52	0.050	+

¹ Textile Institute. *Identification of Textile Materials*. 7 ed. Manchester, England: The Textile Institute, 1975.

² Palenik SJ. Microscopical Examination of Fibres. In: Robertson J, Grieve M, eds. *Forensic Examination of Fibres*. London: Taylor and Francis; 1999; 153–76.

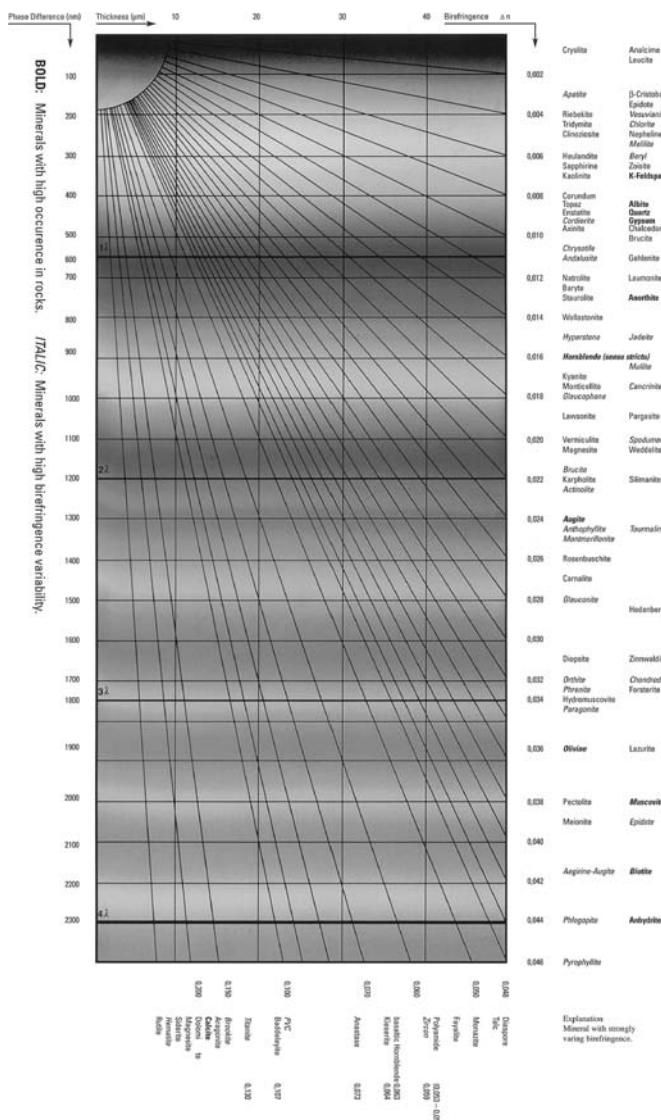
³ Houck MM, Siegel JA. *Fundamentals of Forensic Science*. Oxford: Elsevier Academic Press, 2006.

Fiber	η -parallel	η -perpendicular	Birefringence	Sign of elongation
Modacrylic	1.520–1.535	1.516–1.539		
Dynel	1.535	1.533	0.002	+
SEF	1.534	1.535	-0.001	-
Teklan	1.52	1.516	0.004	+
Verel	1.535	1.539	-0.004	-
Novoloid	1.5–1.7	1.5–1.7		
Kynol(unknown)	1.649	1.649	0	
Kynol(drawn)	1.658	1.636	0.022	+
Nylon	1.546–1.582	1.507–1.526		
Nylon 6	1.568	1.515	0.053	+
Enkalon	1.575	1.526	0.049	+
Nylon 6,6	1.582	1.519	0.063	+
ICI	1.578	1.522	0.056	+
Nylon 11	1.55	1.51	0.04	+
Rilsan	1.553	1.507	0.046	+
Quina	1.546	1.511	0.035	+
Nytril	1.464–1.480	1.464–1.480		
Darvan	1.464	1.464	0	
Olefin	1.52–1.574	1.496–1.522		
Polyethylene	1.556	1.512	0.044	+
Courlene X3	1.574	1.522	0.052	+
SWP	1.544	1.514	0.03	+
Polypropylene	1.52	1.492	0.028	+
Courlene	1.53	1.496	0.034	+
Ulstron	1.530	1.496	0.034	+
Polyacrylostyrene	1.560	1.572	-0.012	-
Polyester	1.586–1.710	1.53–1.589		
Dacron	1.7	1.535	0.165	+
Fortrel	1.72	1.535	0.185	+
Kodel	1.632	1.534	0.098	+
Kodel II	1.642	1.54	0.102	+
PBT	1.688	1.538–1.540	0.148–0.150	+
PCDT	1.632–1.642	1.534–1.542	0.098–0.102	+
PEN	1.862	1.589	0.273	+
PET	1.699–1.710	1.535–1.546	0.147–0.175	+
PTT	1.586	1.566	0.060	+
Terylene	1.707	1.546	0.16	+
Vycron	1.713	1.53	0.183	+
Rayon	1.542–1.553	1.513–1.523		
Cuprammonium	1.553	1.519	0.034	+
Viscose	1.542	1.520	0.022	+
Viscose (high tenacity)	1.544	1.505	0.039	+
Fortisan	1.547	1.523	0.024	+
Fortisan 36	1.551	1.52	0.031	+
Vincel	1.551	1.513	0.038	+

Fiber	η -parallel	η -perpendicular	Birefringence	Sign of elongation
Saran	1.599–1.610	1.607–1.618		
Spandex	1.561	1.56	0.001	+
Sulfar	1.849	1.738	0.111	+
Teflon	1.38	1.34	0.04	+
Triacetate	1.469–1.472	1.468–1.472		
Arnel	1.469	1.468	0.001	+
Tricel	1.469	1.469	0	
Vicara	1.538	1.536	0.002	+
Vinal	1.540–1.547	1.510–1.522		
Vinyon	1.528–1.541	1.524–1.536		
Fibravyl	1.54	1.53	0.01	+
Rhovyl	1.541	1.536	0.005	+
Vinyon HH	1.528	1.524	0.004	+

Appendix C

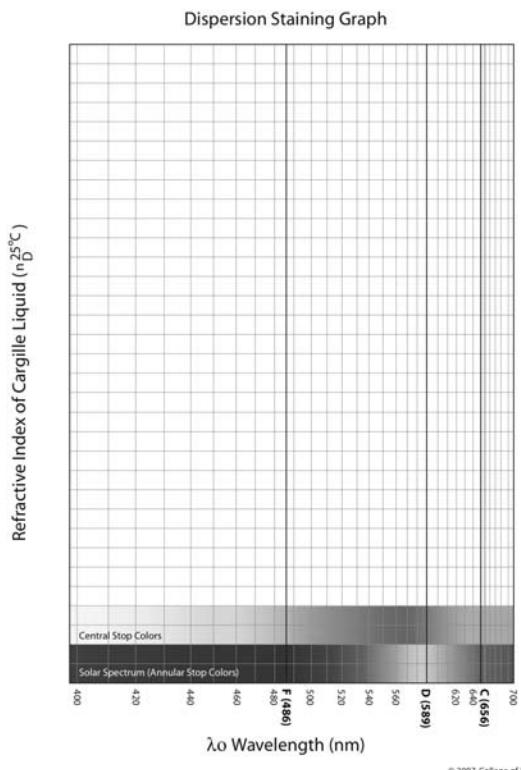
Michel-Lévy Chart



Michel-Lévy chart. A full-color version of this figure can be found in the color plate section of this book.

Appendix D

Dispersion-Staining Graph



Dispersion-staining graph. Reproduced from *Essentials of Polarized Light Microscopy*, John Gustav Delly (2007), with permission of College of Microscopy. A full-color version of this figure can be found in the color plate section of this book.

Mock Case Scenario

OBJECTIVE

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

1. working with others to provide analytical results
2. devising examination schemes using analytical procedures learned throughout previous lab experiments
3. correctly analyzing evidence
4. reaching appropriate results and conclusions

INTRODUCTION

This laboratory experiment draws on the knowledge and skills that you have gained previously. For this exercise the class will be divided into teams. Each team will be given a request for examination form. This is a form that is normally submitted along with evidence when it is brought to a forensic laboratory for analysis. On this form, exhibits are listed and examinations are requested. Each team will obtain their evidence, inventory their samples, and then secure everything in a locked evidence locker. Each team will then divide the requested examinations and complete the analytical work. Each team will examine the evidence as it sees fit, and a single lab report will be prepared by each team.

Procedure

1. You will be assigned to a team.
2. Obtain the ‘Forensic Laboratory Request for Examination’ form (available from <http://www.wileyeurope.com/college/wheeler>) for your team and the assigned evidence (i.e., exhibits) from an investigator.
3. Document the chain of custody (date, time, and person) and condition of the evidence (i.e., packaging and seal) on the ‘Report of Forensic Laboratory Examination’ form and in the notes. Secure the evidence in a locked drawer when not in use.
4. Divide the examination duties so that the analysis can be completed within the two lab periods.

5. Devise appropriate analytical schemes for each type of evidence from previously learned techniques so that you are able to perform the requested examinations. Make sure that *all* appropriate exams are performed for each evidence type. When making a comparison the goal is to either prove an association or to prove a difference.
6. Perform the analysis of the evidence types. Document all testing performed in detailed notes. This may include drawings and photographs. Initial and date all pages.
7. Combine the documentation, notes, drawings, and photographs from all evidence types.
8. Compose a combined lab report of all results.
9. Release your evidence back to an investigator.

REPORT REQUIREMENTS

Your lab report will consist of a final lab case file containing the following information:

1. A completed ‘Report of Forensic Laboratory Examination’ form (available from <http://www.wileyeurope.com/college/wheeler>) that includes:
 - (a) Chain of custody (date, time, via information)
 - (b) Case and lab numbers
 - (c) Victim/suspects names
 - (d) List of evidence received
 - (e) List of examinations requested (statement of problem)
 - (f) Clear and concise results. Using the results of all the evidence exams, state the conclusions that may be reached from the results of the testing. It is important to use short concise statements that point to a conclusion if possible. Refer to the evidence examined using their exhibit number exactly as listed on the ‘Forensic Laboratory Request for Examination’ form.
 - (g) Signature of all analysts and date
2. Return the signed ‘Forensic Laboratory Request for Examination’ form.
3. Attach all documentation, drawings, and notes taken during analysis. This includes the initial exam notes, packaging details, and test procedures used. All data and/or comparisons should be compiled in the notes. Each page must have the student analyst’s initials and date.
4. Conclusions and interpretations: Prepare a discussion stating the result for each evidence type (i.e., glass, hair, fibers). The written discussion should include an explanation of how the results and conclusion were reached, the value of each test performed, and the effect of these results on the investigation.

FORENSIC LAB
Request for Evidence Examination

Investigation Officer: _____

Agency: _____

Address: _____

City: _____ Zip: _____

Phone: _____ Fax: _____

Email: _____

Laboratory #: _____

Case #: _____

Victims(s): _____

Offense: _____

Suspect/Accused(s): _____

Offense Date: _____

Offense City/Country: _____

Exhibits: (Initial exhibits received)

Case History:

Examinations Requested:

Submitting Officer Signature: _____ Date: _____

For Lab use only

Received from:

Received By:

Date/Time:

Laboratory # _____

Case # _____

Re: (v) _____

(s) _____

Report of Forensic Laboratory Examination

Submitted By: _____

Received: Date: _____ Time: _____ Via: _____

Returned To: _____ Date: _____ Via: _____

Material Submitted:

Examination Requested:

Results of Examination:

Signature of Examiner/Date

Signature of Examinee/Date

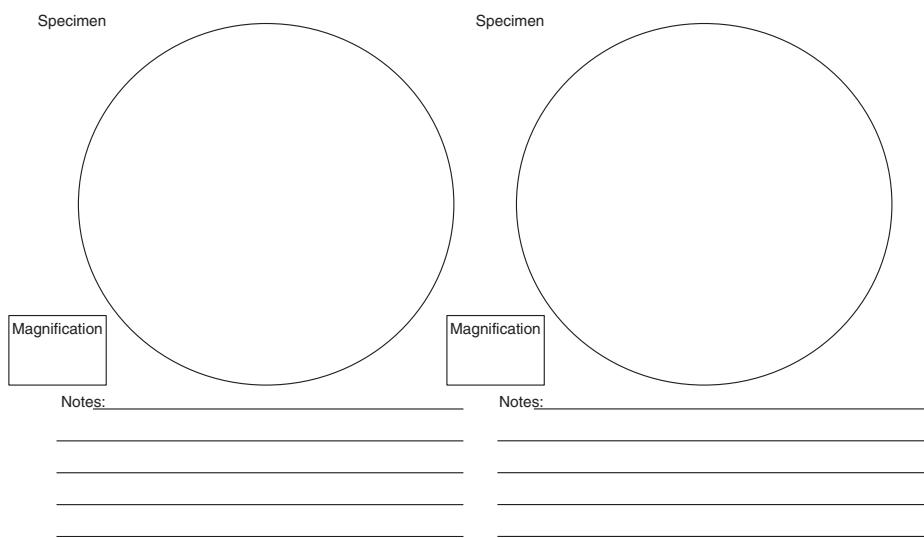
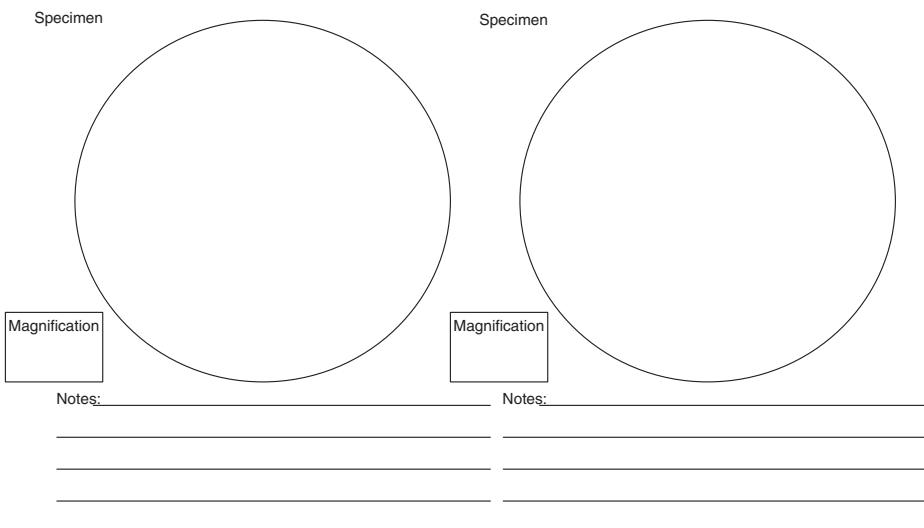
Signature of Examiner/Date

Signature of Examiner/Date

Appendix F

Circle Template

Also available from <http://www.wileyeurope.com/college/wheeler>



Glossary of Microscopy Terms

Aberration	Any disturbance of the rays of light in an optical system such that they can no longer be brought to a sharp focus or form a clear image. See chromatic and spherical aberration.
Achromat	A lens that is corrected for chromatic aberrations at two wavelengths and spherical aberrations at one wavelength.
Amorphous [†]	Lacking a repeating array of crystal lattice points; amorphous materials are isotropic but may show strain birefringence.
Analyzer	A polarizing filter located above the sample and objective. Its orientation is perpendicular to the polarizer.
Angular aperture (AA) [†]	The largest angle between the image-forming rays collected or transmitted by a lens system, e.g., objective or condenser of a microscope.
Anisotropic	Having physical properties that differ according to the direction of measurement. Anisotropic samples possess order that varies along their different axes. These samples have more than one refractive index.
Annular stop	The opaque ring-shaped stop with a central opening placed in the objective back focal plane that is used to give annular stop observations for dispersion-staining.
Aperture	An opening, usually circular, that limits the quantity of light that can pass through the opening.
Apochromatic objective	A lens that is corrected for chromatic aberrations at three or more wavelengths and at two or more spherical aberrations.
Axis, crystallographic [†]	One of several imaginary lines assumed in describing the positions of the planes by which a crystal is bounded, the positions of the atoms in the structure of the crystal, and the directions associated with vectorial and tensorial physical properties.
Back focal plane [†]	A ‘plane’ normal to the axis of a lens in which all back focal points lie.
Barrier filter	A filter used in fluorescence microscopy that suppresses unnecessary excitation light that has not been absorbed by the fiber and selectively transmits only the fluorescence.
Becke Line	An optical halo caused by the concentration of refracted light rays along the edge of the particle.

Becke Line test	A method for determining the refractive index of a sample relative to its mounting medium by noting the direction in which the Becke Line moves when the focus is changed. The Becke Line will always move towards the higher refractive index medium (fiber or mounting medium) when focus is raised and will move toward the lower refractive index medium when focus is lowered. The sample should be illuminated with a narrow cone of light, which can be obtained by closing down the substage condenser.
Bertrand lens	A lens placed between the objective and the ocular usually used to observe a magnified image of the objective back focal plane to examine interference figures. It is sometimes used to observe the lamp filament when setting up Köhler Illumination.
Biaxial crystals	Crystals in the othrorhombic, monoclinic, and triclinic systems. They have three principal refractive indices: α , β , and γ .
Birefringence	The numerical difference in refractive indices for a sample. Birefringence can be determined by subtracting the refractive indices.
Body tube	The portion of the microscope that connects the oculars to the nosepiece that holds the objectives.
Brightfield illumination [†]	The usual form of microscope illumination with the image of the sample on a bright, evenly lighted field; or in vertical illumination, light reflected through the objective by means of prism or semi-reflecting plane glass.
Central stop	The opaque stop placed in the objective back focal plane. This is used to give a central stop for dispersion-staining.
Chromatic aberration	The variation of either the focal length or the magnification of a lens system with different wavelengths of light, characterized by prismatic coloring at the edges of the optical image and color distortion within it.
Cleavage [†]	The property of a crystalline substance of splitting along definite crystal planes.
Comparison microscope	An optical instrument composed of two compound light microscopes connected by an optical bridge. This allows two samples to be viewed at the same time with either transmitted or reflected light.
Compensation	The result of insertion of a compensator or crystal into a light path with retardation that exactly matches the retardation of the sample. Usually conducted when the sample is in the 45° position in the NE corner of the field of view. Complete compensation is said to occur when the colors go to zero order (black).
Compensator	Any variety of optical devices that can be placed in the light path of a polarizing microscope to introduce fixed or varied retardation comparable with the sample. Compensators may employ a fixed crystal of constant or varying thickness or a mineral plate that may be rotated to alter the thickness presented to the optical path (and retardation introduced) by a set amount.

Compensator, full-wave plate (first-order red)	A layer of quartz, selenite, calcite, or an oriented polymer film of the proper thickness to produce a retardation between 530–550 nm. This is approximately the retardation of the first order red color on the Michel-Lévy chart.
Compensator, quarter-wave plate	A thin layer of mica or an oriented polymer film of the proper thickness to produce a retardation of about 130 nm. This is approximately the retardation of the first order gray color on the Michel-Lévy chart.
Compensator, quartz wedge [†]	A wedge, cut from quartz, having continuously variable retardation extending over several orders (usually 3–7) or interference colors. The retardation exactly compensating that of a crystal can be found by pushing in the wedge while counting orders until it reaches a position at which the interference color of the crystal appears black. Retardation can be compensated only when the slow component of the crystal and the wedge are perpendicular.
Compensator, tilting (Berek)	A compensator typically containing a plate of calcite or quartz, which can be rotated by means of a calibrated drum to introduce variable retardation up to about ten orders.
Compound light microscope	An optical instrument that forms a magnified image through the use of a two-step process. The objective forms the magnified real image of the object and the ocular forms the magnified virtual image of the object.
Condenser	A lens or series of lenses, located below the stage of the microscope, which serve to collect and concentrate the light on the sample. There are two basic types of condensers: brightfield and darkfield.
Conjugate foci [†]	In an image-forming system, two fields are said to be conjugate with each other when one or more object fields are simultaneously in focus in a single plane, e.g., in Köhler illumination of the field diaphragm, specimen and ocular front focal plane.
Conoscopic observation	Observations of the back focal plane of the objective by the insertion of an auxiliary lens, removing the eyepiece. The lamp filament, substage aperture diaphragm, objective back focal plane, and ocular focal plane are simultaneously in focus with the viewer's eye.
Constructive interference	See Interference, constructive.
Contrast	The difference in brightness between the light and dark areas of an image.
Critical angle (C) [†]	The angle at which total reflection of a light ray passing from one medium to another occurs. The angle of incidence must be large (55° to 60°).
Crystalline [†]	A substance (usually solid but can be liquid) in which the atoms or molecules are arranged in a definite pattern that is repeated regularly in three dimensions. Crystals tend to develop forms bounded by definitely oriented plane surfaces that are harmonious with their internal structure. They may belong to any of six crystals systems: cubic, hexagonal, tetragonal, orthorhombic, monoclinic, or triclinic.

Curvature of field	An optical distortion where the center of the image is in focus but the edges are out of focus. When corrected, the image is said to be flat. Lenses corrected for this distortion are said to be 'Plan or Plano.'
Darkfield illumination [†]	Incident or transmitted illumination of the specimen by indirect light whereby no direct light is admitted directly to the objective.
Depth of field	The thickness of the optical slice that is in focus at the real intermediate image of a sample.
Depth of focus	The thickness of image at the real intermediate image plane in the microscope.
Destructive interference	See Interference, destructive.
Dichroism	The property of exhibiting different colors, especially two different colors, when viewed in polarized light along different axes.
Diffuse Illumination	See Illumination, diffuse.
Dispersion [†]	The variation of refractive index with color (or wavelength) of light. The spreading of white light into its components colors when passing through a glass prism is due to dispersion, which, in turn, is due to the fact that the refractive index of transparent substances is lower for long wavelengths than for short wavelengths. A measure of dispersion v (nu) is defined as: $v = \frac{n_D - 1}{n_F - n_C}$.
Dispersion-staining	An identification technique using a specialized objective, which uses the differences between the refractive index dispersion of a sample and that of the immersion liquid. A dispersion-staining objective typically has three stops: annular, central, and open. Using an annular stop with the substage iris closed, a sample mounted in a high dispersion medium will show a boundary of a wavelength where the sample and the medium match in refractive index. Using a central stop, the sample will show colors complementary to those seen with an annular stop.
Double refraction [†]	The refraction of light in two slightly different directions to form two rays or vector components. Each ray is polarized and their vibration directions are perpendicular to each other. Furthermore, each ray has a different velocity and therefore a different refractive index. See birefringence.
Epi-illumination	Illumination technique where the light source is placed above the sample causing the objective to act as the condenser and objective.
Excitation filter	The first filter used in fluorescence microscopy; usually a bandpass filter. It produces specific exciting wavelengths from a high-energy light source, which are capable of inducing visible fluorescence in various substrates.
Extinction points	Orientation of a sample where light emerging from the sample is vibrating at right angles to the preferred direction of the analyzer. These positions are 90° apart and reveal the vibration directions of each sample. These directions will parallel the vibration directions of the two polars when the sample is extinct.

Extinction, oblique [†]	Extinction is oblique if the vibration directions are oblique to the long direction of the crystal.
Extinction, parallel [†]	Extinction is parallel if the vibration directions are parallel and perpendicular to the long direction of the crystal.
Extinction, symmetrical [†]	Extinction is symmetrical if the vibration directions bisect a prominent angle.
False Becke Line [†]	A second bright line that moves in the direction opposite to the Becke Line. It is usually observed with thick particles or when the refractive index difference between particles and mounting medium is large at the low index side of the interface.
Fast ray [†]	The fast ray or fast components for a crystal corresponds to the lower refractive index.
Field diaphragm	A variable aperture located in or near the light source.
Field of view	The maximum area visible when looking through the oculars of a microscope. Usually measured in millimeters.
Fluorescence microscope	An optical instrument using filter sets composed of excitation and barrier filters. These are oriented so that when used in conjunction with a high-energy light source, fluorescent images can be viewed.
Fluorite objective [†]	This objective is corrected both for spherical aberration and chromatic aberration at two wavelengths.
Focal length	The distance from the center of the surface of a lens or mirror to its focal point.
Focal point	A point at which rays of light converge or from which they appear to diverge after refraction in a lens or reflection from a mirror.
Full wave plate	See Compensator, full wave plate.
Illumination, diffuse [†]	A good quality illumination of lower intensity than Köhler or Nelsonian illumination because a ground glass is interposed between the lamp filament and the microscope condenser. The illuminator and lamp filament have few or no adjustments, although a field diaphragm is helpful.
Illumination, Köhler	An illumination technique that produces a bright, uniform field of view. The condenser is focused so that the conjugate image of the light source is in the back focal plane of the objective lens.
Illumination, Nelsonian [†]	High intensity illumination in which the lamp filament is imaged in the plane of the specimen. A ribbon filament or arc lamp is required to give uniform illumination; the lamp must be focusable; the filament position must be adjustable in all directions; sometimes called ‘critical’ illumination.
Illumination, oblique [†]	Illumination obtained by shading part of the condenser lens or by throwing the condenser film placed in that plane.
Image, real [†]	Image formed when the incident and reflected rays converge in front of the mirror. The image that would register on a ground glass screen or photographic film placed in that plane.

Image, virtual [†]	Image formed when the incident and reflected rays converge behind the mirror. The image would appear by construction to be in a given plane, but a ground glass screen or photographic film placed in that plane would show no image.
Immersion objective [†]	A microscope objective that is used with a liquid of refractive index greater than 1.00 between the specimen and objective and usually between the specimen and substage condenser. Immersion objectives are used when a numerical aperture greater than 1.00 is desired, because this cannot be achieved with a dry objective.
Inherent color	The color of a sample under visible light. Often referred to as the true color of the sample. Not to be confused with the interference color of the sample.
Interference color [†]	The colors exhibited by a sample caused by removal of visible wavelengths while viewed under cross polars. If a crystal is not at extinction, the emerging vector components recombine in the vibration plane of the analyzer. Since one component is retarded, interference causes the image to appear colored by destroying some wavelengths of light, their colors being subtracted from white light. The remaining light consists of other colors, which form the image of the crystal. These are called ‘interference’ or ‘polarization’ colors.
Interference, constructive [†]	The retardation of two light beams by exactly one wavelength or even multiples of one wavelength. The two waves reinforce each other, resulting in brightness.
Interference, destructive [†]	The retardation of two light beams exactly an odd number of half wavelengths, resulting in darkness as the two waves are perfectly out of phase.
Interference figures	Light patterns that emerge from a crystal when viewed with conoscopic illumination and under crossed polars. The conoscopic pattern of extinction positions of a crystal superimposed on the pattern of interference colors corresponding to the full cone of directions by which the crystal is illuminated, each direction showing its own interference color.
Isogyres [†]	In a uniaxial interference figure, the two black bars that form a cross and represent the pattern of extinction positions of the crystals. In a biaxial interference figure, the two black curved bars (brushed) that intersect the isochromatic curves (lemniscates) and represent the pattern of extinction positions of the crystal.
Isotropic	Substances that are identical in all directions; showing a single refractive index. For example, isotropic scattering of light by a substance entails that the intensity of light radiated is the same in all directions. These samples have only one refractive index.
Köhler illumination	See Illumination, Köhler.
Lambda zero (λ_0) [†]	In dispersion-staining, the wavelength at which both particle and liquid have the same refractive index.

Light microscope	A microscope that employs light in the visible or near-visible portion of the electromagnetic spectrum.
Magnification, empty [†]	An increase in the size of an image with no additional increase in detail.
Magnification, maximum useful (MUM) [†]	The maximum magnification necessary to resolve detail. Magnification in excess of MUM gives no additional resolving power. It can usually be estimated as being 1000 times the NA of the objective.
Melatope [†]	The center of rotation of the isogyres in biaxial interference figures representing the point of emergence of rays that, in the crystal, travel along the optic axes.
Michel-Lévy chart	A chart relating thickness, birefringence, and retardation so that any one of these variables can be determined when the other two are known.
Micrometer (μm) [†]	The usual unit of length for light microscopical measurements ($1 \mu\text{m} = 10^{-3} \text{ mm}$): It is still often referred to by its former name, ‘micron.’
Microphotograph [†]	A small, microscopic photograph, in which the image is minified; it requires enlarging or the use of a lens system in order to view it (see also photomicrograph).
Microscopy [†]	The application of any tool or technique helpful in characterizing microscopic objects.
Monochromatic light [†]	Light composed of one wavelength. It may be obtained by the use of a laser or by gaseous discharge tubes in combinations with proper filters. An approximation is obtained by interference filters or monochromators.
Nanometer (nm) [†]	The usual unit of linear measurement with the electron microscope and of measuring ultraviolet and visible light wavelengths. ($1 \text{ nm} = 10^{-9} \text{ m}$ or about $4 \times 10^{-8} \text{ in}$). Replaces the former name, ‘millimicron’ ($\text{m}\mu$).
Negative crystals [†]	A uniaxial crystal is optically positive if $\varepsilon < \omega$. A biaxial crystal is said to be optically positive if $\gamma - \beta < \beta - \alpha$.
Negative fibers	A fiber whose refractive index for light perpendicular to the long axis of the fiber, n_{\perp} , exceeds the refractive index of light parallel to the length of the fiber, n_{\parallel} , $n_{\parallel} > n_{\perp}$
Nelsonian illumination	See Illumination, Nelsonian.
Nicol prism [†]	A polarizing element made of two pieces of calcite specially cut, ground, polished, and cemented. A transmitted beam splits into two polarized components, one of which is refracted into and absorbed by the asphalt mount. The remaining polarized beam is transmitted.
Numerical aperture (NA)	The numerical measure of the light gathering ability of a lens. NA determines the resolving power of a microscope.
Ocular (eyepiece) [†]	The lens that provides the second step of magnification in a compound microscope. The lens system closest to the eye, usually 10X.

Omega vibration direction (ω) [†]	Any vibration direction in the plane of the a axes for uniaxial crystals.
Opaque [†]	Material through which light cannot pass. Opaque to transmitted light, that is, 100 % absorbing throughout the visible light range.
Optic axial angle [†]	The acute angle between two optic axes and a biaxial crystal in the plane of α and γ . It is constant for and characteristic of any particular substance.
Optic axial plane [†]	The plane containing the optic axes as well as α and γ .
Optic axis [†]	In crystallography, the direction through an anisotropic crystal along which unpolarized light travels without suffering double refraction. Uniaxial crystals have one optic axis; biaxial crystals have two optic axes.
Optic sign [†]	For uniaxial materials, by definition, the sign is positive if the value $\epsilon - \omega$ is negative. For biaxial materials, the sign is positive if γ is the acute bisectrix and negative if α is the acute bisectrix or positive if $\gamma - \beta > \beta - \alpha$ and $\gamma - \beta < \beta - \alpha$.
Orthoscopic observation	Normal observations made with a microscope. When the field diaphragm, ocular front focal plane, and sample are in simultaneous focus with the viewer's eye.
Parfocal objective [†]	Objectives that are mounted so that only small adjustment of the bodytube and stage is necessary to focus after changing from one objective to any of the others. They are mounted in such a way that the second conjugate plane is in the same position on the optical axis of the microscope for each objective. Objectives used on a rotating nosepiece are usually parfocal. Eyepieces are also parfocal within any given manufacturer's series.
Phase annulus	The transparent ring at the front aperture of the condenser of a phase contrast microscope that limits illumination of the sample.
Phase contrast microscope	A form of interference microscopy that transforms differences in the optical path of a sample into differences in amplitude in the image. This produces higher contrast allowing small differences to be easily viewed.
Phase plate	A transparent plate located in the rear focal plane of a phase contrast microscope, which reduces the amplitude of background waves and advances or retards the phase of the component relative to the diffracted waves.
Photomacrography [†]	Photographs in the magnification range between conventional macrophotography and photomicrography or say 1X to about 30X. Special lenses with built-in irises designed for small magnification ranges are usually used and without an ocular.
Photomicrograph [†]	A large photograph of a small object as viewed through a microscope, the image of which is enlarged or magnified (see also microphotograph).
Plane polarized light	Light that is vibrating in one plane.

Pleochroism	The optical property of samples exhibiting different colors when viewed in plane-polarized light at different orientations.
Polarized light microscope	A microscope that contains a polarizer and an analyzer, which produces polarized light or crossed polars for sample viewing.
Polarizer	A filter that allows light waves to pass in only one direction. It is located between the light source and the sample.
Polars [†]	Two identical polarizing elements in a polarizing microscope. The polar placed between the light source and substage condenser is called the polarizer; the polar placed between the objective and ocular is called the analyzer. The vibration directions of the two polars may be crossed 90° to achieve ‘crossed polars;’ slightly uncrossing one polar gives ‘slightly uncrossed polars;’ removing the analyzer results in ‘plane-polarizing light.’
Positive crystals [†]	A uniaxial crystal is optically positive if $\epsilon > \omega$. A biaxial crystal is said to be optically positive if $\gamma - \beta > \beta - \alpha$.
Positive fiber	A fiber whose refractive index for light parallel to the length of the fiber, n_{\parallel} , exceeds that of the refractive index of light perpendicular to the length of the fiber, n_{\perp} ; the elongation is said to be positive, $n_{\parallel} > n_{\perp}$
Privileged direction (of a polarizer)	The direction of vibration to which light emerging from a polarizer has been restricted.
Quarter wave plate	See Compensator, quarter wave plate
Quartz wedge	See Compensator, quartz wedge
Real image	See Image, real.
Reflection	Light that leaves a surface at an angle equal to the incident angle.
Refraction	The change in the speed of light as it passes from one medium to another medium of a different refractive index.
Refractive index	The ratio of the velocity of light in a vacuum to its velocity in a transparent medium. Refractive index generally increases with the atomic number of the constituent atoms. A high density or atomic number elements usually results in a high refractive index. An optical property used to distinguish different transparent samples.
Relief	The degree of contrast observed between a particle and the medium into which it has been mounted.
Resolving power [†]	The ability to distinguish two small points in an image with their diffraction discs not overlapping more than half their diameter. The actual distance of one of the double refracted rays behind the other as they emerge from an anisotropic particle. It depends on the difference in the two refractive indices, $n_2 - n_1$ and the thickness.
Retardation (Δ)	The reduction in the velocity of the slow ray as compared to the fast ray of light produced by a birefringent sample. Often refers to the interference color produced under crossed polars, which corresponds to the x-axis of the Michel-Lévy chart. Retardation is equal to the thickness multiplied by the birefringence (difference in refractive index) of the sample.

Sign of elongation [†]	The elongation of a substance in relation to refractive indices. If it is elongated in the direction of the high refractive index, it is said to be positive; if it is elongated in the direction of the low refractive index, it is negative.
Slow ray [†]	The slow vibration direction of a crystal corresponding to the higher refractive index.
Spherical aberration	An optical distortion caused because a spherical lens or mirror does not focus parallel rays to a point, but along a line. Therefore, off-axis rays are brought to a focus closer to the lens than are on-axis rays.
Stage micrometer	Usually a stage scale with divisions 10 µm (0.01 mm) apart. This is used to calibrate the scale found on oculars so that measurements can be made on the microscope.
Stereomicroscope	An optical instrument that uses two optical paths to produce three dimensional images.
Temperature coefficient of refractive index [†]	The change in refractive index (<i>n</i>) with temperature. The degree of variation of <i>n</i> depends on the composition of the substance and the state of aggregation, e.g., whether it is a solid or a liquid. It is usually about 100X larger for liquids than for solids and about -0.0005°C for liquids.
Translucent [†]	Transmission of light in such a way that image-forming rays are irregularly refracted and reflected.
Transparent	Material that can transmit visible light.
Tubelength, mechanical [†]	The distance from the shoulder of the objective to the upper end of the drawtube. The mechanical tubelength for biological and most polarizing microscopes is 160 mm; Leitz formerly had a 170 mm tubelength.
Uniaxial crystals [†]	Anisotropic crystals in the tetragonal and hexagonal systems having one unique crystallographic direction and either two (tetragonal) or three (hexagonal) directions that are alike and perpendicular to the unique directions.
Unpolarized light [†]	A bundle of light rays having a common propagation direction but different vibration directions.
Virtual image	See Image, virtual.
Wavelength	The distance of one cycle of an electromagnetic wave (the distance between two points at which the phase of a wave is the same). It is usually measured in nanometers, or formerly in Angstrom units (1 nm = 10 Å).
Working distance [†]	The distance between the top of the coverslip and the nearest portion of the objective.

[†] McCrone WC, Delly JG. *The Particle Atlas*. 2 ed. Vol 4, Ann Arbor, MI: Ann Arbor Science, 1973.

Author's note: Although we have collected many terms here we have focused on the basic microscopy terms. Additional terms are available in the glossary online from the *McCrone Atlas of Microscopic Particles*, Westmont, IL, 60559: McCrone Associates, Inc.; 2005 [updated 2005; cited 2007 November 27]; Available from: <http://www.mccroneatlas.com/>.

Index

- Abaca fiber
 microscopic characteristics of 217
 optical properties of 331
 uses of 220
- Abbe refractometer 35–6
- Absorbance equation 309
- Aberration 347
- Absorption spectrum 298, 300–1, 308
- Acetate fiber
 melting point 316
 optical properties 333
 polymer composition 228
- Achromatic objectives 347
- Acrylic fiber
 melting point 316
 optical properties 333
 polymer composition 229
 types of 333
- Actinolite fiber
 optical properties 331
- Acute bisectrix (Bx_a) 257, 261, 354
- Additive retardation (addition) 54–5, 234, 257–8, 260
- Akund fiber
 microscopic characteristics of 216
 uses of 220
- Alumina silicate glass 194
- Anidex fiber
 polymer composition 220
- Amosite fiber 219
 optical properties 331
- Amphetamine 278–9, 282–3
- Anagen phase 151, 180, 186–8, 190
 root 180, 186–8
- Analyzer 36, 39–44, 46–7, 49–50, 52, 254, 259, 347, 350, 352, 355
- Angular aperture (AA) 15–6, 347
- Animal fibers 210–1, 217–8, 220, 331
 morphological properties of 217
 optical properties of 331
- Animal hair see specific animal also
 examinations 149–50, 152–158, 160–8
 medulla 154
 root 161
- scale patterns
 coronal 153–4
 imbricate 153–4
 spinous 153–4
- Anions, tests for 265, 268, 270
 carbonate 270
 chlorine 270
 nitrite 270
 sulfate 270
- Anisotropic 41, 43–4, 46, 48, 50, 53–4, 251, 256, 347, 354, 356
- Annular stop 31–2, 36, 347, 350
- Antelope hair 153, 161
 color banding 161
 root shape 161
 scale pattern 161
- Anthophyllite fiber
 optical properties 331
- Aperture 347
 angular 15–6, 347
 numerical 14–6, 257, 353
 substage 19, 50, 195, 255, 349
- Apochromatic objective 347
- Aramid fiber
 melting point 316
 optical properties 333
 polymer composition 229
 types of 333
- Arch
 filament 94–5
 plain 104–5
 tented 104–5
- Asbestos fiber 210, 212–3
 microscopic characteristics of 218
 optical properties of 331
- Axis, crystallographic 347
- Azlon fiber
 melting point 316
 optical properties 333
 polymer composition 228
- Back focal plane 4, 13, 15–6, 42, 71, 255–6, 347, 351
- Ballistics see firearms

- Barrier filter 59, 62–3, 347, 351
 Bear hair
 medulla type 163
 scale pattern 163
 Beaver hair
 medulla type 162
 Becke line method 30–2, 34, 36, 48–51, 54, 73, 193–4,
 196–7, 210, 219, 222–3, 231, 233, 260, 347,
 348, 351
 Beer-Lambert Law 309
 Bertrand lens 16, 19, 36, 39–40, 42, 255–7, 260–1, 348
 benzphetamine 278, 283
 Biaxial crystals 251, 253, 355–7, 259–262, 348, 354,
 355
 Biaxial interference figures 258, 261, 352–3
 Billingham test 215, 220, 224
 Binders 144, 300, 325
 infrared data 300
 Biological samples 69, 289, 291
 Birefringence 348, 355
 crystal/mineral 251, 254, 257, 270
 equation 43
 equation of fiber 49
 fiber 48–9, 53–4, 56, 210–1, 219, 230–1, 233, 238,
 331–5
 polarized light and 41, 43–4, 46
 Body tube 3, 348
 Borosilicate glass 194, 326
 Braid
 Solid braid 84
 Diamond braid 84
 Twisted braid 84
 Breech mark 116, 120
 Brightfield illumination 348
 Buckling 151, 170–1
 Burn test 215, 220–2, 224, 231, 235
 Caliber 116
 Calibration
 ocular 20–3
 Camel fiber
 microscopic characteristics of 218
 Camel hair
 medulla type 162
 scale pattern 162, 218
 Caribou hair
 color banding 161
 root shape 161
 scale pattern 161
 Carpet
 examinations 84–87
 knitted 84
 tufted 84–5
 worksheet 90
 woven 84–5
 Cartridge case 115–6, 118
 Cashmere fiber
 microscopic characteristics of 218
 Cat hair
 medulla type 163
 root shape 161, 163
 scale pattern 163
 Cations, tests for
 ammonium 270
 calcium 270
 potassium 270
 sodium 269
 Cellulose-based fibers
 types of 228
 Central stop 31–2, 36, 348, 350
 Chemical-based fibers
 types of 228–30
 Chinchilla hair
 medulla type 162
 Chlordiazepoxide HCl 278
 Christmas Tree stain 290–1
 Chromatic aberration 348
 Chrysotile fiber
 optical properties 218–9, 331
 Class characteristics 77, 83, 111–3, 116–7,
 131, 133
 Cleavage 251, 348
 Cobalt thiocyanate 278, 281
 Cocaine 278–9, 281, 285
 Codeine 278
 Coir fiber
 microscopic characteristics of 217
 uses of 220
 Cold shock 96–7
 characteristics 97
 Color
 assessment 307–8, 311
 fiber characteristics 209–11, 230
 glass characteristics 193, 326–7
 hair characteristics 150–6, 160–4, 180–1, 187
 inherent 352
 interference 43, 48, 54, 257, 268, 352
 light properties 32, 43–4, 234
 paint characteristics 143–5, 325
 soil characteristics 243–5, 251
 Color tests 124–5, 277–9
 antimony 124
 barium 125
 Cobalt Thiocyanate 278, 281
 copper 127
 Diphenylamine 126
 Dithiooxamide (DTO) 127
 Duquenois-Levine 278, 280
 Harrison-Gilroy 125
 lead 124
 Mandeline 278, 282
 Marquis 278, 282
 Mecke 278, 283
 modified Greiss 125–6
 nitrates 125
 Sodium Rhodizonate 124
 Comparison microscope 348
 fiber 238
 firearms 117
 parts diagram 119

- hair 182
tool marks 113
Compensation 348
Compensator 39,-40, 53–4, 211, 231
full-wave plate (first-order red) 54, 349
quarter-wave plate 54, 349
quartz wedge 54, 349
tilting (Berek) 54, 349
Compound light microscope 13–38, 349
optical path 15
parts diagram 17
Concentric fractures 202–3
Condenser 13–5, 38, 41–2, 59, 61, 67, 69–70, 256–7, 310, 349
Confirmatory test 43, 139, 265, 277, 290
Conjugate foci 16, 349
Conoscopic observation 16, 50, 251, 255–7, 347
optical path 256
Contamination
of microcrystalline tests 268, 278
of refractometer 35
Contrast 69–70, 132, 325, 349, 355
Controlled substances see specific drug
Convolution 43, 210, 216
Cortex 150–1, 153, 155, 160, 180, 187
Cortical fusi 152–3, 180, 188–9
Core 104–5
Cotton fiber
microscopic characteristics of 216, 270
optical properties of 43, 331
Cow hair
medulla type 163
root shape 161,163
Coyote hair
color banding 164
medulla type 164
scale pattern 164
Crimp 161–2, 210
Crocidolite fiber 219
optical properties 332
Critical angle 349
Cross-section
fiber 210–2
hair 151, 169–2, 180, 215–7, 220, 227, 230–1, 238
paint 144, 326
rope 85
wood 139
Crystalline 43, 48, 54, 251, 349
Crystallographic axis 347
Curvature of field 350
Cuticle 150–2, 155, 160, 170, 180, 187, 217
types of 152–3
Cystolithic hair 138
Darkfield illumination 350
Darvan fiber
optical properties 333
Deer hair
color banding 161
root shape 161
scale pattern 153, 161
Delta 104–6
Delustering agnet 211
Density
equation 195
glass 193, 195, 318
hair 151, 170, 180
minerals 243, 245–6
refractive index and 33
Depth
of field 3–4, 6, 13, 39, 60, 68, 325, 350
of focus 6, 325, 350
Diamond braid 84
Dichroism 350
Diphenylamine (DPA) 126
Dithiooxamide test 123, 127
Dispersion 31, 194, 350
equation 31
plot 31, 317
Dispersion staining 29, 31–2, 219, 350
colors 32
graph 339
objective optical path 32
Distance determination 123, 126
DNA 104, 186
and hair 187–9
mitochondrial 187–8
nuclear 187–8
Dog hair
medulla type 163
root shape 161, 163
Double refraction 350
Drugs see specific drug
Duct tape examinations 83, 86
worksheet 91
Duquenois-Levine test 139, 278, 280
Dye 209, 211, 220, 230,-1, 227–8, 311
Ejector marks 116
Elastoester fiber
polymer composition 230
Elk hair
color banding 161
root shape 161
scale pattern 161
Emission spectroscopy 299, 309, 323, 326
Energy Dispersive X-ray (EDX) 324–6
Epi-illumination 350
Ermine hair
color banding 163
medulla type 163
scale pattern 163
Excitation filter 39, 62–3, 350
Extinction see microscopic characteristics for specific fibers also
oblique 351
parallel 44, 351
points 41, 43, 210, 212, 302, 311, 350
symmetrical 351
Extraordinary ray (e-ray) 43, 38, 252, 254

- False
 Becke line 351
 negative 279
 positive 279
 Fast ray 49, 54–5, 257, 351
 Ferret hair
 medulla type 164
 scale pattern 164
 Fiber see specific fibers also
 animal 217
 birefringence of 48–9, 53–4, 56, 210–1, 219, 222, 230–1, 233, 238, 331–5
 cellulose-based 228
 chemical-based 228
 classification of 210
 color 209–11, 230
 comparisons 237–8
 cross sections of 211, 230
 dyes and pigments 211, 230
 examinations 209, 219
 fluorescence 231
 FTIR analysis of 297, 301
 man-made 210, 227–236
 melting points (hot stage) of 315–6
 Microspectrophotometry of 307, 311
 mineral 218
 natural 210–221
 optical properties of 331, 333
 refractive index of 210, 219, 230, 331, 333
 sign of elongation of 211, 220, 231, 331, 333
 solubility of 311, 221, 231
 worksheet 214
- Field diaphragm 14, 16, 350
 Field of view 3, 6, 13, 35, 39, 43, 60, 112, 117, 181–2, 194, 238, 351
- Filament
 aged 94–5
 broken 966–7
 burnout 94–5
 characteristics of 95–7
 cold shock 94, 96–7
 discoloration 96
 distorted 95–6
 examinations 93–102
 halogen 94
 hot shock 95–6
 worksheet
 double filament 101
 single filament 100
- Fingerprint
 components of 104
 examinations 103–110
- Firearm
 cartridge cases 115–6, 118
 class characteristics 116
 distance determination 123, 126
 examinations 113–121
 firing pin 115–6, 122, 325
 gunshot residue (GSR) 122–130
 individual characteristics 116
 primer 116, 122–3
- projectiles 115–6, 122
 propellants 115, 122–3
 shotshell 122
 Firing pin 115–6, 122, 326
 Flat glass 201
 Flax fiber
 microscopic characteristics of 216–7
 optical properties of 331
 uses of 220
 Float glass 201
 Fluorescence Microscope 59–66, 211, 215, 220, 227, 230–1, 237–8, 308, 351
 optical path 62
 parts diagram 64
 Fluorite objective 351
 Fluorocarbon fiber
 melting point 316
 optical properties 333
 polymer composition 230
 Focal length 5–6, 16, 231, 351
 Focal point 14, 351
 Fourier Transform Infrared Spectrophotometer (FTIR)
 297–306
 fiber 297, 301
 functional group locations 299
 paint 144, 300
- Fox hair
 color banding 163
 medulla type 163
 scale pattern 136
 types of 163
 Full wave plate 349, 351
- Gauge 116
 Glass
 breakage 193
 colorants 193, 327
 components of 193, 326–7
 cracks (fractures)
 concentric 202–3
 radial 202–3
 density 193, 195
 elemental 326–7
 examinations 193–208
 refractive index 30–1, 193–5, 317–8
 types of 194
 Wallner markings 203–4
- Glass fiber
 optical properties 333
 polymer composition 230
- Goat hair
 medulla type 164
 root shape 164
 scale pattern 164
- Gold chloride 284–5
 Greiss test, modified 126
 Grooves 116
 Ground hog hair
 color banding 164
 medulla type 164
 scale pattern 164

- Gunshot residue
 distance determination 123, 126
 elemental 325
 examination 122–130, 325
- Hair
 animal 160–168
 DNA 186–192
 examinations 149–192
 human 169–177
 known 176
 unknown 177
 general microscopic characteristics 149–159
 structure of 150
 worksheet 159
- Harrison-Gilroy test 123, 125
- Hartmann Net graph 317
- Hematoxylin-Eosin 290, 292
- Hemp fiber
 microscopic characteristics of 216–7
 optical properties of 331
 uses of 220
- Heroin 278, 284–5
- Herzog test 215, 220, 224
- Hog hair 164
- Horse fiber
 microscopic characteristics of 218
- Horse hair
 medulla type 164
 root shape 164
- Hot shock 95–6
 characteristics 96
- Hot stage
 fibers 315–6
 glass 315, 317–8
- Human hair
 artificial treatments 151, 179–81
 color 150–6, 160–4, 180–1, 187
 cortex characteristics 150–1, 153, 155, 160, 180, 187
 cuticle characteristics 150–2, 155, 160, 170, 180, 187,
 217
 diseases 181
 environmental and acquired traits 181
 examinations 149–192
 medulla characteristics 150–2, 154–5, 170–1, 180,
 187–8
 pigmentation characteristics 151, 153, 155, 180,
 187–8
 racial group characteristics 169–72
 root characteristics 150–1, 155, 160–3, 171, 180,
 186–8
 shaft characteristics 150–1, 154–5, 160–4, 170–1,
 180–1, 188
 somatic origin characteristics 149, 169, 171, 178, 181
 tip characteristics 150–1, 154, 161, 164, 171, 180–1
- Illumination
 diffuse 16, 351
 Köhler 16, 18, 351
 Nelsonian 16, 351
 oblique 193–5, 197, 351
- Image 3–4, 13–5, 39, 41–2, 61, 67, 69, 112, 255, 323
- real 351
 virtual 2, 352
- Immersion objective 290, 352
- Impressed action marks 116
- Impression evidence
 fingerprints 103–110
 firearms 115–121
 shoe print 131–6
 tire print 131–6
 tool mark 111–4
- Incandescence 93, 96
- Indicatrix 251
- Individual characteristics 77, 83, 111, 116,
 131
- Inherent color 41, 43, 352
- Ink examinations 307, 311
- Interference
 color 43, 48, 54, 257, 268, 352
 constructive 299, 352
 destructive 67, 70, 299, 352
- Interference figures
 biaxial 251–3, 255–8, 261, 348
 uniaxial 251–4, 256–60, 356
- Isogynes 257–8, 352
- Isotropic 41, 43, 46, 48, 218, 252–3, 255,
 352
- Jute fiber
 microscopic characteristics of 216–7
 optical properties of 331
 uses of 220
- Kapok fiber
 microscopic characteristics of
 216
 optical properties of 331
 uses of 220
- Kenaf fiber
 Optical properties 331
- Knitted carpet 84
- Köhler illumination 16, 18, 351
 Setting up 18
- Laminated glass 202
- Lamp filaments see filament
- Lands 116
- Latent print 107
- Leaded glass 194, 326
- Light microscope 13–20, 326
- Llama fiber
 microscopic characteristics of 218
- Loop
 radial 105
 ulnar 105
- Lumen 210, 216, 220
- Lynx hair
 color banding 163
 medulla type 163
 scale pattern 163
- Lyocell fiber
 optical properties 333
 polymer composition 228

- Magnification 5
 empty 353
 maximum useful (MUM) 353
 total 6
- Man made fibers see specific fiber also
 cellulose-based 228
 chemical-based 228
- Mandeline reagent 278, 282
- Marijuana
 color test 278, 280
 examinations 137–9
 microscopic characteristics 138
- Marquis reagent 278, 282
- Mecke reagent 278, 282
- Medulla 150–2, 154–5, 160–4, 170–1, 180, 187–8
 types of 154
- Medullary Index 150, 154
- Melamine fiber
 polymer composition 230
- Melanin see pigment
- Melatope 257–8, 353
- Metallic fiber
 polymer composition 230
- Methamphetamine 278, 282
- Melting points 316, 319
- Michel-Lévy chart 54, 210–1, 220, 231, 337
- Microchemical tests see color test
- Worksheet
 known 272
 negative 273
 unknown 374
- Microcrystalline tests
 for antimony 124
 for barium 124
 for lead 124
 for nitrites 125
- Microscopy terms 347
- Microspectrophotometer 307–314
 fibers 311
 ink 311
 optical path 310
 paint 311
- Mineral fibers
 optical properties of 210, 218
- Mink hair
 color banding 162
 medulla type 162
 scale pattern 162
- Minutia
 Bifurcation 106
 Short ridge 106
 Ridge ending 106
 enclosure 106
- Mitochondrial DNA 187–8
- Mock case scenario 341
- Modacrylic fiber
 melting point 316
 optical properties 334
 polymer composition 229
- types of 334
- Mohair fiber
 microscopic characteristics of 218
- Monochromatic light 34, 299, 353
- Moose hair
 color banding 161
 root shape 161
 scale pattern 161
- Morphine HCl 278, 283
- Mountain lion hair
 color banding 164
 medulla type 164
 scale pattern 164
- Mounting samples
 dry mount 25–7
 wet mount 25–8
 scale casts 25–8
- Munsell color value 244, 281
- Muskrat hair
 color banding 162
 medulla type 162
 scale pattern 162
- Nanometer (nm) 353
- Natural fibers
 animal 217–8
 mineral 218–9
 plant 216–7
- Negative crystals 353
- Negative retardation (subtraction) 53–4, 211, 220, 231, 256, 354, 356
- Nicol prism 353
- Normal aged lamp 94
- Normal lamp burnout 94
- Normal new lamp 93
 characteristics 95
- Novoloid fiber
 melting point 316
 optical properties 335
 polymer composition 230
- Nuclear DNA 187
- Numerical aperture (NA) 14, 16, 257, 353
- Nylon fiber
 melting point 316
 microscopic characteristics 229
 optical properties 334
 polymer composition 228
 types of 334
- Nytril fiber
 optical properties 333
 polymer composition 230
- Objective
 achromatic 347
 apochromatic 347
 centering 18
 dispersion-staining 29, 31–2, 219, 350
 fluorite 351
 immersion oil 352
 parfocal 354
- Oblique illumination method 193–5, 197, 351

- Ocular (eyepiece) 3–6, 13–6, 20–2, 39, 41–2, 59–63, 67–9, 112, 117, 182, 210, 238, 255–7, 310, 348–9, 351, 353–6, 20–22
calibration
- Olefin fiber
melting point 316
optical properties 334
polymer composition 230
types of 334
- Omega vibration direction (ω) 252, 354
- Opiates 278, 283
- Opossum hair
color banding 164
root shape 164
scale pattern 164
- Optic axial
angle 256, 354
plane 354
- Optic axis 252–4, 257, 260–1, 354
- Optic sign 251, 256–7, 259–61, 354
- Optical properties
of biaxial minerals 255
of glass 194
of man-made fibers 333
of natural fibers 331
of isotropic minerals 252
of uniaxial minerals 254
- Ordinary ray (o-ray) 43, 48
- Orthoscopic observation 16, 256–7, 354
setting up 18
- Otter hair
color banding 162
medulla type 162
scale pattern 162
- Ovoid bodies 152–3, 163, 180
- Oxycodone HCl 278, 283
- Paint
components of 144
cross sections 144
elemental 323, 325–6
examinations 143–8
FTIR 297, 299–301
absorption band locations-binders 300
absorption band locations-pigments 301
microspectrophotometry 307–9, 311
pigments 300
solubility 144–5
- PBI fiber
polymer composition 230
- Phase annulus 67, 69–71, 354
- Phase contrast microscope 67–74
optical path 70
- Phase plate 67, 69–71, 354
- Physical match
examinations 77–82
using edge shape 78
using surface features 78
using mirror images 78
- Pigment
hair 151, 153, 155, 180, 187, 188
paint 144
infrared data 300
- Plain arch 104–6
- Plane polarized light 42, 49, 217–9, 228–9, 354
- Plant fibers
morphological properties of 216
optical properties of 351
uses of 220
- Pleochroism 41, 44, 230, 238, 311, 355
- Polarized light 47–9, 53, 210, 217–9, 229, 254–6, 268
- Polarized light microscope 39–58, 238, 355
optical path 42
parts diagram 45
- Polarizer 39–44, 49, 254, 355
- Polyacrylostyrene fiber
optical properties 334
- Polyamide fiber see nylon
- Polyester fiber
melting point 316
microscopic characteristics 229
optical properties 334
polymer composition 229
types of 334
- Positive crystals 355
- Positive fiber 355
- Presumptive test
for drugs 277, 279
for semen 290
- Primer
firearms 115–6, 122–3, 326
paint 144
- Privileged direction (of a polarizer) 355
- Procaine 281, 284–5
- Propoxyphene 277, 283
- Projectile 115–6, 122
- Propellant 115, 122–3
- Quarter wave plate 39, 54, 349, 355
- Quartz wedge 37, 54–5, 349, 355
- Rabbit fiber
microscopic characteristics of 218
- Rabbit hair
medulla type 162
scale pattern 162
- Raccoon hair
color banding 162
medulla type 162
scale pattern 162
- Racial group microscopic characteristics 169–70, 172
- Radial fractures 202–4
- Ramie fiber
microscopic characteristics of 217
optical properties of 331
uses of 220
- Rayon fiber
melting point 316
microscopic characteristics 228
optical properties 334
polymer composition 228
types of 334
- Real image 349, 351

- Reflected light 3–5, 61
 Reflectivity equation 309
 Refraction 5, 26, 29–30, 350, 355
 Refractive index 21, 26, 30–1, 355
 Becke line method 30, 34, 41, 43, 48–9, 53–4, 194, 197, 210, 219–22, 231, 238, 252–5, 348,
 density and 33
 dispersion staining method 31, 36, 350
 Emmons double variation method 317–8
 equation 30
 equation of dispersion 31
 negative 355
 oblique lighting method 193–7
 of isotropic materials 48, 352
 of anisotropic materials 48, 347
 phase contrast method 67–94
 positive 353
 temperature and 33
 Relative refractive index 31
 Relief 26, 49, 251, 254, 317, 355
 Resolving power 14, 16, 355
 Retardation
 additive 54–5, 234, 257–8, 260
 equation 43
 negative 53–4, 211, 220, 231, 256, 354, 356
 Rib markings see Wallner lines
 Rifling action marks 116
 Rope
 braided 84
 examinations 83–4
 twisted 84
 worksheet 89
 Sable hair
 medulla type 163
 scale pattern 163
 Saran
 melting point 316
 optical properties 335
 polymer composition 230
 Scale casts 25, 28, 155–6, 161–4, 217
 Scale patterns see specific animal/human hair also
 coronal 153–4
 imbricate 153–4
 spinous 153–4
 Scanning Electron Microscope (SEM) 323–330
 glass 326
 gunshot residue 326
 paint 325
 Seal hair
 medulla type 162
 scale pattern 162
 Semen see sperm
 Sheep hair
 medulla type 162
 scale pattern 162
 Shoe print examinations
 casting of 133
 class characteristics 131
 examinations 131–6
 Individual characteristics 131
 printing of 134
 Shotgun shell 122
 Shouldering 151–2
 Sign of elongation 53–5, 209–11, 227, 230–1, 331, 333, 356
 Silica quartz glass 194
 Silk fiber
 microscopic characteristics of 218
 optical properties of 331
 Simple hair 138
 Sink float method 195, 198
 Sisal fiber
 microscopic characteristics of 216–7
 optical properties of 331
 uses of 220
 Skunk hair
 medulla type 164
 scale pattern 164
 Slow ray 49, 54–5, 257, 356
 Snell's Law 30
 Soda-lime glass 326
 Sodium Rhodizonate test 123–4
 Soil
 color 244–5
 components of 245–6
 density 245–6
 examinations 243–264
 minerals 244, 251–5
 moisture content 245
 particle size 245
 texture 245
 Solid braid 84
 Solubility
 fibers 211, 221, 231
 paint 143–5
 Somatic origin of hair
 microscopic characteristics for 169–72
 Spandex fiber
 melting point 316
 optical properties 355
 polymer composition 230
 Species identification
 hair 150, 155, 160–6
 sperm 290
 wood 139
 Sperm
 Christmas Tree stain 291
 examination 289–294
 Hematoxylin-Eosin stain 292
 slide preparation 291
 Squirrel hair
 medulla type 164
 scale pattern 164
 Stereomicroscope 3–12, 356
 Common Main Objective 3–4
 optical path 5
 Greenough 3–4
 parts diagram 7
 Steroids 278, 282
 Subtractive retardation (negative) 53–4, 211, 220, 231, 256, 354, 356

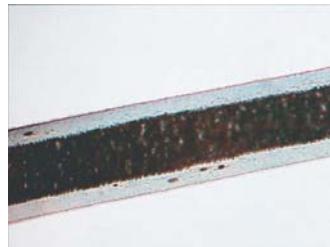
- Sulfar fiber
optical properties 335
polymer composition 230
- Sunn fiber
microscopic characteristics of 217
uses of 220
- Surface dye 211
- Synthetic fiber see man-made fiber
- Telogen phase 151
root 181, 186–9
- Temperature coefficient of refractive index 33, 194, 198, 356
- Tempered glass 201
- Tented arch 104–5
- Thermal microscopy examinations 315–322
- Tire print
casting of 133
class characteristics 131
examinations 131–6
individual characteristics 131
printing of 134
- Tool mark
casting of 113
class characteristics 111
examinations 111–4
individual characteristics 111
- Total magnification 6
- Transmitted light equation 309
- Tremolite fiber
optical properties 331
- Triacetate fiber
melting point 316
optical properties 335
polymer composition 228
types of 335
- Tubelength, mechanical 356
- Tufted carpet 84–6
types of 86
- Twist
S (left laid) 84, 220
Z (right laid) 84, 220
Right 116
Left 116
- Twist test 220
- Twisted braid 84–5
- Type line 104
- Uniaxial crystals 251–60, 356
Uniaxial interference figures 257, 260
Unpolarized light 356
- Vicara fiber
optical properties 333, 335
- Vinal fiber
melting point 316
optical properties 335
polymer composition 230
- Vinyon fiber
melting point 316
optical properties 335
polymer composition 229
types of 335
- Virtual image 5, 352
- Wallner lines 202, 204
- Whorl 104–5
plain 105–6
central pocket 105–6
double loop 105–6
accidental 105–6
- Wolf hair
medulla type 164
- Wood
hard 139
soft 139
- Wool fiber
microscopic characteristics of 217–8
optical properties of 331
- Working distance 4–7, 31, 210, 231, 356
- Worksheets
carpet 90
circle template 345
double filament 101
duct tape 91
fiber 214
hair 159
human hair known 176
human hair unknown 177
microchemical known 272
microchemical negative 273
microchemical unknown 274
report of forensic laboratory examination 344
request for evidence examination 343
rope 89
single filament 100
- Woven carpet 84–5



a) cortical fusi



b) double medulla



c) ovoid bodies



d) public buckle



e) shouldering



f) pigment orientation

Several microscopic characteristics of hair that can be noted to 'individualize' a hair sample.



a)



b)



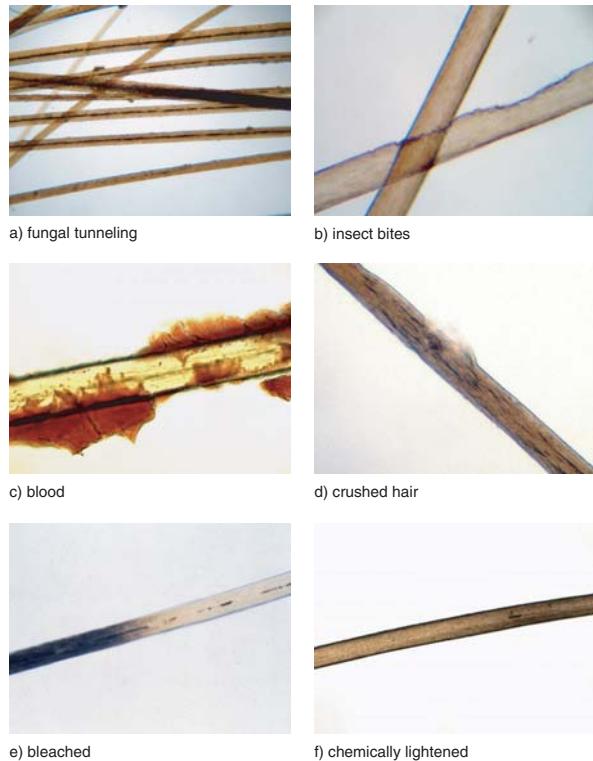
c)



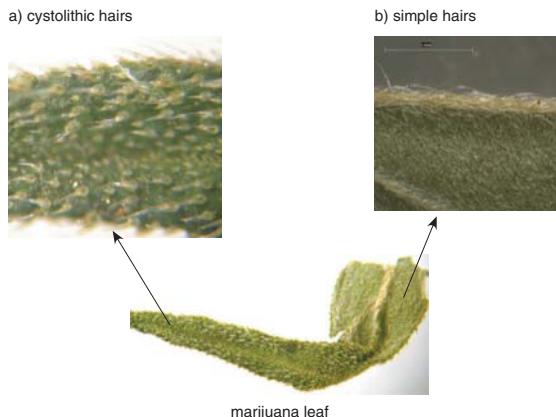
cross-sectional shapes



Some hair characteristics are associated with anthropological groups: a) Caucasian; b) Negroid; and c) Mongoloid.



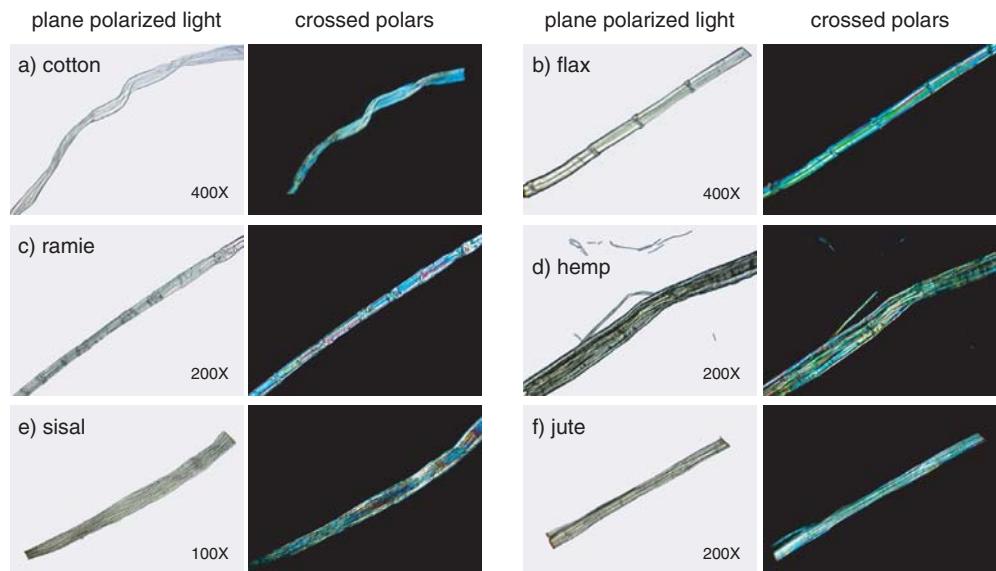
Acquired hair characteristics may be obtained through environmental conditions or by artificial treatments:
 a) fungal tunneling (viewed as dark irregular spaces) are air pockets that are caused by fungal growth; b) insect bites are found on hair that has been discarded for a time period (i.e., dustballs); c) blood can leave colored material along the hair shaft; d) the type of damage to a hair can also sometimes be determined, for example, this hair has a crushed portion; the color of hair can be chemically changed by e) bleaching or f) dying.



A marijuana leaflet showing the location of cystolithic and simple hairs. a) Cystolithic hairs are found on the top of marijuana leaves while b) simple hairs are found on the bottom of leaf. Both must be present in a sample for a positive identification.



The split field of view as seen on the comparison microscope. This allows comparison of known and unknown samples within the same viewing field.

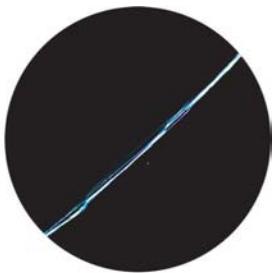


Common natural fibers under plane polarized and crossed polarized light.

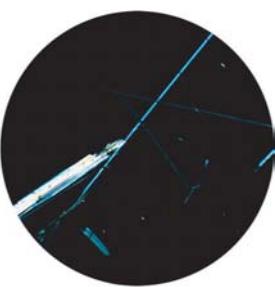
plane polarized light



crossed polars



a) chrysotile

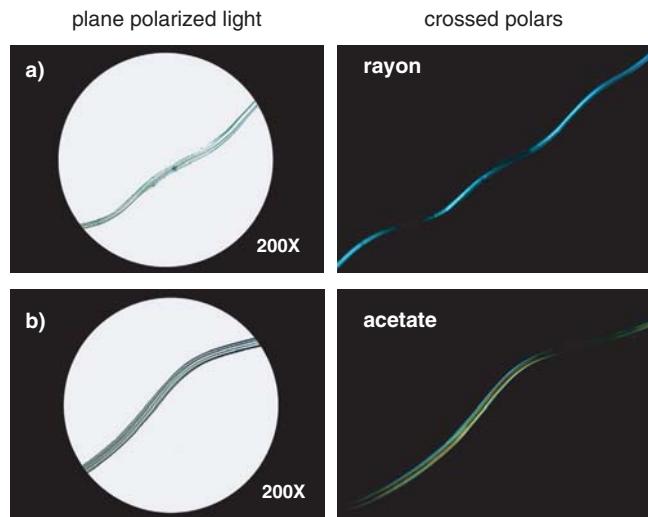


b) amosite

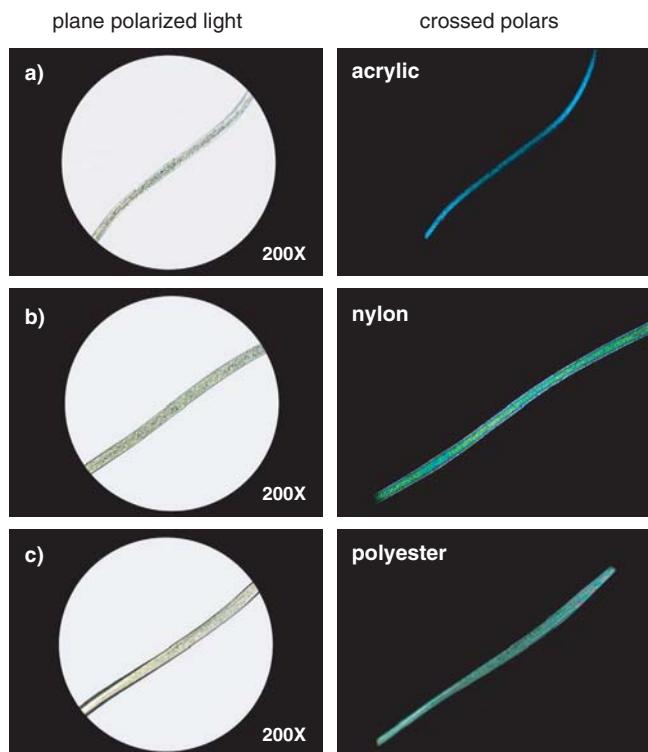


c) crocidolite

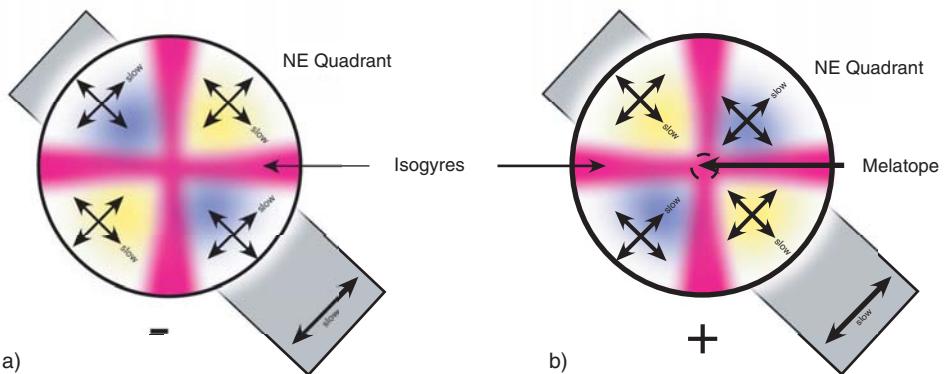
Common asbestos fibers under plane polarized and crossed polarized light.



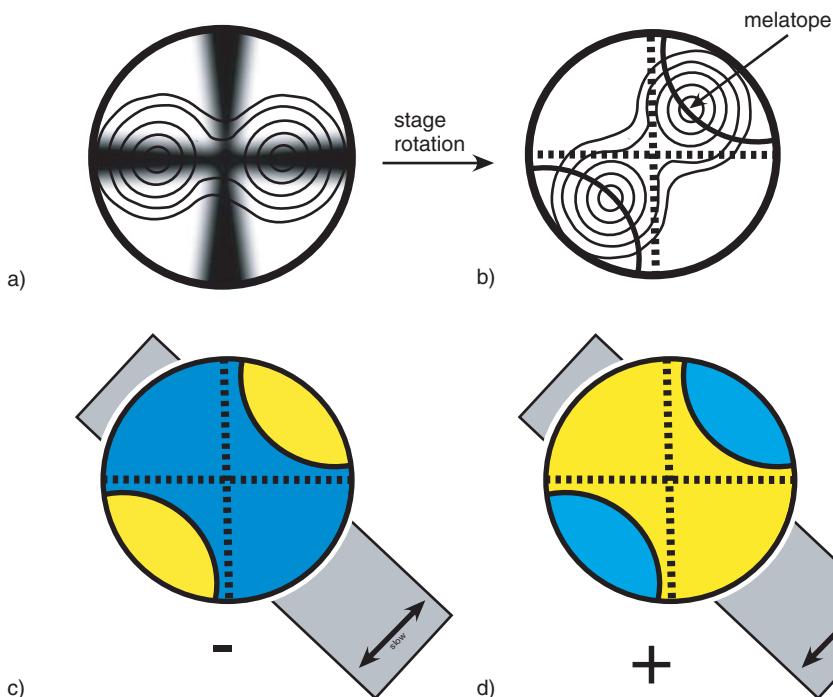
Common cellulose-based polymer fibers under plane polarized and crossed polarized light.



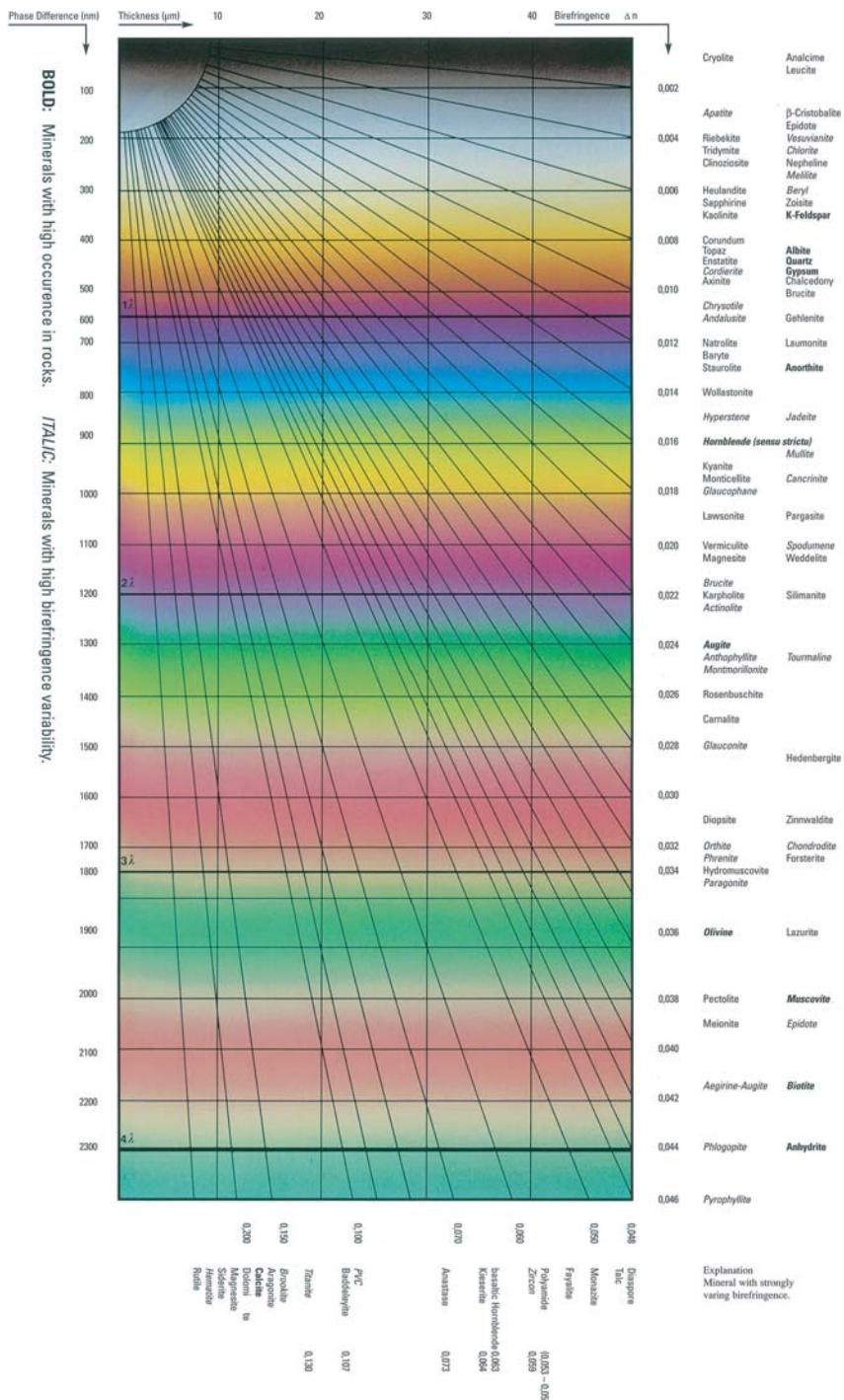
Common chemical-based polymer fibers under plane polarized and crossed polarized light.



A typical interference figure for a uniaxial mineral. The isogyes appear first order red upon insertion of the first order red plate. a) A negative mineral will appear yellow in the NE quadrant because subtraction has occurred (slow rays of mineral and accessory plate are perpendicular). b) A positive mineral will appear blue in the NE quadrant because addition has occurred (slow rays of the mineral and accessory plate are parallel).

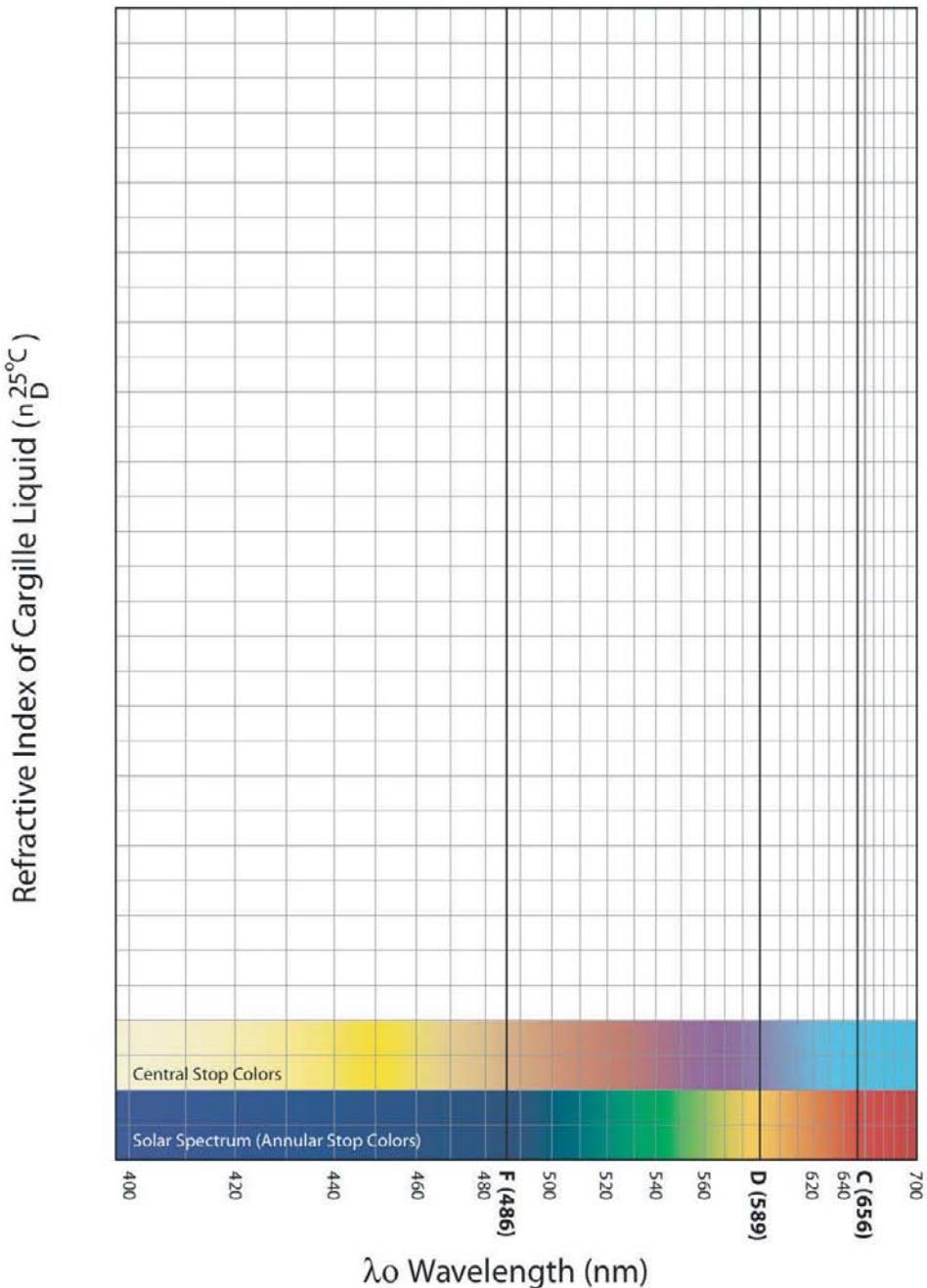


A biaxial interference figure. With stage rotation, the interference figure changes from a) a black cross above interference rings to b) two hyperbolic isogyes above each melatope. Upon insertion of the first order red plate the center of the crosshairs in b) turn c) blue for a negative mineral and d) yellow for a positive mineral.



Michel-Lévy chart.

Dispersion Staining Graph



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Dispersion-staining graph. Reproduced from *Essentials of Polarized Light Microscopy*, John Gustav Delly (2007), with permission of College of Microscopy.