Washington State Patrol

Crime Laboratory Division

Materials Analysis
Natural Materials Training Manual

August 2018

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1 INTRODUCTION

1.1 PURPOSE AND SCOPE

This manual contains an outline for training and/or assessing a forensic scientist in the area of natural materials. The various study segments should be covered in the order presented.

This manual endeavors to promote and maintain consistency and quality among forensic scientists performing natural materials analyses across the Crime Laboratory Division. Certain inherent aspects of natural materials analysis prohibit the establishment of a rigid set of standard procedures to cover every case. Sufficient latitude should be given to allow for independent thought and individual freedom in selecting alternative courses of action. Upon completion of this training program, the trainee will be thoroughly familiar with the options available to perform an examination of most types of evidence that may be received.

1.2 EXPECTATIONS

The trainee is expected to have successfully completed the Primary Foundation Manual Module 1 and the following study segments from the Primary and Secondary Foundation Manuals: Imaging and Visualization, Evidence Recovery, Evidence Screening and Evaluation of Trace Evidence, Basic Practical Microscopy, Advanced Microscopy, Special Applications in Microscopy, Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDX), and Microchemical Methods.

Trainees who have prior related training and experience may be able to progress through the training program at an accelerated pace or skip certain study segments. The required documentation of such related training and/or experience will be left to the technical lead(s) or their designee.

The instructor must be experienced in the area of natural materials analysis. The instructor's casework and courtroom experiences, both prior and present, provide a unique aspect to the trainee's learning process that is impossible to duplicate in this training program. The instructor will share such experiences with the trainee. The instructor will also discuss with the trainee the training and reference materials (if any) available on the FLSB Portal. Although the trainee's primary interaction will be with the assigned instructor, this program promotes and encourages discussions with other experienced examiners. When possible, the trainee should also take outside courses in natural materials analysis.

The trainee will maintain a notebook or multiple notebooks throughout the duration of this training program and will record notes and observations for each study segment. The trainee notebook should be maintained in a neat and current fashion and should be present during conversations with the trainer. Upon completion of training, the trainee will maintain the training notebook for the duration of their career.

The trainee should be continuously evaluated throughout the training for comprehension and competency in theoretical knowledge, basic practical skills, and critical thinking skills. Training is progressive and continuously builds on and reinforces prior learning. Deficiencies on any of the training steps may occur during the course of the training and should be rectified. It is important that these deficiencies be openly and promptly discussed among the trainee, trainer, technical lead, and/or supervisor, as appropriate. Repeating training steps and testing may be necessary to satisfactorily complete this training program.

In order to successfully complete this training program the trainee must, after completion of all topic areas, successfully complete a closed book written exam passed with 80%, a competency exam passed with a 100%, and an oral testimony exam with a pass/fail. The completion of these steps will be documented on a training checklist located at the end of this manual. The competency exam will take the form of a mock case, which will include a draft report. The oral testimony exam may either be a full moot court or an oral examination of testimony type questions between the trainer and the trainee. Supervised casework is optional and dependent on the trainee's repertoire of subdisciplines as well as performance on mock casework.

The trainer is responsible for writing an interoffice communication (IOC) to the trainee's supervisor when the trainee has successfully completed the Vehicle Lamp Training Manual. The trainee's supervisor will maintain copies of training IOCs and authorizations in their files.

1.3 ORGANIZATION OF THE TRAINING MANUAL

This training manual consists of several study segments, each covering different aspects of natural materials analysis. The study segments are organized in a specific manner to build on each other. Understanding the components and classification of botanical materials comes first with a study segment on plant physiology and anatomy. The next topic covers general methods for analysis of natural materials with a study segment on sample preparation. Analysis then expands into the common types of samples that may be encountered with study segments on wood and paper followed by foods and food products. The final study segment is putting everything learned together with a segment on natural materials casework. This segment will cover types of case questions, the significance of analyses, reviews of previous cases, performing a series of mocks cases, and learning court preparation.

Each study segment is comprised of five sections:

Objectives – Summarize the purpose of each study segment.

Topic Areas – Designates topics to be included in the study segment.

Readings – Lists the reference materials that should be read to complete the study segment.

Study Questions – Lists questions that assist the trainee in comprehension of the readings, promotes active discussion between the trainer and trainee, and documents understanding of the topic areas. Written answers to these questions will be maintained in the training notebook as documentation of training.

Practical Exercises – Hands on activities that are designed to provide the trainee first-hand experience with the main concepts of each study segment. Data or written explanation for each exercise must be maintained in the training notebooks.

1.4 SAFETY

Good chemical safety practices should be employed.

2 PLANT PHYSIOLOGY AND ANATOMY

2.1 OBJECTIVES

- To introduce the trainee to a variety of botanical materials and terminology to serve as a foundation for the examination of such materials.

2.2 TOPIC AREAS

1. Classification
    a. Taxonomy
        i. Botanicals
            1. Fungi
            2. Algae
            3. Bryophytes
            4. Lichens
            5. Pteridophytes
            6. Gymnosperms
            7. Angiosperms
    b. Usage
2. Plant Cells
    a. Organelles
        i. Plastids
        ii. Nucleus
        iii. Vacuole
    b. Ergastic Substances
        i. Starch
        ii. Crystals
        iii. Lipids
        iv. Silica
        v. Mucilages
        vi. Protein Bodies
        vii. Tannins
    c. Cell Wall
        i. Structure
        ii. Types
            1. Parenchyma
            2. Collenchyma
            3. Schlerenchyma
3. Tissues
    a. Leaves
    b. Stems
    c. Influorescences
    d. Flowers
    e. Fruits
    f. Seeds

g. Roots
h. Rhizomes
i. Veins
j. Meristems
k. Epidermis
    i. Stomata
    ii. Trichomes

2.3 READINGS

1. Coyle HM **(2005)** *Forensic Botany*, CRC Press, New York. (Read chapters 1-4, 6).
2. Kaufman PB **(1989)** *Plants: Their Biology and Importance*, Harper and Row, Publishers, Inc., New York. (Read chapters 1-5, 17, 20-25, 30-32).
3. Mauseth JD **(1988)** *Plant Anatomy*, Benjamin/Cummings Publishing Company, Inc., California. (Read chapter 2).
4. Taiz L and Zeiger E **(1991)** *Plant Physiology*, Benjamin/Cummings Publishing Company, Inc., California. (Read chapter 1).
5. Winton AL, Moeller J, and Winton KB **(1916)** *The Microscopy of Vegetable Foods*, Second Edition, John Wiley & Sons, New York. (Read "The Principal Histological Elements" pp 20-27; "Morphology of Organs" pp 28-45).

2.4 STUDY QUESTIONS

1. Describe different plant classification schemes.
2. What is the difference between plant taxonomy and plant systematics?
3. What is the difference between plant anatomy and plant morphology?
4. Define plant phylogeny. What was the key characteristic used for plant phylogeny prior to DNA analysis?
5. Give the scientific name and family of Poison Oak, Poison Ivy, and Poison Sumac.
6. List the major divisions of the Plant Kingdom.
7. List the major families of the Pinophyta Division.
8. Why are the Rosaceae, Leguminaceae, Poaceae, Cucurbitaceae important plant families?
9. Draw and label a plant cell that includes the following: primary cell wall, secondary cell wall, plasma membrane, nucleus, vacuole, and plastids.
10. Draw a parenchyma, collenchyma, and a sclerenchyma cell. Note the differences in the primary and secondary walls between the 3 cell types.
11. Draw a cross-sectional view of simple pit and a bordered pit that includes the primary walls, secondary walls, middle lamella, pit membranes, pit canal, pit chamber, and inner aperture.
12. List and describe the functions of the various plant organelles.
13. Define the different types of plastids. In what tissues are they typically found?
14. What part of a plant cell may have starch grains? In what parts of a plant are these organelles typically found?
15. What are the different types of ergastic substances and where are these substances located within a cell/tissue/organ?

16. Describe the chemical composition and morphology of different crystals founds in plants. Where are they found?
17. What is the difference between a cystolith, a phytolith, and a stegmata?
18. What is the chemical composition of the primary and secondary walls of a plant cell?
19. What are the morphological, anatomical, and developmental differences between Angiosperms and Gymnosperms?
20. What is the difference between a fruit and a vegetable, botanically speaking?
21. Define the different types of inflorescences?
22. Define the different types of fruits?
23. Describe the 5 different types of stomatal complexes?
24. What is a guard cell and where would you find one?
25. What is the difference between adaxial and abaxial?
26. Where are oxalate crystals found?
27. What is the relationship between plasmodesmata, the primary pit field, and pits?
28. What are the structural, morphological, and chemical differences between the primary wall and the secondary wall?
29. What is the relationship between sclerenchyma cells, sclereids, fiber elements, vessel elements, tracheids, tracheary elements, vessels, and fibers?
30. What is a perforation plate and in what types of tissue would it be observed?
31. What types of cells make up phloem tissue?
32. List the distinguishing morphological characteristics between monocots and dicots for leaves, flowers, pollen, stems, roots, secondary growth (e.g. wood).

2.5 PRACTICAL EXERCISES

1. Obtain a known sample set from your trainer. The sample set should include different tissues and different major categories of botanicals. Photograph and/or draw each sample and label the features that are used to identify the type of tissue(s) and possible classification of the sample. Classification may be scientific and/or usage. Some samples may require stereomicroscopic examination in addition to visual examination.
2. Observe demo on how to dry plants using a plant press.
3. Collect a set of reference samples (fresh and for pressing) of the plants present. Take photographs of the areas and plants sampled. Use gardening shears or a fresh scalpel to remove reference samples from plants. Reference samples may include: leaves, stems, roots, cones, needles, anthers (pollen), fruits, seeds, flowers, and in some cases whole plants. Photograph and/or draw each reference sample and label the features that are used to identify the type of tissue(s) and possible classification of the sample.
4. Obtain a set of unknowns from your trainer. Photograph and/or draw each sample and label salient features. Identify the type of tissue(s) and possible classification of each sample. Classification may be scientific and/or usage. Some samples may require stereomicroscopic examination in addition to visual examination.

3 SAMPLE PREPARATION

3.1 OBJECTIVES

- To introduce the trainee to a variety of sample preparation methods for microscopy work with botanical materials.

3.2 TOPIC AREAS

1. Mounting
   a. Whole
   b. Smear
   c. Squash
   d. Section
2. Medium
   a. Water
   b. Cargille Liquids
   c. Glycerin
   d. Solubility
3. Microchemical Tests/Stains

3.3 READINGS

1. Palenik S **(1988)** "Microscopy and Microchemistry of Physical Evidence" in *Forensic Science Handbook Volume II*, Ed. Richard Saferstein, Prentice Hall, New Jersey, pp 161-208. (Read "Morphology" pp 172-173; "Microchemical Tests" pp 176-183; "Biological Substances" pp 189-197).
2. Ruzin SE **(1999)** *Plant Microtechnique and Microscopy*, Oxford University Press, New York. (Read chapter 7 "Staining" pp 87-119, chapter 9 "Special Methods" pp127-136, and chapter 11 "Histochemistry and Cytochemistry" pp 145-176).
3. Winton AL, Moeller J, and Winton KB **(1916)** *The Microscopy of Vegetable Foods*, Second Edition, John Wiley & Sons, New York. (Read "Reagents" pp 8-10; "Preparation of Materials for Examination" pp 12-19).

3.4 STUDY QUESTIONS

1. What methods may be used to prepare samples for microscopic examination?
2. What stains and microchemical tests may be used on food evidence?
3. Why should tap water be used instead of deionized or distilled water?

3.5 PRACTICAL EXERCISES

1. Hand Cut Sections
   a. Collect a variety of fresh plant materials. Be sure to include different parts of the plant as well as major divisions and families (Gymnosperms, Monocots, Dicots).
   b. Prepare cross-sections and longitudinal sections. Wash samples with tap water and a soft artist's brush in a small watch glass. Use a double-edge razor blade (preferably Teflon-coated). Wetting the blade with water before cutting tissue will allow the sections to float onto the blade rather than being compressed. Cut sections directly onto a wetted

microscope slide. Observe with a stereomicroscope and a light microscope. If air bubbles are problematic, then add glycerin mounting medium (60% glycerin/40% ethanol) and add a cover slip. Place the slide on a warmed hot plate (not hot!) until bubbles cease. Observe the samples with a stereomicroscope and light microscope. Make observations with different optics, including brightfield, polarization, and phase contrast.

c. Freeze a portion of the materials in liquid nitrogen, then quickly remove and cut thin sections with a pre-chilled razor blade.

2. Casting of Leaf Veins

   a. Select 4 different dicot leaves and 1 monocot leaf.

   b. Cover the upper surface with clear nail polish. Once the polish sets, gently lift off the cast and observe under a stereoscope. Repeat with the lower surface of the leaves. Compare and contrast the upper and lower surfaces from the same leaves and from different leaves.

3. Epidermal Peels from Angiosperm Leaves

   a. Select leaves from at least 4 plants from different Angiosperm families. Some suggestions are:

      i. Acanthaceae: Bear's breeches (Acanthus mollis), Nerve Plant (Fittonia verschaffeltii), Polka Dot Plant (Hypoestes phyllostachya)

      ii. Brassicaceae: Sweet Alyssum (Lobularia maritima), Broccoli or Cabbage (Brassica oleracea), Dyer's Woad or Glastum (Isatis tinctoria)

      iii. Caryophyllaceae: Carnation (Dianthus caryophyllus), Sweet William (Dianthus barbatus), Common Chickweed (Stellaria media)

      iv. Convolvulaceae: Sweet Potato or Yam (Ipomoea batatas), Morning Glory (Ipomoea purpurea)

      v. Cucubitaceae: Cucumber (Cucumis sativus), Watermelon (Citrullus lanatus)

      vi. Liliaceae: Tiger lily (Lilium columbianum), Daffodil (Narcissus sp.), Tulip (Tulipa sp.)

      vii. Magnoliaceae: Tulip Tree (Liriodendron tulipifera), Southern Magnolia (Magnolia grandiflora)

      viii. Malvaceae: Kenaf (Hibiscus cannabinus), Hibiscus (Hibiscus rosa-sinensis), Cotton (Gossypium hirsutum)

      ix. Poaceae: Bermuda grass (Cynodon dactylon), perennial ryegrass (Lolium perenne), St. Augustine grass (Stenotaphrum secundatum)

      x. Ranunculaceae: Larkspur (Consolida ambigua), Buttercup (Ranunculus sp.), Clematis (Clematis caracasana)

      xi. Rubicacea: Coffee (Coffea arabica), Gardenia (Gardenia jasminoides), Egyptian Star Cluster (Penta lanceolata)

      xii. Solanaceae: Peppers (Capsicum sp.), Tomato (Solanum lycopersicum), Petunia (Petunia sp.)

   b. Prepare epidermal peels of the 4 different leaves by using a new scalpel blade or double sided razor blade to gently cut a nick in the leaf. Then peel the epidermis back. Mount the epidermal peel in glycerin mounting medium (60% glycerin/40% ethanol) and add a cover slip. Place the slide on a warmed hot plate until bubbles cease. Repeat and then make cross sections prior to adding the cover slip.

   c. Place on a microscope and observe under brightfield, phase contrast, and polarization optics. Observe the cuticle, including the shape and distribution of parenchyma cells, the

stoma pattern, and the trichome patterns on the abaxial and adaxial sides of the leave. Are there starch grains present? Are there any oxalate crystal or silica phytoliths

d. Prepare epidermal peels of the 4 different leaves by warming the leaves in 5% aqueous nitric acid in separate small beakers. The leaves should slowly disintegrate, and the cuticles may be found floating on the surface. Gently remove each cuticle and mount it in glycerin mounting medium (60% glycerin/40% ethanol) and add a cover slip. Place the slide on a warmed hot plate until bubbles cease. Repeat and then make cross sections prior to adding the cover slip.

e. Place on a microscope and observe under brightfield, phase contrast, and polarization optics. Observe the cuticle, including the shape and distribution of parenchyma cells, the pattern of stomata, and the trichome patterns on the abaxial and adaxial sides of the leave. Are there starch grains present? Are there any calcium crystals or silica phytoliths?

4. Tissue Clearing by Removal of the Cytoplasm

   a. Obtain a variety of dried plant materials to be cleared. Be sure to include different parts of the plant as well as major divisions (Gymnosperms, Moncots, Dicots). Follow the directions for “Clearing Tissues with NaOH and Chloral Hydrate” on page 128 of Ruzin, 1999. For some samples, omit the Fast Green counter stain (step 9). Mount samples in Permount. NeoClear may be used as a xylene substitute. Observe the samples with a stereomicroscope and light microscope. Make observations with different optics, including brightfield, polarization, and phase contrast.

   b. Chloral hydrate is a schedule 4 controlled substance. Have an analyst qualified in Seized Drugs prepare a stock solution of chloral hydrate. Once in solution, chloral hydrate is not controlled and can be kept on shelf.

5. Tissue Clearing by Modifying Refractive Indices of Cytoplasmic Components

   a. Obtain a variety of fresh plant materials to be cleared. Be sure to include different parts of the plant as well as major divisions (Gymnosperms, Moncots, Dicots). Follow the directions for “Clearing Tissues Without Removing Cytoplasmic Components” on page 129 of Ruzin, 1999. Observe the samples with a stereomicroscope and light microscope. Make observations with different optics, including brightfield, polarization, and phase contrast. Make observations before and after staining.

6. Smears and Squashes

   a. Smears are made from viscous foods containing fine droplets and or particles which can be spread to give a thin translucent layer. Prepare separate slides of mustard and ketchup by placing a small globulet on one end of a slide. Draw another clean slide across the surface of the globulet at a forty-five degree angle. Add one drop of water and gently, without applying pressure, place a coverslip over the smear. Examine each smear with the light microscope at 100X to 400X. Sketch or photograph what you see.

   b. Squashes are prepared when the particle size is usually greater than 180 µm. Obtain a bean. Hydrate the bean in a small Petri dishes using tap water until material is moist and soft (overnight). Place a small amount of the sample in the middle of a slide, lay a clean microscope slide across the sample at right angles, and press down and twist until material appears translucent. Pull the slides apart, add a drop of tap water, and install a 22 mm square coverslip. Label the slides and sketch/photograph structures you see using plane and crossed polars via the PLM.

7. Perform the Iodine Test on several samples of starches and non-starches. Observe staining via 200-400 X using brightfield microscopy. Carefully watch the depth of color penetration over time, what happens?

   a. Background – Iodine staining is a classic method used to identify the presumptive presence of starch and in some cases proteins.

b. Test Target – Starch

c. Formulation

   i. Working Solution: 3 grams iodine, 1.5 grams potassium iodide, and 100 ml distilled water

d. Procedure

   i. Place particles on slide, and add cover glass. Add one or two drops of solution to the slide via capillary action. The starch will stain quickly, so if photography is needed have camera ready. Be careful not to over stain.

e. Interpretation

   i. Starch will stain blue to purple and will continue to a near black color over time.

8. Perform the Trypan Blue Test on a variety of plant sources, including intact starch such as corn and wheat. Crush a small amount of different breakfast cereals on a slide and stain with trypan blue. What are the results? What do you see? Photograph your results.

   a. Background – trypan blue is good for detecting gelatinized/cooked starch as seen in breakfast cereals. It will not stain intact birefringent starch.

   b. Test Target: Differential Stain

   c. Formulation

      i. 0.25 gr into 100ml of distilled water or 40% - 90% aqueous commercial dye; if aqueous dye concentration is unknown prepare a 0.5% and dilute if this is too strong

   d. Procedure

      i. Add 1 to 2 drops to preparation on slide

      ii. wait a couple of minutes,

      iii. remove excess and add coverslip

   e. Interpretation

      i. Damaged starch granules: blue, no birefringence

      ii. Starch which has lost granular structure (e.g. starch present in wafers and extruded products): pale blue

      iii. Intact starch: unstained, birefringent

      iv. Protein: blue

      v. Lignified cellulose: dark blue

      vi. Cellulose: pale blue

4 WOOD AND PAPER

4.1 OBJECTIVES

- The trainee will learn the basic methods in wood identification.
- The trainee will learn basic methods for comparing wood related products.

4.2 TOPIC AREAS

1. Hardwoods
2. Softwoods
3. Monocots
4. Pulp and Paper

4.3 READINGS

1. Hoadley BR **(1990)** *Identifying Wood, Accurate Results with Simple Tools*, Taunton Press, Newtown, Conn. (Read chapters 1-6, chapters 10-12, and Appendices I-IV).
2. Meier E **(2015)** *Wood! Identifying and Using Hundreds of Woods Worldwide*. Publisher: author. (Read chapter 1, chapters 3-5, and pp 39-47 of chapter 6).
3. Parham RA and Gray RL **(1982)** *The Practical Identification of Wood Pulp Fibers*, Tappi Press, Atlanta, GA. (Read chapters 1-2, appendices A-B).
4. Wiedenhoeft A **(2006)** "Wood evidence: proper collection, documentation, and storage of wood evidence from a crime scene" *Evidence Technology Magazine* 4(3): 28-37.

4.4 STUDY QUESTIONS

1. What are rays?
2. What cell types are in a ray?
3. What is ray fleck?
4. What is underlying cause of grain direction?
5. What is the difference between heartwood and sapwood? Can this difference be observed in forensic samples?
6. What distinguishes a hard wood from a softwood?
7. How is wood from a monocot different that hardwoods and softwoods?
8. Give an example of a monocot wood.
9. What is the difference between wood and lumber?
10. What are the limitations on species identification of wood?
11. Discuss the relationship of earlywood, latewood, springwood, and summerwood to growth rings.
12. What are the 3 principal planes used to classify a wood.
13. What are resin canals?

4.5 PRACTICAL EXERCISES

1. Examination of Gross Features
    a. Macroscopic gross features in wood can often be examined with the unaided eye. Obtain a set of wood standards (blocks of lumber) of both softwoods and hardwoods. Observe the grain of wood, smell, and color. Look at all sides. For each block, locate the cross,

radial, and tangential sections. If present, describe the patterns of pores, rays, and resin canals. Are tyloses present? Take photographs and write down your observations. Discuss your findings with your trainer.

b. Obtain a set of wood samples (native state – branches, twigs) of both softwoods and hardwoods. Observe the color and texture of the bark. Are there lenticels and bud scars present? Can you see any rings? Describe the sapwood and heartwood. Take photographs and write down your observations. Discuss your findings with your trainer.

2. Microscopic Examinations

a. Examine reference slide collections of softwoods and hardwoods. Review the slides with a stereomicroscope and a light microscope. For each slide, identify the cross, radial, and tangential sections. Note the size of any vessels or resin canals present. In tangential sections, describe the following: resin canals, spiral wall thickenings of longitudinal tracheids, presence and type of longitudinal parenchyma. In cross sections: describe the following: resin canals, diameter of tracheids, and growth ring transitions. In radial sections, describe the following: ray tracheids, ray parenchyma ends, and ray parenchyma cross field pitting. Did you observe any bordered pits or crystals? In which woods and which sections? Take photographs and write down your observations. Discuss your findings with your trainer.

3. Preparing Wood Samples

a. Use a stereomicroscope to determine the orientation of the some softwood and hardwood lumber blocks. Cut off samples (approximately 1 cm x 1 cm x 2.5 cm) using a small saw such that each side of the sample is either a radial, tangential, or a cross section. Boil the samples until they sink to the bottom of a beaker. Attempt to cut hand sections and also try a rotary microtome (if available). Be sure to note the orientation of each section. Stain the sections for 5 minutes in 1.0% aqueous Safranin O in a porcelain spot dish. Rinse briefly in water, and then mount sections in glycerin jelly on a microscope slide. Add a coverslip and observe with a light microscope. For permanent slides, run stained sections through a dehydration series and then mount in Norland Optical adhesive, Cytoseal, or Meltmount. Compare your sections to the reference collection and key them out with wood identification keys. Write down your observations for each slide, and discuss your findings with your trainer.

b. Repeat this process with a wooden toothpick fragment and a wooden Popsicle stick fragment. Were you able to obtain all 3 types of sections? Could you determine the type of wood?

4. Practical Experience

a. Obtain 6 samples of lumber blocks from your trainer. Prepare radial, tangential, and cross sections of each sample and observed with a light microscope. Identify the samples to the lowest level possible on the scientific classification scheme using your macroscopic and microscopic observations and the wood identification keys. Take photographs and write down your observations. Discuss your findings with your trainer.

b. Obtain 6 wood fragments from your trainer. These fragments may be too small to make all 3 types of cross sections. Take photographs and write down what macroscopic observations you can make. Repeat with a stereomicroscope. Determine, if possible, the radial, tangential, and transverse (cross) planes. Discuss your findings with your instructor. Prepare radial, tangential, and cross sections of each sample and observed with a light microscope. Identify the samples to the lowest level possible on the scientific classification scheme using your macroscopic and microscopic observations and the wood identification keys. Take photographs and write down your observations. Discuss your findings with your trainer.

5. Examination of Pulped Wood

a. Examine reference slide collections of softwoods and hardwoods. Review the slides with a stereomicroscope and a light microscope.

b. Observe and discuss differences between wood samples versus paper samples

6. Fiber Analysis of Paper and Paperboard

a. Obtain samples of paper and paperboard. Macerate and observe different cell types.

5 FOOD AND FOOD PRODUCTS

5.1 OBJECTIVES

- To identify the common food starches using polarized light microscopy and stains.
- To characterize and identify the common grains components of food and food products.

5.2 TOPIC AREAS

1. Herbs & Spices
2. Condiments
3. Tea and Coffee
4. Cereal Grains
5. Flours
6. Fruits and Vegetables
7. Starch
    a. Chemistry
    b. Color Tests
        i. Iodine Test
        ii. Trypan Blue Test
    c. Morphology
    d. State
        i. Unprocessed Overall shapes
        ii. Processed
        iii. Gelatinization

5.3 READINGS

1. Bock JA, Lane MA, and Norris DO **(1988)** *Identifying Plant Food Cells in Gastric Contents for the use in Forensic Investigations: A Laboratory Manual*, U.S. Department of Justice, National Institute of Justice, Washington D.C.

2. Jackson BP and Snowdon DW **(1990)** *Atlas of Microscopy of Medicinal Plants, Culinary Herbs, and Spices*, CRC Press; Boca Raton.

3. Winton AL, Moeller J, and Winton KB **(1916)** *The Microscopy of Vegetable Foods*, Second Edition, John Wiley & Sons, New York. (Read “The Principal Histological Elements” pp 20-27; “Morphology of Organs” pp 28-45).

5.4 STUDY QUESTIONS

1. How should food evidence be stored? Why?
2. What methods may be used to collect food evidence?
3. How would collect and package vomit samples off a floor?
4. When is storing food evidence (gastric contents, stains, intact food) at room temperature or a refrigerator okay?
5. How would collect crumbs and powders off a kitchen counter top?
6. How would collect a liquid residue off a kitchen counter top?
7. Should formalin be added to an intact food (e.g. a green bean or a broccoli floret)?
8. Chemically speaking, what is starch? How is it formed?

9. Where is starch found in its natural state?
10. What food sources have starch?
11. Describe the morphology of starch.
12. What is the gelatinization process?
13. How do ungelatinized starch grains appear under crossed polars? With the 1st order red plate?
14. How do gelatinized starch grains appear under crossed polars?
15. Describe how the iodine test works for starch.
16. Describe how the trypan blue test works for starch.
17. What are the distinguishing features between starches from different sources?
18. Are there any instrumental methods used to identify starch? What are the limitations?
19. Define a cereal grain.
20. What are the most common cereal grains?
21. What is flour?
22. What is meal?
23. What is malt?
24. What non-cereal grain plant sources may be used to make flour?
25. What are some examples of foods that are made from flour?
26. What is the basis for identifying food grain traces by light microscopy?
27. Define the following using botanical terms: fruit, seed, vegetable, stem, leaf, root.
28. Describe the different classes of fruits and give examples.
29. Give examples of foods that come from stems, leaves, and roots.
30. Define parenchyma, collenchyma, and schlerechyma and give food examples.
31. Define epidermis, trichomes, stomata, guard cell and give food examples.
32. Describe the different cells types that may be found in plant veins.
33. Describe the different types of cell inclusions and give food examples.
34. What's the difference between and herb and a spice?
35. What are common morphological features seen in herbs and spices?
36. What are common condiments?
37. What does chloral hydrate do to plant cells?
38. When is it appropriate to use chloral hydrate?

5.5 PRACTICAL EXERCISES

1. Characterization and comparison of starch grains
    a. Individually mount and compare the starch samples (including corn, wheat, potato, tapioca, arrowroot, pea, rice, banana, sago) from the reference collection. The comparisons will include grain size in um, hilum shape and location, striations, outside edge shape, and extinction appearance of 'Maltese cross'.
2. Starch in food products

a. Obtain the following food products and identify the starch if possible, remember to try staining methods too and photograph your starch: glazed doughnut, plain bagel, white bread, wheat bread, raw potato, potato flakes, cooked French fry, cooked pizza crust vs. uncooked pizza crust, beans (pinto, kidney) - uncooked then cooked at increasing temperatures to examination gelatinization, Lucky Charms™ cereal.

b. Find starch in 5 different foods of your choose and characterize.

3. Characterization of flour

a. Obtain the following flours (you may need to purchase some) and examine by stereobinocular microscopy. Obtain glass sample vials and deposit 1-2 grams into each and label for the reference collection. Prepare permanent samples of each specimen on microscope slides using Norland optical adhesive and label each slide for the reference collection. Photograph each sample and annotate specific botanical features of each. Refer to all cited literature and any suggested internet sources for guidance.

b. Flours – Barley; Buckwheat; Bulgur,; Corn; Cornmeal; Couscous,; Farina; Oats; Oat bran; Oats, rolled; Rice, brown; Rice, white; Semolina; Wheat germ; Wheat

4. Characterization of ingredients in breakfast cereal

a. Obtain the following cereals for the reference collection (you may need to purchase some). Carefully read the list of ingredients on the label. Crush an individual flake/nugget/morsel onto a microscope slide and examine the particles using the stereoscope. Particle pick all of the different ingredients you can see to another slide. Identify the grains. Use the 1-Naphthol Test to test for the presence of sugar. Use iodine stain and trypan blue to test/identify starch species and if the starch is modified /cooked/gelatinized. What other ingredients can you identify, such as salt, or calcium carbonate in Cheerios?

b. Cereals – Coaco Puffs, Lucky Charms, Quaker Toasted Oats, Trix, Cheerios, and Post Grape Nuts.

5. Characterization of bread, cakes, cookies, donuts, and muffins.

a. Obtain the following fresh food products. You only need crumbs from the following items, but make sure the crumb is not a topping treat, raisin, nut or chocolate nibble. Crush a dried crumb to a microscope slide, add water, a coverslip and identify. Masticate a small piece of the same food 30 times and place a small dollop on a slide with coverslip and a drop of water (this simulates gastric contents somewhat, we will discuss this more in a latter lesion). Has the chewing made it harder to identify the ingredients? Remember to try the stains, and photograph and annotate your identifications.

b. Fresh Food Products – White bread; Multi-grain bread; Wheat bread; Rice cakes; Variety of cookies, donuts, muffins and cakes.

6. Collect the following foods for later examinations

a. Purchase the following fresh food at your local grocery store (remember to not buy all at one time, so they do not spoil):

b. Foods: Apple; Banana; Beans (lima, peas, pinto); Beets; Carrot; Celery; Cherry; Citrus (orange or lemon); Corn; Cucumber; Fig; Grapes and Raisins; Green Beans (pod portion); Lettuce; Mushrooms; Onions; Pear; Peppers; Pineapple; Strawberry; Tomato.

7. Slide preparation methods for masticated samples

a. Prepare samples from the foods listed in question 1. Here are some following options for preparation:

i. Chew a sample 40 times and spit the pulp out into a sample vial (preferred).

ii. Mascerate with a razor blade in some water.

iii. Place a sample in a glass vial with water and shake/vortex.

b. Observe the masticated samples with PLM.

8. Examination and identification of food unknowns

   a. Examine the unknown food samples supplied to you by your instructor and identify by comparison to known samples (from above or others).

9. Use of Chloral Hydrate as a clearing agent.

   a. Chloral hydrate is a schedule 4 controlled substance. Preparation of chloral hydrate – soln prepared by Consub. Once in solution, chloral hydrate is not controlled and can be kept on shelf.

   b. In the microscopical examination of vegetable materials, distinctive, often diagnostic structures are specific cell types and crystals such as calcium oxalate. These can be best observed using a clearing agent which removes cell substances such as starch, volatile oils, chlorophyll, protein and resins, all of which obscure cell structure (See the Botanical Traces coursebook by Shane, Section entitled - Plant Microtechnique and Staining, for additional clearing agents to try).

   c. Examine lettuce to see stomata and guard cells.

   d. Examine Basil and Cinnamon: Prepare for microscopical examination by clearing each with the chloral hydrate solution. Place a small amount of material on separate slides and add several drops of chloral hydrate, then add coverglass. Gently warm over alcohol lamp by waving the slide over a very low flame. As soon as bubbles start to appear add more chloral hydrate and warm again. You may need to repeat this phase several times. Then remove the coverglass and add either a solution of chloral hydrate plus glycerol or a 65:35 glycerine/alcohol solution to prevent crystallization of the chloral hydrate, and then add another coverglass. Examine the sample using plane polarized light and compare to the Atlas of Microscopy by Jackson and Snowdon, pp12-13 (basil) and pp. 62-63 (cinnamon). Do you see the acicular crystals in cinnamon?

10. Preparation of Standards

    a. Prepare permanent microscope slides of the solid spices, herbs and condiments listed below. Prepare two slides of each. The first one will be a direct mount in Norland Optical adhesive without prior preparation. The other slide is made by the clearing agent Histo-clear and following the steps outlined in the previous lesson.

    b. Some Common Spices and Herbs – Anise; Basil; Bay; Chives; Cinnamon; Clove; Cummin; Dill; Ginger; Mace; Nutmeg; Parsley; Peppermint; Rosemary; Sage; Thyme; Mustard; Black and red pepper.

    c. Add to reference collection.

    d. Some Common Condiments – Bacon bits; Barbecue sauce ; Cocktail sauce ; Fry sauce ; Horseradish ; Hot sauces based on chili, including Tabasco sauce ; Ketchup; Lime and Lemon juice ; Mayonnaise and salad cream ; Mixed pickle ; MSG ; Mustard ; Pepper ; Ranch sauce ; Relish ; Salsa ; Salt ; Soy sauce ; Steak sauces such as A1, Heinz 57, and HP Sauce ; Sugar ; Chili sauce ; Tabasco sauce ; Tartar sauce; Vinegar ; Wasabi ; Worcestershire sauce.

11. Obtain unknowns from trainer and examine and compare.

6 EVIDENTIARY SIGNIFICANCE AND MOCK CASEWORK

6.1 OBJECTIVES

- To develop an understanding of the significance and limitations of natural materials exams
- To ensure appropriate documentation and report writing skills
- To ensure appropriate techniques and confidence for court presentation

6.2 TOPIC AREAS

1. Assessment of Submitted Evidence
2. Types of Exams
3. Technical Manual Requirements
4. Report Wording
5. Court Testimony

6.3 READINGS

1. Natural Materials Chapter from the current Materials Analysis Technical Procedures Manual

6.4 STUDY QUESTIONS

1. What types of evidence are submitted for natural materials analysis?
2. How should natural materials analysis evidence be stored?
3. What conclusions may be reached from a natural materials analysis?
4. What information should be included in your notes?
5. What information should be included in a report?
6. What types of evidence are submitted for natural materials analysis?
7. How should natural materials evidence be stored?
8. What conclusions may be reached from a natural materials analysis?
9. What information should be included in your notes?
10. What information should be included in a report?
11. How does cognitive bias come in to play?

6.5 PRACTICAL EXERCISES

1. Review at least 3 case files. A representative file from each analyst should be included in the mix. Consider requesting files from archives in order to review a sufficient number of case files. Note the wording of observations, worksheets, and what printouts were included. Note how the conclusion(s) were documented.
2. Work at least 5 natural materials analysis mock cases as if they were real cases. These cases should be realistic in the type of evidence submitted. At least one of the mock cases should include a wood identification, a comparison of 2 materials, and food analysis. Follow the requirements of the Technical Manual and include a draft report.
3. Perform at least 3 practice technical reviews. These reviews may be on copies of active natural materials analysis case files prior to the actual case files being technical reviewed by a qualified analyst or on mock botanicals analysis case files created for this exercise.
4. Discuss with other natural materials analysts any court testimony experiences they have had.

5. Observe court testimony in natural materials analysis if possible.
6. Participate in an oral practice session to practice giving verbal answers to court type questions for natural materials analysis.

7 NATURAL MATERIALS TRAINING CHECKLIST

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Plant Physiology and Anatomy				
	Reading			
	Study Questions			
	Exercises			
Sample Preparation				
	Reading			
	Study Questions			
	Exercises			
Wood and Paper				
	Reading			
	Study Questions			
	Exercises			
Food and Food Products				
	Reading			
	Study Questions			
	Exercises			
Evidentiary Significance and Mock Casework				
	Reading			
	Study Questions			
	Exercises			
Written Test				
Competency Exam				
Oral Testimony Exam				