OSBI Trace Laboratory Training Manual

Revision12, Effective Date 12-31-2025

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Introduction

The Trace Evidence Laboratory of the Criminalistics Division (CSD) of the Oklahoma State Bureau of Investigation (OSBI) is part of an accredited full service laboratory system responsible for the analysis of samples from a suspected crime. This training manual is intended to provide an analyst with the skills and information needed to perform analysis of submitted samples. Each section of this manual lists a specific goal and the tasks that a trainee should complete in order to achieve this goal. The training will be assessed using written and oral examinations as well as competency examinations.

At the conclusion of training the trainee should have the following:

1. Knowledge of the principles and practices of forensic trace analysis as they relate to the analysis of the different types of case materials.
2. Knowledge of the theory and application of instrumentation and specialized techniques used to examine different types of trace evidence.
3. The skills and ability to perform accurate forensic analysis independently and proficiently, to accurately document the findings of all analysis in accordance with the appropriate policies and procedures, and to accurately generate a report on those findings.

Parts of this Training Manual have been taken from a variety of sources, including but not limited to: Scientific Working Group for Material Analysis, Virginia Department of Forensic Science, Texas Department of Public Safety, Hamilton County Coroner's Office (Ohio), Scientific Working Group for Gunshot Residue, Technical and Scientific Working Group for Fire and Explosions.

Instrumentation

The Trace Evidence Laboratory utilizes many different instruments in the analysis of evidence. The instrumentation sections of the Training Manual will educate the analyst on the different instruments and their uses in the analysis of evidence. The training will include reading assignments, possible written assignments, hands-on experience, and practical exercises with each instrument.

A variation of instruments is used for different types of analysis; therefore, it is not necessary to complete the training on all instruments before an Analyst is released for casework in a particular area of Trace Analysis.

Trace Analysis

To analyze individual pieces of evidence the Criminalists in the Trace Evidence Laboratory may utilize different instruments in the analysis of evidence. The analysis sections of the Training

Manual will educate the analyst how to incorporate the various instruments into the analysis of specific types of evidence. The training will include reading assignments, possible written assignments, hands-on experience, and practical exercises with each technique.

Evaluation of Training

This Training Manual includes many practical exercises. Successful completion of the exercises requires the analyst to complete the exercises according to the directions in the written exercise. Not all exercises will conclude in the final identification of an item and may be a prerequisite for the next exercise.

Many training sections include analyzing samples and/or practice/simulated cases, all results of the practice samples/cases must reflect results of past testing, or a reason for differing results will be documented. Reasoning could include age and deterioration of sample. If reason is due to uncertainty or hesitation to make identification or inclusion of multiple samples, more training may be required to increase confidence of trainee. At a minimum, trainer and trainee will discuss the trainee's reasoning.

Documentation of training exercises, training samples (Form 1 or equivalent, as approved by Technical Manager) and reports generated from practice cases must be archived in the analyst personal folder on the QA server.

A competency test is given with most sections. To pass a competency, the obtained results will match the expected results, which will be maintained by the Technical Manager. The trainee must complete the test using instrumentation appropriate for the item being tested:

GSR & Elemental – SEM/EDS
Paint – Microscopy, FTIR, MSP & SEM
Fibers – Microscopy, FTIR & MSP
Ignitable Liquids – GC/MS
Physical Fit – Microscopy
Pressure Tape & Adhesive Analysis – Microscopy, FTIR, MSP, & SEM

If an analyst has previously passed a mock trial in the Trace Evidence Unit and has previous experience testifying in court, the analyst may be given an oral examination in lieu of a second mock trial. If given a mock trial, two or more Senior Criminalists and the Technical Manager or designee will be present during the mock trial and will be using the "Mock Trial Evaluation Form" for grading. Requirements for passing include a minimum score of a 2 on all points and approval

from the Technical Manager. Any score lower than a 2 must have a written explanation for the score.

This training manual can be modified by the Technical Manager for re-training purposes, including an analyst that is returning to Trace Analysis from another discipline or an analyst that needs retraining in a specific area for remedial reasons.

Once released for casework, it is up to the analyst to seek further training for the maintenance of skills and expertise. The Technical Manager will periodically send out articles for the analyst to read; those articles are to be documented on the Additional Reading/Training form.

The finalized Training Notebook will be kept by the analyst at the OSBI laboratory. Upon termination or transfer to another unit, the Training Notebook will be scanned and uploaded into the analyst's individual folder on the QA server.

Orientation to OSBI

If the analyst has previously completed the following Introductory section, it is not necessary for the analyst to complete this section again.

Goals

- To ensure the trainee is familiar with forensic science and the different types of forensic services
- To familiarize the trainee with criminal and civil laws that pertain to forensic chemistry
- To familiarize the trainee with the OSBI Trace Quality System
- To introduce the trainee to the OSBI laboratory management systems
- To introduce the trainee to courtroom dynamics and behavior in the courtroom

General Knowledge of Forensic Science – This section is intended to provide the analyst with a broad overview of forensic science and the types of analysis performed by the OSBI.

Date	Literature
	DeForest P.R. **What is Trace Evidence?** Forensic Examination of Glass and Paint, Analysis and Interpretation. B. Caddy, ed., 2001, pages 1-4

Applicable Criminal and Civil Law and Procedures – This section is intended to familiarize the analyst with criminal and civil law procedures that are applicable to forensic science

Date	Literature
	Haggerty II, M.D. **Confrontation and the Preliminary Hearing**. *Q & A: The Newsletter of the Criminal Law Section.* Vol. 4, Issue 3, May-June 2006, pages 22-31
	Woodson, M. **Relevance and Reliability: What All Expert Testimony Needs**. *Oklahoma Bar Journal,* Vol. 79, No. 7, March 2008, pages 543-545
	Calhoun, M. C. **Scientific Evidence in Court: Daubert or Frye, 15 Years Later**. *Legal Backgrounder,* Vol. 23, No. 37, August 22, 2008, pages 1-4

Quality System Overview – This section is intended to familiarize the analyst with the OSBI Trace Quality system.

Date	Literature/Tasks
	OSBI *Trace Evidence Quality Assurance Manual*

Miscellaneous – The section is intended to introduce the analyst to the laboratory management systems.

Date	Literature/Tasks
	OSBI CSD QMA 2, Evidence Management Requirements, and observe evidence submitting procedures
	OSBI CSD QMA 3, Evidence Packaging and Sealing Requirements, and observe evidence sealing and handling procedures
	The analyst will be shown where to locate the BEAST LIMS system and a brief overview will be given
	The analyst will observe checking out, inventorying and analyzing of a minimum of 5 cases
	The analyst will be shown where to locate Chemical Inventory and a brief overview will be given

Testimony & Presentation of Evidence in Court – This section is intended to provide the analyst with knowledge of acceptable courtroom attire and behavior when called upon to testify.

Date	Literature
	OSBI Policy 108
	Shelton Hon., D.E., Barak, G., Kim, Y.S., **A Study of Juror Expectation and Demands Concerning Scientific Evidence: Does the "CSI Effect" Exist**. *Selected Works (www.works.bepress.com).* February 2007, pages 331-368
	Advanced Expert Witnessing for Forensic Laboratory Scientists online course (https://criticalvictories.com/forensic/), if available

Date	Tasks
	Discuss courtroom testimony and presentation of evidence with trainer
	Discuss bringing and opening evidence in court
	Review documentation when leaving evidence in court, BEAST
	Review a witness critique form
	Observe a Criminalist from the Trace Evidence laboratory giving testimony

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Liquid Nitrogen Safety

Liquid Nitrogen is the liquefied form of nitrogen gas. When nitrogen is in the gas phase, it is mostly inert gas that is colorless, odorless, and tasteless. In the liquid phase, nitrogen is very cold (-196 C or -320 F), which makes it ideal for keeping things cool. Because of its extremely cold temperature, any exposure to your skin can cause severe frostbite. On vaporization, liquid nitrogen expands by a factor of almost 700, so 1 liter of liquid nitrogen becomes 24.6 cubic feet of nitrogen gas. This can cause explosion of a sealed container, or it can displace oxygen in the room and cause suffocation without warning. Liquid nitrogen should always be stored in a vented container in a well-ventilated room. Oxygen may condense on the surface of liquid nitrogen causing it to be highly reactive with organic materials. This can cause ordinarily noncombustible materials to burn rapidly when it comes in contact with oxygen enriched liquid nitrogen. When handling liquid nitrogen, always wear thermal gloves and a protective face shield. Never dispose of liquid nitrogen by pouring it on the floor as it could displace enough oxygen to cause suffocation. Nitrogen gas is colorless and odorless; the cloud that forms when liquid nitrogen is poured on the floor is condensed water vapor from the air, not nitrogen gas.

Goals

- To become knowledgeable of the hazards of liquid nitrogen
- To learn the safety precautions to utilize when handling liquid nitrogen
- To learn how to properly use/transfer liquid nitrogen

Literature Reading

Date	Literature
	Safety Data Sheet for liquid nitrogen
	OSBI Policy 121.1 Appendix I, personal protective equipment required when handling liquid nitrogen

Articulate and/or Provide Written Answers

Date	Tasks
	Discuss the following with trainer
	Why is liquid nitrogen used in the laboratory?
	What are three hazards of liquid nitrogen?
	What personal protective equipment must be used when handling liquid nitrogen?
	What is the proper type of container to use to transport liquid nitrogen?
	What first aid is necessary if liquid nitrogen spills on skin or eyes?
	How do you report a liquid nitrogen injury if it occurs?
	Why is there a safety release valve on the cryogenic cylinder?
	Why must you never use a tight-fitting cap on a dewar of liquid nitrogen?

Tasks

Date	Tasks
	Use of the Instrument
	Trainer will demonstrate the proper technique for transferring liquid nitrogen from the cryogenic cylinder to a small dewar.

Evaluation of Training

	Demonstrate how to properly transfer liquid nitrogen

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Trace Evidence Searching, Collecting, and Documenting

Goals

- To gain knowledge of proper packaging and storage of evidence to prevent the loss or contamination of evidence.
- To document the case in the Laboratory Management System
- To take detailed case notes to include abbreviations and common symbols, procedures conducted, photographs, and drawings/sketches
- To become skilled at search, recovery, and preservation of trace evidence both in the laboratory and outside the laboratory

Literature Reading

Date	Literature
	TR-8 Collection of Trace Evidence
	TR-18 Digital Photography
	Review the following sections of the Trace Quality Assurance Manual
	Section 6: Abbreviations
	Section 4: Handling Physical Evidence in the Trace Lab
	Section 9: Examination Documentation
	Review the following sections of the OSBI CSD Quality Manual
	Physical Evidence QP 1 Evidence Intake
	Physical Evidence QP 2.1 Evidence Handling
	Physical Evidence QP 2.3 Evidence Storage and Maintenance
	Physical Evidence QP 3 Evidence Transactions
	QP 16.2 Contents of Case Records
	QP 29 Criminalistics Statistics
	Saferstein, R. Criminalistics: An Introduction to Forensic Science. 10th edition, 2011, pages 34-47 (Conducting a Systematic Search for Evidence to end of chapter), 342 (Collection & Preservation of Fiber Evidence), 347-348 (Collection & Preservation of Paint Evidence)

	Palenik, S. Microscopy and Microchemistry of Physical Evidence. Forensic Science Handbook. Vol. II, Saferstein, R. Ed., 1988, pages 160-168
	Gaudette, B.D. The Forensic Aspects of Textile Fiber Examination. Forensic Science Handbook. Vol. II, Saferstein, R. Ed., 1988, pages 214-221
	ASTM International, E 1492-05, Standard Practice for Receiving, Documenting, Storing and Retrieving Evidence in a Forensic Science Laboratory. pages 1-2
	ASTM International, E 1459-92 (2005), Standard Guide for Physical Evidence Labeling and Related Documentation. pages 1-2
	Flinn, L. L. Collection of Fiber Evidence Using a Roller Device and Adhesive Lifts. *Journal of Forensic Science.* Vol. 37, Issue 1, 1992, pages 106-112
	Springer, F. Collection of Fibre Evidence from Crime Scenes. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 101-115
	Scientific Working Group for Materials Analysis (SWGMAT) Trace Evidence Recovery Guidelines. *Forensic Science Communications*. Vol. 1, No. 3, October 1999, www.fbi.gov (PDF: Trace Evidence Recovery Guidelines SWGMAT)
	Robertson, J., Roux, C. Transfer, Persistence and Recovery of Fibres. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 89-100
	Fong, W., Fiber Evidence: Laboratory Methods and Observations from Casework. *Journal of Forensic Science.* Vol. 29, No. 1, January 1984, pages 55-63
	Masakowski, S., Enz, B., Cothern, J.E., & Rowe, W.F. Fiber-Plastic Fusions in Traffic Accident Reconstruction. *Journal of Forensic Science.* Vol. 31, No 3, July 1986, pages 903-912
	Chewning, D.D., Deaver, K.L., Christensen, A.M. Persistence of Fibers on Ski Masks During Transit and Processing. *Forensic Science Communication.* Vol 10, No 3, July 2008 (PDF: Evidence Search & Recovery)
	Spencer, R. Significant Fiber Evidence Recovered from the Clothing of a Homicide Victim After Exposure to the Elements for Twenty-Nine Days. *Journal of Forensic Science.* Vol. 39, No. 3, May 1994, pages 854-859
	Palmer, R., The Retention and Recovery of Transferred Fibers Following the Washing of Recipient Clothing. *Journal of Forensic Science.* Vol. 43, No. 3, 1998, pages 502-504

	Photography
	Groffy, R.L., Scale Selection and Placement. International Association for Identification, Iowa Division, www.iowaiai.org/scale_selection_and_and placement.html , downloaded 06-23-14 (PDF: Photography)
	Staggs, S., Lighting Methods for Copy and Evidence Close-up Photography. Crime Scene and Evidence Photographer's Guide. www.crime-scene-investigator.net (Excerpts taken from the book, 2nd edition, found on Crime Scene Investigator website, downloaded 06-23-14) (PDF: Photography)
	Reading for Practical Exercise 1
	Roux, C., Langdon, S., Waight, D., Robertson, J. The Transfer and Persistence of Automotive Carpet Fibres on Shoe Soles. *Science & Justice*. 1999, Vol. 39, No. 4, page 239-251
	Scott, H. G. The Persistence of Fibers Transferred During Contact of Automobile Carpets and Clothing Fabrics. *Canadian Society of Forensic Science Journal*. 1985, Vol. 18, No. 4, pages 185-199
	Reading for Practical Exercise 2
	Robertson, J., Roux, C. Transfer, Persistence and Recovery of Fibres. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 95-115.

Tasks

Date	Tasks
	Discuss the following with trainer
	Significance and use of trace evidence type of trace evidence integrity of evidence
	Documentation Case file documentation per laboratory policy to include notes containing: a. date, initials, item description, unique identifier, sketches, measurements, sub-itemizing and images b. chain of custody c. labeling items and packages d. record maintenance, storage and security e. review of the trace matrix in the laboratory management system

	Photographs a. overall, including packaging & excluding packaging b. close up, with and without scale c. naming of image files d. combining photographs 1. into one image 2. into one document and labeling within document
	Contamination and loss practices a. limiting contact between items and individuals b. appropriate protective apparel c. limiting evidence handling and exposure to contaminants b. collecting, packaging and sealing in appropriate packaging c. controlled environments d. clean equipment and work surfaces e. separation of evidence from different sources by location and/or time f. documenting any situation which could have contaminated or compromised the evidence g. consideration of associated evidentiary items from other disciplines
	Crime Scene/Field Evidence a. site and item identification b. observations and documentation c. searches/collection
	Detection, collection and preservation techniques a. criteria for selection of technique and processing sequence b. recording techniques c. visual searches, with or without magnification, to include oblique lighting d. using the most direct and least intrusive collection methods: picking, tape lifting, lifting with exception of tape, scraping, vacuuming and cutting e. appropriate packaging for wet items, use of temporary packages, and proper packaging material f. collection of small (microscopic) items, in lab and in field
	Questioned versus known samples, and collection of representative known samples
	Laboratory examination report writing a. description of items received and methods of analysis used b. examination results and conclusions c. transfer of evidence for further analysis

	Skills Demonstration by Trainee (Perform all techniques at least one time)
	Contamination and loss prevention techniques when manipulating evidentiary items
	Visual searching, using alternate light sources
	Stereomicroscopic searching
	Evidence recovery by picking
	Evidence recovery by tape lifting
	Evidence recovery by scraping
	Evidence recovery by cutting
	Practice Exercises Document your findings as practice cases in the training LIMS system and prepare reports of your findings.
	Complete Practical Exercise 1
	Complete Practical Exercise 2
	Search clothing from at least one mock case using the unaided eye and low power magnification. Document and collect the trace evidence using an appropriate method
	Locate paint smears/abrasions on at least two different items (i.e. clothing, bumper, and door). Document and collect the smears/abrasion
	Collect loose debris from clothing mock using the scraping method. Document and collect the debris using an appropriate technique
	Collect and preserve questioned and known samples from at least three objects, including at least one prying type tool
	Search one motor vehicle and collect and preserve trace evidence (questioned and known samples) using appropriate methods
	Casework
	Observe the documentation included on at least 3 cases where items were searched for evidence
	Observe the processing of two cases, including all documentation necessary

This section does not have a time limit, as opportunities for such training is limited. Completion of this section is not a requirement for an analyst to be released for casework. If this section is not completed before being released for casework, a second authorization will be required once this section is completed.

	Processing Outside the Laboratory
	West Virginia University Online Class "Crime Scene Investigation", if available
	Observe the processing of two scenes outside the laboratory, and the completion of the crime scene memo for both scenes

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Evidence Searching, Collecting & Documenting

Sample Analysis

Date	Tasks
	Review of practical exercises and reports prepared correctly with accurate results

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Fourier Transform Infrared Spectroscopy (FTIR)

Goals

- To learn the theory of FTIR and how to use it in the analysis of submitted samples.
- To familiarize the analyst with how to maintain the FTIR instrument and ensure that it is working properly by using quality assurance and quality control methods.
- The analyst will learn how to prepare samples for analysis, analyze samples using FTIR, and interpret data from this type of analysis.

Literature Reading

Date	Literature
	Protocol TR-6 (Fourier Transform Infrared Spectroscopy)
	Bell, S. Spectroscopy. Forensic Chemistry. pages 149-159
	Bell, S. Infrared Spectroscopy. Forensic Chemistry. pages 161-169
	Thermo Scientific, Introduction to Fourier Transform Infrared Spectroscopy, internal publication, 2007
	Smith, Brian C. Fundamentals of Fourier Transform Infrared Spectroscopy. 1996, pages 1-53, 87-130
	Coleman, P. B., Ed., Practical Sampling Techniques for Infrared Analysis. 1993, pages 44-52, 146-160, 256-273
	Reffner, J. A., Martoglio, P. A. Uniting Microscopy and Spectroscopy. Practical Guide to Infrared Microspectroscopy. Humecki, H. J., Ed., 1995, pages 41-75
	Optional Reading
	Griffiths, P.R. and de Haseth, J.A. Fourier Transform Infrared Spectrometry. 1986
	Bellamy, L. J. The Infrared Spectra of Complex Molecules. 1954
	Syzmznski, H. A. Interpreted Infrared Spectra. 1967
	Cook, B.W., Jones, K. A Programmed Introduction to Infrared Spectroscopy. 1972
	Miller, R.G.J. Laboratory Methods in Infrared Spectroscopy. 1965
	FBI training course, *Infrared Spectroscopy for Trace Evidence.* March 13-17, 2006

Articulate and/or Provide Written Answers

Date	Tasks
	Theory of FTIR
	Theory of FTIR
	What two conditions must be present for infrared absorption to occur
	The differences between transmission and reflectance modes
	How the interferometer functions
	Why a background spectrum is collected
	Define the following: Wavelength Frequency Dipole moment Harmonic vibration Fundamental vibration Interferometer Overtones Data spacing Interferogram Zero path difference (ZPD)
	Describe the electromagnetic spectrum and what are the upper and lower limits of the infrared region of the electromagnetic spectrum
	What is the purpose of the laser
	Controls
	When the ValPro System Qualification is to be performed
	The proper corrective procedure when a ValPro System Qualification on the bench fails
	When a background spectrum will be collected
	Misc.
	State the absorption region for the following functional groups O-H N-H C=O C-O C-Hn C≡N

	N-O2
	Why is the “bench” needed
	When would “bench” need aligning
	What gas is used to purge the system
	Why is the purge used
	What type of library can be used for an identification
	Discuss different sample types and when microscope/micro-compression cell, ATR, or KBr pellet would be used
	Microscope
	Why is the MCT detector cooled with liquid nitrogen
	What is the benefit of using the MCT detector with the microscope attachment and not the DTGS detector
	What is the range of an MCT detector and what is the limiting factor which dictates how low it will detect
	What are interference fringes, why do they occur, and how can they be avoided
	How does the amount of pressure applied affect samples in the micro-compression cell
	Why would KBr be added with samples when using the micro-compression cell
	When is a sine wave produced when using the micro-compression cell
	What is one way to eliminate the sine wave
	When should an alternative to the micro-compression cell be used
	ATR/Bench
	What is ATR
	What is the range of the ATR accessory
	What is the size of the sampling area
	What is the crystal material we use; what other materials are available; what are the advantages and disadvantages
	Which is more significant: the size of the sample or the size of sampling; and why
	What is the difference in the data collected from a single reflection system versus a multiple reflection system
	What is the ATR correction and when and why is it used
	What is the range using the Bench

Tasks

Date	Tasks
	Maintenance
	Demonstrate performance of the ValPro System Qualification using an appropriate standards
	Demonstrate analysis of Polystyrene standard on microscope
	Use of the Instrument
	Demonstrate preparation of a sample for analysis for the ATR
	Demonstrate collection of background scans
	Demonstrate collection of scans using the ATR accessory
	Demonstrate sample preparation using the micro-compression cell
	Demonstrate collection of scans using microscope/micro-compression cell
	Spectral Interpretation
	Review instrumental data and discuss what is and is not acceptable for casework analysis
	Determine if CO_2 and/ or H_2O are present in the scans
	Demonstrate proper report writing

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Gas Chromatograph/Mass Spectrometer

Goals

- To learn the theory of gas chromatography and gas chromatography mass spectrometer and how to use it in trace analysis.
- Describe the capabilities and limitations of the instrument
- To familiarize the analyst with how to maintain the gas chromatograph mass spectrometer instrument and ensure that it is working properly by using quality assurance and quality control methods.
- The trainee will learn how to interpret data from this type of analysis.

Literature

Date	Literature
	Protocol TR-1 (Gas Chromatograph Mass Spectrometer for Trace Analysis)
	Basic Operation of the Gas Chromatograph (Appendix I of Training Manual)
	Basic Operation of the Mass Spectrometer (Appendix II of Training Manual)
	Bell, S. *Forensic Chemistry.* pages 192-200 (PDF: GC and MS)
	Hugel, J., Meyers, J. A. & Lankin, D. C. **Analysis of the Hallucinogens, Mass Spectrometry**. Handbook of Forensic Drug Analysis. 2005, pages 176-185
	American Society for Mass Spectrometry. **What is Mass Spectrometry Handout**, ASMS, www.asms.org, 2013 (PDF: What is mass spectrometry ASMS)
	McLafferty, F. W. & Turecek, F. Interpretation of Mass Spectra, 4th edition, 1993, chapters 1-2
	Pavia, D. L., Lampman, G. M., Kriz, G. S. **Mass Spectrometry**. Intro to Spectroscopy. 2001, pages 390-398 and 446-448
	Saferstein, R. **Forensic Applications of Mass Spectrometry**. Forensic Science Handbook. Vol. I, 2nd edition, pages 117-144, 154-156
	Optional Reading
	McLafferty, F. W. & Turecek, F. Interpretation of Mass Spectra, 4th edition, 1993, chapters 3-4

Articulate and/or Provide Written Answers

Date	Tasks
	Theory of Gas Chromatography
	The theory of the injection port
	The theory of expansion volumes and how to determine the appropriate injection volume
	The theory of split ratios and why it is used with the instrument
	The theory of the method used for analysis, IL-20 method and/or any other methods used for analysis
	The theory of retention time and how it applies to gas chromatograph analysis
	Theory of Mass Spectrometry
	Theory of capillary columns used in the mass spectrometer
	Theory of splitting of the molecule and the function of the ion source
	Theory of the quadrupole mass filter and how it functions
	Theory of electron multiplier
	Controls
	When solvent blank runs are to be run
	When a positive control is to be run
	Why are positive and negative controls used
	The proper corrective procedure if contamination occurs during a casework reagent blank run
	Describe the importance of tuning. Explain the data on the daily tune report, and explain the information provided by differences in daily tunes.
	What is PFTBA and why/how is it used in the mass spectrometer
	When the Tune Eval/Autotune and the air/water quality checks are to be run
	The proper corrective procedure if erroneous assignment of mass values to fragments in a sample, standard, or autotune
	The proper corrective procedure for the failure to produce a satisfactory positive control spectra
	The proper corrective procedure if contamination peaks are found on the autotune (m/e 18, 44, etc.)
	The suggested range for the Isotope Ratios (69/70, 219/220, 502/503) in an autotune
	How other values (mass assignments, peak width and EM volts) found on autotune report disclose how mass spec is performing

	How high is too high a background? What could cause a high background? How would you troubleshoot this problem
	Misc.
	When does the septum (Merlin-Microseal) have to be changed? And what precautions must be taken when changing it
	What syringe must be used with Merlin-Microseal
	When changing of the liner and septa is required
	When changing of the gold seal is required
	When cleaning of the injection port is required
	What is the solvent delay; why is it important; what is "hidden" in solvent delay
	Why is the base peak significant
	The theory of a mass spectral library and where the libraries come from
	When does a standard have to be run
	What information has to be retained with a new standard
	What information about the standard is listed on each mass spectra printout

Tasks

Date	Tasks
	Maintenance (www.chem.agilent.com for instrument manuals)
	Demonstrate how to record maintenance performed in the instrument maintenance log
	Demonstrate syringe replacement
	Demonstrate proper wash and waste bottle volumes
	Demonstrate changing liner and septum
	Demonstrate changing gold seal, cleaning injection port and septa nut
	The trainer will demonstrate the autotunes available and provide discussion on the criteria for acceptance of the daily autotune
	Demonstrate how to autotune instrument, interpret the data and save the PDFs
	Demonstrate how to perform an air/water check
	Demonstrate how to vent the mass spec
	Demonstrate how to disassemble and clean ion source, replace filaments, and reassemble
	Demonstrate how to pump down the mass spec
	Demonstrate how to change a column

	Use of the Instrument
	Properly prepare a minimum of 5 samples in auto-sampler vials
	Demonstrate how to properly load a minimum of 5 samples for analysis on the GCMS, including entering information in the Sample Table
	Demonstrate the proper use of controls
	Demonstrate running a sequence
	Software, Data Management and Spectral Interpretation
	The trainer will demonstrate data management and how case data is organized on the instrument
	Demonstrate saving sequence data in the proper location (folder), minimum of 5
	The trainee will review instrumental data and discuss with the trainer what is and is not acceptable for casework analysis. A minimum of 5 will be reviewed, but all of the following points must be included: Peak separation Acceptable/unacceptable chromatography Background subtraction Complete spectrums Extra/absent ions in a spectrum Comparison of retention time TIC -define EIC-define Comparison of TIC and EIC
	Properly interpret the data obtained and explain how to apply this data if further analysis is required

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Gas Chromatograph Appendix I

Basic Operation of the Gas Chromatograph

In order to achieve maximum resolution between similar compounds on the gas chromatograph (GC), basic understanding of certain variables should be understood. Clear separation must be obtained for the mass spectrometer (MS) to distinguish between a mixture of molecular compounds and a single molecular compound. The capillary gas chromatograph has four methods to separate different molecules: pressure, length of column, type of column, and temperature. By making use of all of these variables together, most compounds will separate well.

Pressure regulation is controlled at the injection port and can be varied to meet certain applications by a feature called EPC (Electronic Pneumatic Control). There are three modes EPC can be used to separate types of molecules in a sample: split, splitless, and pulsed split.

Split Mode: During a split injection, a liquid sample is introduced into a heated inlet where it vaporizes rapidly. A small amount of the vapor enters the column while the major portion exits from the split / purge vent. Split injections are primarily used for high concentration samples when you can afford to lose most of the sample out of the split / purge vent. It is also used for samples that cannot be diluted.

Splitless mode: In this mode, the purge valve is closed during the injection and remains so while the sample is vaporized in the liner and transferred to the column. At a specified time after the injection, the purge valve opens to sweep any vapors remaining in the liner out the split vent. This avoids solvent tailing due to the large inlet volume and small column flow rate. Since the entire sample gets transferred onto the column, this mode is primarily used for samples of low concentration.

Pulsed Split: The pressure pulse modes increase inlet pressure just before the beginning of a run and returns it to the normal value after a specified amount of time. The pressure pulse sweeps the sample out of the inlet and into the column faster, reducing the chance for sample decomposition in the inlet. This is helpful for large molecular weight compounds that tend to linger around in the inlet and thus tend to get purged in other EPC modes. This method can also help to increase sensitivity by placing a larger amount of sample on the column while decreasing the possibility of samples staying in the injection port and causing contamination.

Expansion Volume: During injection, the solvent vaporizes. If the vapor exceeds the volume of the glass inlet liner, it may contaminate metal lines and parts within the injector, which can subsequently result in carry over or contamination of future injections.

Expansion volumes for different pressures or inlet liner sizes can be calculated using the following formulas:

Samples and solvent expansion volume = $\frac{nRT}{P}$

n= # of moles of solvent and sample = $\frac{\text{volume (ml) x density (gm/ml)}}{\text{MW (grams/mole)}}$

T = absolute temperature of injector (K)

P = column head pressure (Atm) + 1 Atm

R = gas law constant = 82.06 cc atm/mole x deg. K

Volume of inlet liner = $\pi r^2 L$

r = radius (cm)

L = length (cm)

The 4mm inlet liner has a 990 µl volume. Solvent expansion volumes are not to exceed the capacity of the liner. Presence of carrier gas in liner diminishes volume available by approximately ½.

The expansion volumes were determined using a 1.0 µl injection volume, a 300°C injection port temperature and a head pressure of 5, 10 and 15 psig.

Solvent Expansion Volumes in µl at Various Head Pressures

Solvent	Density (g/ml)	MW (g/mole)	5 psig	10 psig	15 psig
Carbon Disulfide	1.2632	76.13	582 **	464	386
Methanol	0.7914	32.04	867 **	691 **	386
Ethanol	0.7893	46.07	852 **	471	392
Pentane	0.6262	72.15	299	239	198

***Under these conditions, the size of the vapor cloud exceeds the liner's effective internal volume. Generally, reduce backflash by injecting a smaller amount of liquid. Temperature, pressure, and choice of solvent also affect vapor cloud size.*

The resolving power of a column can be dependent on the length of the column. The longer the column, the greater the resolving power. Longer columns allow for more interaction from each molecule in the sample with the stationary phase. Resolution between similar compounds with small differences can be achieved by increasing the length. Columns can be purchased in many lengths, but the growing trend is toward smaller columns since larger columns are more expensive and greater resolution by other factors can compensate for less resolution from the column.

Capillary columns are a glass tube with a silianized coating containing different functional groups. Hundreds of different types of columns exist, each with a different type of functional group that will separate different compounds of interest. Some stationary phases can select for nonpolar, polar, and even compounds with lone pairs of electrons. The silianized layer thickness has a

direct effect on the retention and elution temperature for each sample compound. Thicker films retain compounds longer by maximizing the amount of time the compounds spend in the stationary phase. Thinner films allow compounds to pass through the column faster, most likely with less separating ability.

Temperature variations in the injection port and on the column are important for separation by capillary gas chromatography. The injection port is usually set at 300°C, which vaporizes samples upon introduction to the GC. The injection port is not part of the column; it's a chamber where the liquid sample can change into the gas phase before entering the column. The oven, that contains the column, is relatively cool at a starting temperature of 250°C less than that of the injection port. This is hot enough to allow the volatile solvent to remain as a gas, but cold enough to cause the less volatile compounds to return to a liquid state. Once the sample becomes a liquid, it deposits itself on the column and won't migrate until the oven temperature heats up to the compounds boiling temperature. When the sample is once again in its gas phase, it travels through the column's stationary phase and mobile phase, jumping between the two. Separation has occurred through the difference in boiling points and through the amount of time different molecules spend in the stationary phase while traveling through the column.

By utilizing these four variables, separation of compounds can be achieved in most cases. Observation of the retention time is valuable in determining the identity of a compound, by comparison with a known standard. Changing just one of these variables can influence the retention time of a compound. The chromatograph should not contain wide peaks, since two or more compounds could resemble one peak. Ideally, sample concentration should be enough to give a single, narrow peak.

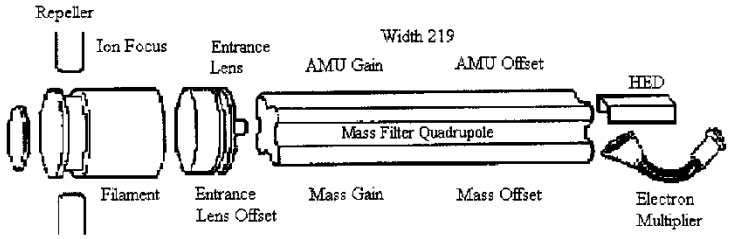
References:

1. Missouri State Highway Patrol Forensic Laboratory Chemistry Training Manual.
2. Hewlett-Packard GC/MS Product Software, August 1996.
3. Restek.com Solvent Expansion Calculator

Mass Spec Appendix II

Basic Operation of the Mass Spectrometer

In order to interpret a tune report, basic understanding of the MS is necessary. The following is a brief explanation of how the Electron Impact (EI) ionization method works. The molecules come off of the GC column and are subjected to electron bombardment, which causes them to fragment and become charged. A repeller is used to direct the ions to the focusing lenses in the ion source and then to the quadrupole mass filter. The mass filter allows selected ion masses to reach the detector. It separates ions based on their masses allowing only ions of a specific mass to reach the detector at a given time. The quadrupole filters by applying to each pair of quadrupole rods, a combination of radio frequency (Rf) and direct current (DC) voltages. One rod pair receives Rf voltage 180 degrees out-of-phase with the other pair, while an equal but opposite DC potential is applied to each rod pair. Under these conditions, at any particular set of Rf and DC voltage values, only ions of a specific mass to charge (m/z) will traverse the length of the open space between the rods. All other ions are neutralized as they strike the surface of the rods. During a typical run, the MS scans for masses ranging from 40 – 550 atomic mass units (amu). It scans for each mass unit in that range starting at the highest amu, working downward, throughout the duration of the run, with the exception of the solvent delay in which the MS is turned off. For instance, at the beginning of the scan, the mass filter selects only for masses of 550 amu, then it selects for masses of 549, and so on. This whole selection process takes place about three times a second. After each ion passes through the quadrupole, it is amplified by the electron multiplier, before reaching the detector. The detector counts the ions of each mass and plots the data on the mass spectrum (abundance vs. mass size). The quadrupole mass filter can select ions in two modes: Scan and SIM. Scan mode selects ions in the whole mass range specified, whereas SIM selects for specific mass units. The Scan mode has a lower sensitivity since most of the ions in a sample collide with the quadrupole rods. However, since samples are generally unknown, the filter mode utilized at the OSBI is the Scan mode to detect the entire spectrum of ions.

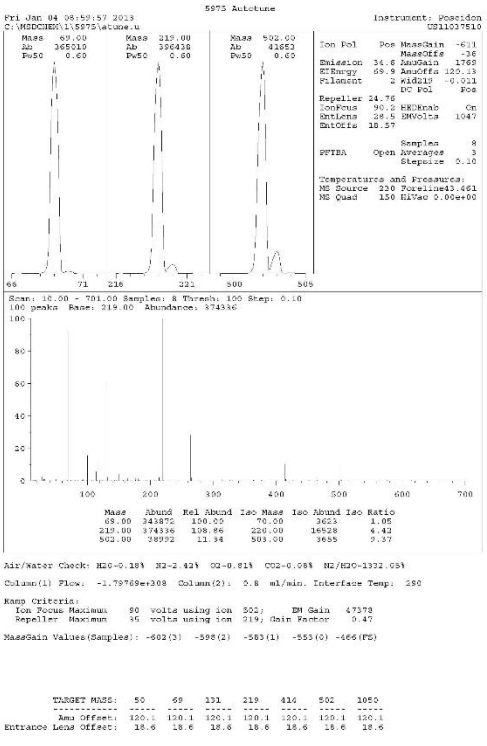


Tuning

Tuning is the process for optimizing the performance of the Mass Selective Detector (MSD). The goal of tuning is to maximize sensitivity while maintaining acceptable resolution (the ability to distinguish between a mass and its isotope), ensuring accurate mass assignment, and providing the desired relative abundance's across the spectrum. The Mass Spec (MS) uses Perfluorotributylamine (PFTBA) because its mass spectrum has ions in the low (69), medium (219), and high (502) mass range. The mass spectrum of PFTBA is shown on the bottom of the

tune report. The instrument looks specifically for masses 69, 219, and 502 in the spectrum of PFTBA, and plots these values along a mass axis (the x-axis of the mass spectrum). The instrument aligns its internal mass axis to match the PFTBA mass axis. Resolution is determined by the ability of the instrument to distinguish two peaks, one mass unit apart. This resolution is displayed on the tune report by the peaks in the upper left hand corner. The peak graph displays the mass of the peak, the abundance of that ion, and the peak width at 50% of the height (Pw50), as shown in the upper left portion of the graph. The x-axis of this graph is the mass assignment. By increasing the Pw50, the area at the base of the graph increases, and a larger range is allowed for the selected mass assignment (i.e. 69, 219, or 502). If made too large, the base area could encompass a range more than its peak range and label the isotope mass with the selected mass. This would achieve greater sensitivity, but the resolution would be very low since it could not distinguish the selected mass from its isotope. It is for that reason that the peak width must be between 0.4 and 0.6. While viewing the tune report, look for clear separation between the selected mass and its isotope.

On the upper right of the printout, there are numerous parameters displayed. These parameters are automatically assigned while using autotune to optimize the MSD performance. These values can be manually changed by using manual tune, but is not recommended for normal use.



Ion Pol: This is the polarity of the field lens. A positive field pushes the ions out of the ion source. (5975)

Emission: The amount of current running through the filament. The higher the current the greater the electron bombardment but decreases a filament life. Too low of a current will result in less ionization and reduced sensitivity.

ElEnrgy: The electron energy of the electron leaving the filament. (5975)

Filament: The MS contains two filaments in case one burns out.

Repeller: Sets the voltage of the repeller (part of the ion source). The repeller is a positive potential that repels the ions, pushing them out of the source. If the repeller is set too low, too few ions will leave the source, resulting in poor sensitivity and poor high mass response. If it is set to high, too many ions at too high a velocity will leave the source. This results in poor mass filtering and poor low mass resolution.

IonFcus: Sets the voltage of the ion focus lens (part of the ion source). Ion focus affects Ion abundance. Generally, the offset is ramped during the tuning to find the ion focus offset that results in the best ion abundance.

EntLens: Refers to the entrance lens gain, a value used to determine a mass dependent voltage that is applied to the entrance lens. The entrance lens is the final lens through which ions pass before they enter the mass filter quadrupole. Typically, during tuning, the entrance lens voltage is ramped to find the setting that provides the maximum abundance.

EntOffs: This is a constant voltage that is applied to the entrance lens. Increase the offset to increase abundance of ions at low masses without substantially decreasing the abundance of ions at high masses.

PFTBA: The status of the valve containing the PFTBA. This valve will open and close automatically for tuning.

MassGain: Sets the value of the mass axis gain, which is a multiplicative factor used in the equation to calibrate the mass axis. Mass gain adjusts the reported value of a given mass to the correct number. The mass that appears in a report has had a linear correction applied to it. This may be thought of as a calibration curve where the uncorrected mass is plotted along the x-axis and the reported mass is plotted along the y-axis. The calibration curve is a straight line with a slope that is proportional to the mass gain. Mass gain has a greater effect on mass assignments at the high end of the mass scale than at the low end.

MassOffs: This is an additive factor used in the equation to calibrate the mass axis.

AMUGain: Atomic mass unit gain affects the width of the mass peak by adjusting the ratio of DC voltage to RF voltage on the mass filter. A higher value gives narrower peaks, but affects peaks at high masses more than those at low masses.

AMUOffs: This affects the width of the mass peaks by adjusting the ratio of DC voltage of the mass filter quadrupole. A higher value gives narrower peaks at all masses.

Wid219: Affects the width of the mass peak at 219 amu. The value entered for this parameter is approximately the value of the correction applied at mass 219. For instance, if a peak width adjustment has been performed and the values are: Mass 69 Pw0.60, Mass 219 Pw0.63, Mass 502 Pw0.60, then entering a value of –0.03 for the Wid219 parameter, followed by a peak width adjustment, should result in the peak widths of all masses being set very close to 0.60 amu.

DC Pol: Sets the polarity of the direct current applied to the quadrupole mass filter. This parameter is set at the optimum polarity at the factory and should not be changed for normal use.

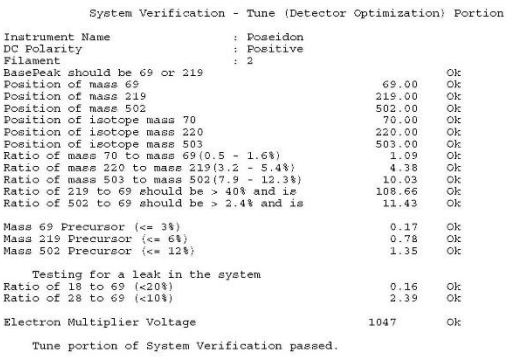
HEDEnab: The High Energy Dynode sets the voltage to focus the ions into the detector, which is located off-axis, hidden from photons and electrons coming from the source. The optimal HED voltage depends on the electron multiplier setting. Thus, the electron multiplier voltage is usually set first. Then the HED voltage is ramped to determine the setting that provides the greatest abundance. The older instruments assigned a value to this parameter, which use to be called X-ray lens, however the HP 5975 MSD does not have an X-ray lens and just indicates “on” or “off”.

EMVolts: The electron multiplier increases the abundance of all ions in the scan range going to the detector.

Samples: The log2 of the number of samples to be taken and averaged at each mass during a scan.

Averages: The number of profile scans to be averaged for each scan reported.

Stepsize: The mass axis increment used for a profile Scan. The larger the number, the faster scans are taken, at a cost of resolution.



System Verification - Tune (Detector Optimization) Portion

Instrument Name	: Poseidon		
DC Polarity	: Positive		
Filament	: 2		
BasePeak should be 69 or 219			Ok
Position of mass 69		69.00	Ok
Position of mass 219		219.00	Ok
Position of mass 502		502.00	Ok
Position of isotope mass 70		70.00	Ok
Position of isotope mass 220		220.00	Ok
Position of isotope mass 503		503.00	Ok
Ratio of mass 70 to mass 69(0.5 - 1.6%)		1.09	Ok
Ratio of mass 220 to mass 219(3.2 - 5.4%)		4.38	Ok
Ratio of mass 503 to mass 502(7.9 - 12.3%)		10.03	Ok
Ratio of 219 to 69 should be > 40% and is		108.66	Ok
Ratio of 502 to 69 should be > 2.4% and is		11.43	Ok
Mass 69 Precursor (<= 3%)		0.17	Ok
Mass 219 Precursor (<= 6%)		0.78	Ok
Mass 502 Precursor (<= 12%)		1.35	Ok
Testing for a leak in the system			
Ratio of 18 to 69 (<20%)		0.16	Ok
Ratio of 28 to 69 (<10%)		2.39	Ok
Electron Multiplier Voltage		1047	Ok

Tune portion of System Verification passed.

Temperatures and Pressures:

MS Source/MSQuad: Displays temperature settings for the Source and Quadrupole.

Foreline: The pressure between the rough pump and the diffusion pump. This area will either state the pressure of the foreline if the MSD uses a diffusion pump or the speed of the turbo pump.

HiVac: Displays the high vacuum pressure.

On the Display of the mass spectrum of PFTBA, other parameters are listed:

Scan: 10.00-700.00 amu is the scan range during the tune. Typically, when drug samples are scanned, this parameter is approximately 40-550 amu.

Samples: The log2 of the number of samples to be taken and averaged at each mass during a scan. If the number is 8, log2 would be 256 scans.

Threshold: Abundance’s below this value will be ignored for scanning. This determines what signal will be accepted as peaks.

Base: Shows the base peak in the sample.

Abundance: Abundance of the base peak.

Tune Evaluation (Tune Eval)

Tune evaluation is a way of verifying the performance of the MSD. First, it will evaluate the most current autotune (ATUNE.U) parameters and when the evaluation is complete, a system verification report is printed.

If all parameters of the autotune are within the predetermined limits, set by Agilent, they will be listed as "OK." If all parameters pass, the instrument can be used for casework. If any of the parameters fail, the reason for failure must be determined and corrected. The Autotune and Tune Evaluation must be run again and pass all parameters before casework can be analyzed on the instrument.

References:

1. Missouri State Highway Patrol Forensic Laboratory Chemistry Section Training Manual.
2. Hewlett-Packard GC/MS Product Software, August 1996.
3. **www.agilent.com** (01/15/2013)

Mass Peaks of Common Contaminants

Mass(es)	Compound General Classification	Potential Source
18, 28, 32, 40, 44	Air	H_20, N_2, O_2, Ar, CO_2
18	Cleaning Solvents	Water
31		Methanol
47, 83, 85		Chloroform
77		Benzene or Xylenes
91,92		Toluene
105,106		Xylenes
43,58		Acetone
85		Freons
73, 147, 207, 222, 281, 295, 341, 355, 429	Dimethylpolysiloxane	Septum or Column bleed
41, 43, 55, 57, 71, 85, 99	Hydrocarbons	Fingerprints or pump oil
149	Phthalates	Plasticizers in tubing, vials, caps, samples

Microspectrophotometry (MSP)

Goals

- To gain knowledge of the theory of the microspectrophotometer and how to use it in the analysis of submitted samples.
- To demonstrate the MSP is working properly by using quality assurance and quality control methods.
- The trainee will learn how to prepare samples for analysis, analyze samples using MSP, and interpret data from this type of analysis.

Literature Reading

Date	Literature
	OSBI Trace Evidence Protocols TR-19 Microspectrophotometer Analysis
	MSP operations manual (user manual)
	Saferstein, R. *Criminalistics: An Introduction to Forensic Science*. 10th edition, 2011, pages 132-137 (Spectrophotometry)
	CRAIC Technologies, **How a UV-visible-NIR Microspectrophotometer Works**. (PDF: MSP)
	Cousins, D. R. **The Use of Microspectrophotometry in the Examination of Paints**, *Forensic Science Review*. Vol. 1, No. 2, Dec. 1989, pages 142-162
	Martin, P., **Forensic Applications of Ultraviolet-Visible-Near Microspectroscopy**. CRAIC Technologies, 2004 (PDF: MSP)
	Kubic, T.A., King, J.B., and I.S. DuBey, "Forensic Analysis of Colorless Textile Fibers by Fluorescence Microscopy," Microscope, 31, 1983, pp. 213-222.
	Ploem, J.S; Tanke, H.J., Introduction to Fluorescence Microscopy, Oxford Science Publications, 1987, Chapters 1 through 3.
	Optional Reading
	CRAIC Technologies, **Mathematical/Statistical Functions Available in CRAIC Lambdafire**. 2014. (PDF: MSP)
	CRAIC Technologies, **Microspectroscopy: An Introduction to Theory and Forensics Applications**. (PowerPoint presentation given to OSBI 2014, located in MSP folder)
	Eyring, M.B. **Visible Microscopical Spectrophotometry in the Forensic Sciences**. Forensic Science Handbook. Vol. 1, Saferstein, R. Editor, 2nd Edition, 2002, pages 322-388

	Hartshorne, A.W. and Laing, D.K., "Microspectrofluorimetry of Fluorescent Dyes and Brighteners on Single Textile Fibres: Part I--Fluorescence Emission Spectra," Forensic Science International, Vol. 51, 1991, pp. 203-220.
	Hartshorne, A.W. and Laing, D.K., "Microspectrofluorimetry of Fluorescent Dyes and Brighteners on Single Textile Fibres: Part II: Colour Measurements," Forensic Science International, Vol. 51, 1991, pp. 221-237.
	Hartshorne, A.W. and Laing, D.K., "Microspectrofluorimetry of Fluorescent Dyes and Brighteners on Single Textile Fibres: Part III: Fluorescence Decay Phenomena," Forensic Science International, Vol. 51, 1991, pp. 239-250.
	Morgan, S.L., Nieuwland, A.A., Mubarak, C.R., Hendrix, J.E., et. al., "Forensic Discrimination of Dyed Textile 369 Fibers Using UV-Vis and Fluorescence Microspectrophotometry," Proceedings of the European Fibres Group. 370 Annual Meeting, Prague, Czech Republic; May 25, 2004. Microsoft Word - Bartick_et_al_EuropeanFiberesGroup_2004.doc (sjsu.edu)

Articulate and/or Provide Written Answers

Date	Tasks
	Theory of MSP
	Define the following: a. Color intensity b. Hue c. Color, visible spectrum d. Dark scan e. Didymium f. Fluorescence MSP g. MSP h. Reference scan i. Reflectance MSP j. Sample scan k. Spectrophotometer l. Transmission MSP m. UV MSP n. VIS MSP
	What are the components of a microspectrophotometer
	Can the MSP be used to identify dyes?
	What colors are poor candidates for analysis by MSP
	Discuss light absorption versus light transmission
	What quality control measures are required prior to casework using MSP
	How many spectra should be collected from a sample
	What determines if additional spectra should be collected from a sample
	What sample color will give the least amount of information in the spectra
	What characteristics are examined in MSP comparisons?
	Controls
	When must the Photometric and Wavelength Check performed
	If the Photometric and Wavelength Check do not give expected results, what must be done

Tasks

Date	Tasks
	Use of the Instrument
	The trainer will demonstrate the operation of the MSP
	Demonstrate setting up the instrument and running the Photometric and Wavelength Check
	Demonstrate the preparation of sample using hand preparation technique
	Demonstrate the preparation of sample using the microtome
	Acquire spectra from fifteen provided samples, including all layers of the samples; print at least one spectrum from each provided sample and one averaged spectrum
	Demonstrate acquiring spectra using fluorescence
	Acquire spectra from one black and one white sample
	Demonstrate using “Statistics” function on samples
	Exercises *To be completed during fiber training*
	Complete Practical Exercise 29
	Complete Practical Exercise 30
	Complete Practical Exercise 31
	Complete Practical Exercise 32

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Scanning Electron Microscope with Energy Dispersive X-Ray Spectrometry (SEM/EDS)

Goals

- To gain knowledge of the theory of the scanning electron microscope and energy dispersive X-ray spectrometry and how to use it in the analysis of submitted samples.
- To demonstrate the SEM/EDS is working properly by using quality assurance and quality control methods.
- The trainee will learn how to prepare samples for analysis, analyze samples using SEM/EDS, and interpret data from this type of analysis.

Literature Reading

Date	Literature
	OSBI Trace Evidence Protocols TR-12 Carbon Coating of SEM Specimen, TR-15 Elemental Analysis, TR-23 Scanning Electron Microscope with Energy Dispersive Spectrometer (SEM/EDS)
	Appendix III: Basic Operation of the SEM/EDS
	Aztec INCA Operators Manual. Oxford Instruments Analytical
	Egerton, R. Physical Principles of Electron Microscopy, 2005, section 1.5, chapter 2
	Bils, Robert F. Electron Microscopy Laboratory Manual and Handbook, Second Edition, Alpha Editions, Los Angeles, CA. 1993, Pages 185 - 189, 196-198, 205, 287-290
	Chandler, J. A., X-ray Microanalysis in the Electron Microscope. pages 327-356, 425-459, 468-469, 511-518
	Gabriel, Barbara L., SEM: A User's Manual for Material Science, American Society for Metals, 1985, pp. 3-31; 53-71.
	Postek, Michael T., et. al. Scanning Electron Microscopy: A Student's Handbook, Ladd Research Industries, Inc. 1980, pp. 1-38; 47-96
	Video: Tescan SEM Training Part 1 (Located in TESCAN Webinars folder)
	Video: Tescan SEM Training Part 2 (Located in TESCAN Webinars folder)
	Video: Tescan SEM Training Part 3 (Located in TESCAN Webinars folder)
	Video: Tescan SEM Training Part 4 (Located in TESCAN Webinars folder)

Optional Reading

	Goldstein, Joseph I., et al, Scanning Electron Microscopy and X-ray Microanalysis. 1992, 2nd Edition, Sections 2.2, 2.3, 3.2, 3.4, 3.5, 4.2, 4.3, 4.5, 4.6 and 4.8, Chapters 5 and 6, Sections 7.2, 7.3, 13.2, and 13.8
	Goldstein, J. I., Yakowitz, H., Newbury, D. E., Lifshin, E., Colby, J. W., and Coleman, J. R., Practical Scanning Electron Microscopy, Plenum Press, 1975
	Roux, C. 9.2 Scanning Electron Microscopy and Elemental Analysis. Forensic Examination of Fibres. Robertson, J. & Grieve, M. Eds., 1999, 2nd Edition, pages 239-243

Articulate and/or Provide Written Answers

Date	Tasks
	Theory of SEM
	Define Depth of Field Working Distance Resolution
	Describe how magnification is achieved in the SEM
	How is a new filament “seasoned”
	How is the calibration checked on the SEM/EDS
	Explain lens hysteresis and why is it important and how often hysteresis should be cleared
	Describe the various signals produced in the SEM, how they are detected and what they are used for (specific to the SEM only)
	What are the advantages and disadvantages of variable pressure
	Describe the vacuum systems used in the SEM
	Theory of EDS
	Describe the interaction between the electron beam and the specimen.
	Define escape peak and sum peak. What causes sum peaks and how you minimize them
	How does “process time” affect spectral resolution? What are the advantages of increasing or decreasing process time
	What is “dead time”? What happens if it is too high or too low
	Define critical excitation energy. When is it appropriate to use low vs. high KV
	What is meant by EDS resolution?
	Describe peak overlaps and specifically how to deal with Pb/S/Mo; Ti/Ba; Ca/Sb; P/Zr
	What is the approximate detection limit for an EDS system
	What is the difference between quantitative analysis and qualitative analysis
	What are the advantages and disadvantages of operating at a higher KV(i.e. 20-25KV) compared to lower KV (i.e. 5-15KV)
	Controls
	When must the calibrations of the SEM and EDS be checked
	How are the calibrations of the SEM and EDS checked
	Sample Prep
	What is “charging” and how can it be avoided
	How does the size of the area sampled affect the data

Tasks

Date	Tasks
	Use of the Instrument
	The trainer will demonstrate the operation of the SEM
	The trainer will demonstrate how to properly mount and coat a specimen
	Demonstrate sample preparation using the carbon planchette
	Demonstrate image capture and storage procedures
	Demonstrate tungsten filament replacement in the SEM gun head
	Demonstrate adjustment of the aperture using focus wobble
	Demonstrate how to check and/or adjust the stigmation
	Produce Spectrographs of each listed element, noting the position of the peak in relationship to each other Pb/S/Mo Ba/Ti Sb/Ca
	Demonstrate using software overlap features
	Demonstrate how to use hysteresis/degauss
	Mount and carbon coat at least three of the following types of specimens to achieve a proper coat thickness to allow for elemental analysis and imaging a. Paint b. Paper c. Soil d. Glass e. Fiber f. Plastic/Polymer

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

SEM/EDS Appendix III

Basic Operation of the SEM/EDS

The OSBI Trace Evidence Unit has a Tescan VEGA3 Scanning Electron Microscope coupled with an Oxford INCA Energy Dispersive X-Ray Spectrometer. The scanning electron microscope utilizes a focused beam of high-energy electrons that systematically scans across the surface of a specimen. The interaction of the beam with the specimen produces a large number of signals at or near the specimen's surface. These interactions include elastic scattering which produces backscattered electrons and inelastic scattering which produces secondary electrons, characteristic x-rays and several other forms of energy.

The SEM is one of the most versatile instruments available for the examination and analysis of the microstructural characteristics of solid objects. Another important feature of the SEM is the three dimensional appearance of the specimen image, a direct result of the large depth of field, as well as to the shadow-relief effect of the secondary and backscattered electron contrast.

Backscattered electrons provide an extremely useful signal for imaging in scanning electron microscopy. Backscattered electrons respond to composition (atomic number or compositional contrast) as well as surface inclination, crystallography and internal magnetic fields. Secondary electrons are defined purely on the basis of their kinetic energy; that is, all electrons emitted from the specimen with an energy less than 50eV, an arbitrary choice, are considered secondary electrons. Although some backscattered electrons are obviously included in this region, their inclusion in the definition of secondary electrons introduces only a negligible effect. Both backscattered and secondary electrons are detected and used independently to form an image that can be seen on the monitor of the SEM. The backscattered electron detector (BSED) will form an image that has high contrast where the secondary electron detector (SE1) will form what is described as being a grayscale image.

Characteristic x-ray emission is a process by which an atom will stabilize itself following inner shell ionization. X-ray spectroscopy in the SEM/EDS involves the identification of radiation of specific wavelength or energy for elemental analysis of the specimen. When an x-ray strikes the semiconductor crystal, electrons in the crystal absorb a given amount of energy that is then converted to an electrical signal that is emitted and amplified. The strength of the current from the crystal is proportional to the x-ray energy. The amplified electrical pulses from the semiconductor are converted to digital form and processed by a multi-channel analyzer that sorts these signals and counts the number of x-rays at each energy level that strike the crystal. This information is then plotted to form a representative spectrum.

Definitions

Alignment: The arrangement of components on an optical and/or mechanical axis to obtain maximum results and a symmetric image form.

Aperture: A small metal disc containing a central hole used to increase image contrast by eliminating non-image-forming rays along an optical axis.

Backscattered Electrons: Electrons produced by elastic collisions of incident electrons with atoms in the sample. The interaction with the core electrons produces a backscattered signal that contains chemical information, reflected in the brightness of the particle reactive to the atomic number of elements. For example, lead with the atomic number 82 is brighter than iron with the atomic number 26.

Characteristic X-Rays: The portion of the x-ray spectrum produced during the inelastic scattering of beam electrons which identifies the specific atom(s) present

Charging: A build-up of negative charge on a specimen (nonconductor) that produces bright spots and distorts the image in the SEM.

Dead Time: The time in which pulses are not measured, often expressed as a percentage of real or clock time.

Detector: The device used to collect and transmit information from the specimen.

Depth of Field: The range of specimen positions along an optical axis, yet retaining a sharp image at a certain lens strength.

EDS: Abbreviation for Energy Dispersive Spectrometer, which detects characteristic x-rays for compositional analysis.

Electron: A negatively-charged particle of mass approximately 1/1845 of the proton, and of wavelength approximately 5 pm of 5x10-3 nm.

Electron Volt: A unit used to measure the energy of subatomic particles. One electron electric charge equal to -1) across an electric potential of one volt. Abbreviated by eV.

Escape Peak: An artifact peak that appears at an energy equal to the energy of the parent line minus that of the silicon K alpha x-ray, 1.74 keV

Excitation Potential: Also known as Critical Excitation Energy. The minimum x-ray tube potential that can accelerate electrons from filament to target with enough energy to expel an electron from a given level in an atom of a given target element.

Filament: The thin wire (or other material) cathode of the electron gun.

Focus: The point at which the rays of a lens converge.

Gun (Electron Gun): The typical triode used to produce an electron beam in an electron optical instrument.

Pole Piece: A pair of soft iron, conical pieces used to concentrate the electric field of an electromagnetic lens.

Process Time: The length of time spent reducing noise from the x-ray signal coming from the EDS during processing.

Resolution:

Image resolution: The distance between two closest yet distinguishable points in an image.
Spectral resolution: The ability of the detector to separate two adjacent peaks in the energy spectrum.

Saturation: The minimum amount of filament required to give the maximum beam current (brightest illumination) in an electron gun.

Scanning Coils: Electric coils in the SEM column used to deflect the beam in order for the beam to scan back and forth over the specimen.

Scintillator: Target for electrons in the SEM collector–Converts electron energy to light energy.

Scanning Electron Microscope: A microscope that utilizes an accelerated focused electron beam to image particulate samples (such as gunshot residue) at high magnification, with great depth of field, while providing size and shape.

Secondary Electrons: Inelastic interactions where electrons near the surface receive enough energy to exit the sample.

Sum Peak: Interpretation of two or more pulses as one when two photons arrive at the detector almost simultaneously, and the output is a single combined pulse corresponding to the sum of the two photon energies.

Steric Hindrance: The prevention or retardation of inter- or intramolecular interactions as a result of the spatial structure of a molecule.

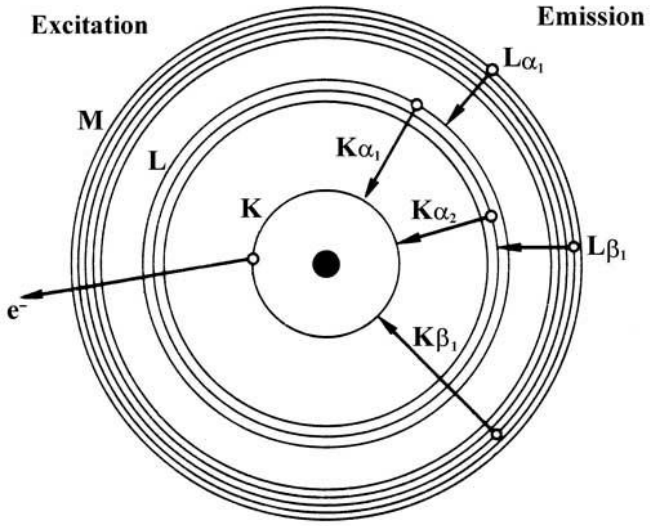
Stub: Specimen mount in the SEM (usually aluminum).

Working Distance: The distance from the final lens to the specimen in the SEM.

Basic Principles

An electron microscope is an optical instrument in which a beam of electrons is used to form a greatly enlarged image of an extremely small object through a series of electromagnetic lenses. Extremely small negatively charged electrons given off by an energized tungsten wire filament can be accelerated by high voltage to produce a coherent beam and can be focused by an electric field. Since electrons are much smaller than air molecules, there must be a high vacuum inside the "column" of electromagnetic cylinders to allow a free path for electrons from the electron gun to the viewing screen.

In the situation of the SEM being coupled with an EDS, characteristic x-rays can also be detected/analyzed. As the beam interacts with the sample and has dislodged an electron from an inner atomic shell, an electron from an outer shell will fill the vacancy. The difference in energies between the initial and final states of the transitional electron will be emitted as x-ray radiation. The various shells of an atom have discrete amounts of energy. This energy difference is a discrete quantity and is characteristic of the atom from which it was released.



Microscopy

Goals

- To gain knowledge of the theory and proper use of various types of microscopes
- To demonstrate proper care and maintenance of various types of microscopes
- The trainee will learn how to prepare samples for analysis by microscopy, analyze samples, and interpret data from this type of analysis.

Literature Reading

Date	Literature
	Petraco, N., Kubic, T. Color Atlas and Manual of Microscopy for Criminalists, Chemists and Conservators. 2004, chapters 1, 2, 3, 4 and 14.
	Egerton, R. Physical Principles of Electron Microscopy, 2005, pages 1-9, 27-33
	McCrone, W.C., Delly, J. G. The Particle Atlas. Vol.I, Edition Two, 1973, pages 3-56
	Bloss, F.D. Optical Crystallography. 1999, The Mineralogical Society of America, chapters 1, 2, 3, 4, and 7
	McCrone, W.C. **Particle characterization by PLM: Part I: No Polars**. *Microscope.* 1982, Vol. 30, 3rd Quarter, pages 185-196 (PDF: Microscopy)
	McCrone, W.C. **Particle characterization by PLM: Part II: Single Polar**. *Microscope.* 1982, Vol. 30, 4th Quarter, pages 315-331 (PDF: Microscopy)
	McCrone, W.C. **Particle characterization by PLM: Part III: Crossed Polar**. *Microscope.* 1982, Vol. 31, 2nd Quarter, pages 187-206 (PDF: Microscopy)

Optional Reading

	Saferstein, R. Criminalistics, An Introduction to Forensic Science. 2011, pages 165-182
	DeForest, P. R. **Foundations of forensic microscopy**. *Forensic Science Handbook*. Vol. I, Saferstein, R. Ed., 2002, 2nd edition, pages 215-319
	McCrone, W. C., McCrone, L. B., Delly, J. B. Polarized Light Microscopy. 2010
	McCrone Research Institute, Particle Manipulation and Sample Preparation for Microanalytical Techniques. 2005
	California Criminalistics Institute, Fibers, Fiber identification and Comparison (M202), Exercise Manual, pages 1-8

Articulate and/or Provide Written Answers

Date	Task
	Describe the operation of the stereomicroscope in layman's terms
	Describe two different stereomicroscope designs
	What is the difference in the image produced by a stereomicroscope versus a compound microscope
	Explain the basic theory of optics and how magnification is achieved
	What is the total magnification of a microscope
	Define "empty magnification"
	Describe polarized light
	Name two ways that the contrast of an image can be increased
	What are the functions of the sub-stage condenser
	Explain retardation as it applies to a birefringent material
	Define extinction, sign of elongation and pleochroism
	What is an immersion objective
	What regular maintenance is necessary to maintain a microscope
	Name the parts of:
	Stereomicroscope
	Polarizing light microscope
	Comparison microscope
	Explain the basic theory of:
	Polarized Light Microscopy (PLM)
	Fluorescence
	Brightlight
	Darkfield
	Reflectance
	Tools
	Describe how to make micro-cover slips
	What main tool is used for particle handling

Tasks

Date	Tasks
	Use of the Instrument
	Demonstrate the proper use of the stereo microscope
	Demonstrate Kohler illumination

	Demonstrate ocular calibration
	Demonstrate centering objectives
	Demonstrate refractive index determinations utilizing the Becke line method
	Demonstrate proper cleaning of the microscope body and optics
	Demonstrate setup and alignment of light microscopes
	Tools
	Demonstrate making micro-cover slips
	Demonstrate the use of a tungsten needle to move small particles
	Demonstrate photomicrography with the digital camera
	Demonstrate making tungsten needles
	Exercises
	Complete Practical Exercise 3 *can be exempted if Forensic Microscopy class taken
	Complete Practical Exercise 4 *can be exempted if Forensic Microscopy class taken
	Complete Practical Exercise 5
	Complete Practical Exercise 6
	Complete Practical Exercise 7
	Complete Practical Exercise 8
	Complete Practical Exercise 9
	Complete Practical Exercise 16
	Complete Practical Exercise 17 *optional
	Complete Practical Exercise 18
	Complete Practical Exercise 19 *optional

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Fiber Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of primarily synthetic and cotton fiber samples along with familiarization of other natural fibers. The trainee must have completed the training module on Microscopy, FTIR, and MSP or be working on concurrently with this training.

Goals

- To become familiar with synthetic fiber and textile history, terminology, and usage including common end uses of different fiber, yarn, fabric and cordage types
- To become familiar with synthetic fiber and textile chemistry and manufacturing processes including chemical compositions, chemical and mechanical treatments, and manufacturing mechanical, dyeing and finishing processes
- To become skilled at search, recovery, preservation, and examination techniques including proper sample handling; packaging and documentation for fibrous materials associated with a variety of substrates
- To gain experience in examination and comparison of textiles for physical fits, physical construction, and synthetic fiber composition;
- To become familiar with identification and/or comparison of cotton and other natural and manufactured fibers by optical, chemical and physical property examinations;

Literature Reading

Date	Literature
	OSBI TR-170 Fiber Analysis
	Introduction to Fibers and Textiles
	ASTM, E2225-21. Standard Guide for Forensic Examination of Fabrics and Cordage
	Scientific Working Group for Materials Analysis (SWGMAT). **Introduction to Forensic Fiber Examination**, Ch. 1 2011 revision (PDF: Fibers)
	David, S. K., Pailthorpe, M. T**. Classification of Textile Fibres: Production, Structure, and Properties**. Forensic Examination of Fibres. Robertson J. & Grieve M. C., Ed., 2nd edition, 1999, pages 1-31

	Saferstein, R. Criminalistics an Introduction to Forensic Science. 10th edition, 2011, pages 330-342
	Petraco, N., Kubic, T. Color Atlas and Manual of Microscopy for Criminalists, Chemists and Conservators. 2004, chapters 7, 8 and 9
	Bresee, R. R. **Evaluation of Textile Fiber Evidence: A Review**. *Journal of Forensic Science.* Vol. 32, No. 2, March 1987, pages 510-521
	Fiber Transfer and Persistence
	Akulova, V., Vasiliauskiene, D., Talaliene, D. **Further Insights into the Persistence of Transferred Fibres on Outdoor Clothes**. *Science and Justice*. Vol. 42, No. 3, 2002, page 165-171
	Masakowski, S., Enz, B., Cothern, J.E., Rowe, W. F. **Fiber-Plastic Fusions in Traffic Accident Reconstruction**. *Journal of Forensic Science.* Vol. 31, No. 3., July 1986, pages 903-912
	Palmer, R. **The Retention and Recovery of Transferred Fibers Following the Washing of Recipient Clothing**. *Journal of Forensic Science*. Vol. 43, No. 3, 1997, pages 502-504
	Robertson, J., Roux, C. **Transfer, Persistence and Recovery of Fibres**. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 89-100
	Taupin, J. M. **Hair and Fiber Transfer in an Abduction Case – Evidence from Different Levels of Trace Evidence Transfer**. *Journal of Forensic Science.* Vol. 41, No. 4, July 1996, pages 697-699
	Casework Familiarization
	Biermann, T. W. **Fiber Finder Systems**. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 135-152
	Farley, M. A. **Legal Standards for the Admissibility of Novel Scientific Evidence**. Forensic Science Handbook, Saferstein, R., Ed., Vol. III., 1993, pages 1-23
	Gaudette, B. **The Forensic Aspects of Textile Fiber Examination**. Forensic Science Handbook, Saferstein, R., Ed., Vol. II, 1988, pages 209-272, Chapters 4 & 5
	Dignan SJ, Murphy KJ. **Fibre evidence from fingernail clippings**. Can Soc For Sci J 2002; 35(1):17-21.
	Grieve, M. C., Dunlop, J., Haddock, P. **An Investigation of Known Blue, Red and Black Dyes Used in the Coloration of Cotton Fibers**. *Journal of Forensic Science.* Vol. 35, No. 2, March 1990, pages 301-315
	Palenik, S. **Microscopy and Microchemistry of Physical Evidence**. Forensic Science Handbook. Saferstein, R., Ed., Vol. II, 1988, pages 161-208
	Robertson, J. **Protocols for Fibre Examination and Initial Preparation**. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 116-134

	Roux, C., Huttuen, J., Rampling, K., Robertson, J. **Factors Affecting the Potential for Fibre Contamination in Purpose-Designed Forensic Search Rooms**. *Science & Justice*. 2001, Vol. 41, pages 135-144
	Microscopy/Polarized Light Microscopy Review
	ASTM, E2228-19. Standard Guide for Microscopical Examination of Textile Fibers
	Palenik, S. **Microscopical Examination of Fibres**. Forensic Examination of Fibres. Robertson, J., Grieve, M. C., Ed., 2nd edition, 1999, pages 153-177
	Scientific Working Group for Materials Analysis (SWGMAT). **Microscopy of Textile Fibers**. www.swgmat.org (PDF: Microscopy Chapter 2011)
	Stoeffler, S. F. **A Flowchart System for the Identification of Common Synthetic Fibers by Polarized Light Microscopy**. *Journal of Forensic Science.* Vol. 41, Vo. 2, March 1996, pages 297-299
	FTIR Microscopy/FTIR-ATR
	ASTM, E2224-19, Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy
	Tungol, M. W., et. al., **Forensic Examination of Synthetic Textile Fibers by Microscopic Infrared Spectrometry**. Practical Guide to Infrared Microspectroscopy. Humecki, H., Ed. 1995, pages 245–285
	UV-Vis Spectroscopy/Microspectrophotometry Review
	Scientific Working Group for Materials Analysis (SWGMAT). **Ultraviolet-Visible Spectroscopy of Textile Fibers**. www.swgmat.org (PDF: UV-VIS Spectroscopy of Textile Fibers Chapter 2011)
	Wiggins, K.G., Holness, J.A., March, B.M. **The Importance of Thin Layer Chromatography and UV Microspectrophotometry in the Analysis of Reactive Dyes Released from Wool and Cotton Fibers**. *Journal of Forensic Science.* Vol. 50, No. 2, 2005, pages 1-5
	Optional Reading
	Laing, D. K., Hartshorne, A. W., Cook, R., Robinson, G. **A Fiber Data Collection for Forensic Scientists-Collection and Examination Methods**. *Journal of Forensic Science.* Vol. 32, No. 2, March 1987, pages 364-369
	Wiggins, K. G., Cook, R., Turner, Y. J. Dye **Batch Variation in Textile Fibers**. *Journal of Forensic Science.* Vol. 33, No. 4, July 1988, pages 998-1007
	Fong, W., Inami, S. H. **Results of a Study to Determine the Probability of Chance Match Occurrences Between Fibers Known to be from Different Sources**. *Journal of Forensic Science.* Vol. 31, No. 1, January 1986, pages 65-72
	Kirkbride, K. P., Tungol, M. W. Forensic Examination of Fibres, Robertson, J., Grieve, M., Ed., 2nd edition, 1999, pages 179-222
	Wiggins, K.G. **Recognition, identification and comparison of rope and twine**. *Science & Justice.* 1995, Vol. 35, No. 1, pages 53-58

	ASTM International. E222402 **Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy**. pages 1-5
	Scientific Working Group on Material Analysis. **Forensic Fiber Examination Guidelines, Chapter 6: Infrared Analysis of Textile Fibers, Spectroscopy**, *Forensic Science Communications* [online]. (April 1999 Revision) http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/april1999/houcktoc.htm (PDF: FBI – Fiber Guidelines)
	West Virginia University Textile and Fibers online course, if available
	McCrone, W.C., Delly, J.G. **Descriptions and Color Photomicrographs**, The Particle Atlas. 1973, Edition Two (352)
	Scientific Working Group for Materials Analysis (SWGMAT). Fiber Daubert (PDF: Fibers) 2012.
	Coxon, A., Grieve, M., Dunlop, J. A Method of Assessing the Fibre Shedding Potential of Fabrics. Journal Forensic Science Society. 1992, Vol. 32, pages 151-158
	Grieve, M. C., Dunlop, J., Haddock, P. S. Transfer Experiments with Acrylic Fibres. Forensic Science International. 1989, Vol. 40, pages 267-277
	Pounds, C. A., Smalldon, K. W. The Transfer of Fibres Between Clothing Materials During Simulated Contacts and their Persistence During Wear, Part I-Fibre Transference. Journal Forensic Science Society. Vol. 15, 1975, pages 17-27
	Pounds, C. A., Smalldon, K. W. The Transfer of Fibres Between Clothing Materials During Simulated Contacts and their Persistence During Wear, Part II-Fibre Persistence. Journal Forensic Science Society. Vol. 15, 1975, pages 29-37
	Pounds, C. A., Smalldon, K. W. The Transfer of Fibres Between Clothing Materials During Simulated Contacts and their Persistence During Wear, Part III-A Preliminary Investigation of the Mechanisms Involved. Journal Forensic Science Society. Vol. 15, 1975, pages 197-207
	Scientific Working Group for Materials Analysis (SWGMAT). Trace Evidence Recovery Guidelines, Part 4 Contamination and Loss. Forensic Science Communications. Vol. 1, No. 3, October 1999, www.fbi.gov (PDF: Trace Evidence Recovery Guidelines SWGMAT 1999)
	Scientific Working Group for Materials Analysis (SWGMAT) Trace Evidence Quality Assurance Guidelines. Forensic Science Communications. Vol. 2, No. 1, January 2000, www.fbi.gov (PDF: Trace Evidence Quality Assurance Guidelines SWGMAT 2000)

Tasks

Date	Tasks
	Observe Casework and Discussion
	Observe 3 cases
	Case #:
	Case #:
	Case #:
	Discuss the following with trainer
	Contamination and loss practices collecting, packaging and sealing in appropriate packaging documenting any situation which could have contaminated or compromised the evidence
	Detection, collection and preservation techniques a. criteria for selection of technique and processing sequence b. recording techniques c. visual searches, with or without magnification, to include oblique lighting and alternate light sources d. using the most direct and least intrusive collection methods: picking, tape lifting, scraping, vacuuming, combing and cutting e. appropriate packaging for wet items, use of temporary packages, and proper packaging material
	Questioned versus known samples, and collection of representative known samples
	Laboratory analyses a. identification b. comparison c. sources d. destructive versus non-destructive testing e. documentation including notes, sketches and images f. maintaining evidence integrity and security
	Laboratory examination report writing a. description of items received and methods of analysis used b. examination results and conclusions
	Cotton analysis
	Skills Demonstration
	Contamination and loss prevention techniques when actually manipulating evidentiary items
	Visual searching

	Stereomicroscopical searching
	Fibrous evidence recovery by: a. picking b. tape lifting c. scraping d. vacuuming e. combing f. cutting
	Detection & identification of cotton
	Exercises
	Complete Practical Exercise 10
	Complete Practical Exercise 12
	Complete Practical Exercise 22
	Complete Practical Exercise 24
	Complete Practical Exercise 25
	Complete Practical Exercise 33
	Complete Practical Exercise 34
	Complete Practical Exercise 35
	Complete Practical Exercise 36

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Fiber Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Analyze 60 samples (20 from 3 of the major fibers classes such as Nylon, Polyester, etc.) using: Polarized Light Microscopy FTIR and Microspectrophotometry as needed to discriminate between samples; Document using Form 1 or equivalent.
	Analyze 10 cotton samples
	Analyze 5 practice cases and record all results; document using LabDev.
	Successfully completed requirements for the following instrumentation:
	Microscopy
	FTIR
	MSP

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Elemental Analysis for Trace Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of samples suspected to contain inorganic elements using the Scanning Electron Microscope with Energy Dispersive X-Ray Spectrometry (SEM/EDS). The trainee must have completed the training module on SEM/EDS prior to completing this training module.

Goals

- To become skilled at using SEM/EDS to identify inorganic elements in samples submitted.
- The trainee will learn how to prepare samples for analysis, analyze samples using SEM/EDS, and interpret data from this type of analysis.

Literature Reading

Date	Literature
	OSBI TR-15 Elemental Analysis Protocol
	Chandler, J. A., 1987. X-ray Microanalysis in the Electron Microscope, North-Holland Publishing Company, sections 4.5.1, 4.5.2
	Woldseth, R., X-Ray Energy Spectrometry. 1973, 1st Edition, pages 1.1-1.20
	E. Margui and R. Van Grieken, 2013. X-Ray Fluorescence Spectrometry and Related Techniques: An Introduction, Momentum Press, Chapters 2 & 4, Chapter 7.5 (PDF)

Articulate and/or Provide Written Answers

Date	Tasks
	Controls
	What quality control/quality assurance measures are performed during analysis
	What must be done when a new lot of carbon cohesive mounting material is acquired
	How often must the carbon cohesive mounting material be analyzed for the presence of interfering elements
	If the cohesive material is found to contain an inorganic element, what must be done

Tasks

Date	Tasks
	Use of the Instrument
	Perform elemental analysis of the 37 element standard. A properly labeled spectrograph of each standard should be produced and placed in the training file
	The trainer and trainee will discuss spectrum labeling
	Analyze the following specimens/samples: Red Phosphorous powder Barium Sulfate Calcium Carbonate Silicon Dioxide Talc Kaolin
	Spectral Interpretation
	Demonstrate how to directly compare spectra from two different samples using the Inca software
	Demonstrate overlapping spectra using the Inca software
	Demonstrate to overlay and reconstruct features of a sample using the Inca software
	The trainer will demonstrate data management and how case data is organized on the instrument
	The trainer will discuss what report and data must be included in the case file
	Properly analyze three unknown samples
	Demonstrate the ability to produce reports in accepted format

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Elemental Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Analyze 25 samples and record all results; document using Form 1 or equivalent.
	Analyze 5 practice cases and record all results; document using LabDev.
	Successfully completed requirements for the following instrumentation:
	SEM/EDS

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Paint Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of paint samples. The trainee must have completed the training module on Microscopy, FTIR, MSP, and SEM or be working on concurrently with this training.

Goals

- To become familiar with the analysis of paint, automotive and architectural
- To become skilled at the analysis of paint samples
- To determine when two samples could have come from the same source or not

Literature Reading

Date	Literature
	OSBI TR-130 Physical Fit Comparison Protocol
	OSBI TR-160 Paint and Polymer Analysis Protocol
	General Background
	Crown, D.A. The Forensic Examination of Paints and Pigments. Charles C. Thomas, 1968, pages v and vi, 3-7
	Petraco, N., Kubic, T. Color Atlas and Manual of Microscopy for Criminalists, Chemists and Conservators. 2004, chapter 10
	ASTM International, E1610-17, **Standard Guide for Forensic Paint Analysis and Comparison**. pages 1-13
	Scientific Working Group on Materials Analysis, Forensic Paint Analysis and Comparison Guidelines. *Forensic Science Communications*. May 2000, http://www.fbi.gov, pages 1-19 (PDF: SWGMAT Forensic Paint Analysis…)
	Thornton, J.I. **Forensic Paint Examination**. Forensic Science Handbook. Vol. 1, 2nd ed., R. Saferstein, ed., 2002, pages 430-473
	Morgans, W.M. Outlines in Paint Technology. 3rd ed., 1990, Chapter 1
	VanHoven, H., and Fraysier, H. **The matching of automotive paint chips by surface striation alignment**. *Journal of Forensic Science.* 1983, Vol. 28, No. 2, pages 463-467
	Ryland, S.G., et. al. **Discrimination of 1990s original automotive paint systems: A collaborative study of black nonmetallic base coat/clear coat finishes using infrared spectroscopy**. *Journal of Forensic Science.* 2001, Vol. 46, No. 1, pages 31-45
	Ryland, S.G., Jergovich, T.A., Kirkbride, K.P. **Current Trends in Forensic Paint Examination**. *Forensic Science Review.* Vol. 18, Issue 97, 2006, pages 98-117

	Lambourne, R. **Paint Composition and Applications – a General Introduction**. Paint and Surface Coatings, Theory and Practice, R. Lambourne ed., 1987, pages 22-39 (PDF: Paint Composition and Applications)
	Automotive Paints and Coatings. G. Fettis, ed., 1995, pages 1-14, 28-38, 55-58, 72-91, 119-144, 148-157
	Thornton, J., Krause, S., Lerner, B., and Kahane, D. **Solubility characterization of automotive paints**. *Journal of Forensic Science.* 1983, Vol. 28, No. 4, pages 1004-1007
	Bentley, J. **Composition, Manufacture and Use of Paint**. Forensic Examination of Glass and Paint; Analysis and Interpretation. B. Caddy, ed., 2001, pages 123-141
	Microspectrophotometry
	ASTM International, E2808-19, **Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis**. pages 1-8
	Kopchick, K.A., and Bommarito, C.R. **Color analysis of apparently achromatic automotive paints by visible Microspectrophotometry**. *Journal of Forensic Science.* 2006, Vol. 51, No. 2, pages 340-343
	Stoecklein, W. **The role of colour and microscopic techniques for the characterisation of paint fragments**. Forensic Examination of Glass and Paint; Analysis and Interpretation. B. Caddy, ed., 2001, pages 143-162
	Scientific Working Group on Materials Analysis, **Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis**. October 2007. http://swgmat.org, pages 1-14 (PDF: SWGMAT Standard Guide for Microspectrophotometry…)
	Infrared Microspectroscopy
	ASTM International, E2937-18, **Standard Guide for Using IR in Paint Examinations.** pages 1-9
	Scientific Working Group on Materials Analysis, **Standard Guide for Using Infrared Spectroscopy in Forensic Paint Examinations**. April 2009, http://swgmat.org, pages 1-20 (PDF: SWGMAT Guidelines for Using Infrared…)
	Suzuki, E.M., and Gresham, W.R. **Forensic science applications of diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS): I, Principles, sampling methods and advantages**. *Journal of Forensic Science.* 1986, Vol. 31, No. 3, pages 931-952
	Suzuki, E.M. **Extended Frequency Infrared Examination of Paints**. *Crime Laboratory Digest.* July 1995, Vol. 22, No. 3, page 94
	Beveridge, A., Fong, F., and McDougall, D., **Use of infrared spectroscopy for the characterization of paint fragments**. Forensic Examination of Glass and Paint; Analysis and Interpretation, B. Caddy, ed., 2001, pages 183-220
	Ryland, S.G. **Infrared Microspectroscopy of Forensic Paint Evidence**. Practical Guide to Infrared Microspectroscopy, Humecki, H. J., ed., 1995, pages 163-243

	Sample Preparation
	Cartwright, et.al. **A microtome technique for sectioning multilayer paint samples for microanalysis.** *Canadian Society of Forensic Science Journal.* 1977, Vol. 10, No. 1, pages 7-12
	McEwen, D.J. and Cheever, G.D. **Infrared microscopic analysis of multiple layers of automotive paints**. *Journal of Coatings Technology.* 1983, Vol. 65, No. 819, pages 35-41
	Wilkinson, J.M., Locke, J., and Laing, D.K. **The examination of paints as thin sections using visible microspectrophotometry and Fourier transform infrared microscopy**. *Forensic Science International.* 1988, Vol. 38, pages 43-52
	Orzechowski, A. **An optical microscopy method to display pigment agglomerates in polymer particles**. *The Microscope.* 1979, Vol. 27, No. 1, pages 5-9
	Derrick, M.R. **Infrared microspectroscopy in the analysis of cultural artifacts**. Practical Guide to Infrared Microspectroscopy, H.J. Humecki, ed., 1995, pages 294-298
	Teetsov, A.S. **Unique preparation techniques for nanogram samples**. Practical Guide to Infrared Microspectroscopy. H.J. Humecki, ed., 1995, pages 417-443
	Welsh, F. S. **A polished paint layer cross section in 30 minutes**. *Microscope.* 1997, Vol. 45, No. 2, pages 37-40
	Groves, E. and Palenik, C.S. **Applications of Blue Light-curing Acrylic Resin to Forensic Sample Preparation and Microtomy**. Journal of Forensic Sciences. 2016, Vol 61, No. 2 (PDF: Applications of Blue Light Curing…)
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Articulate and/or Provide Written Answers

Date	Tasks
	General Background
	Define the following: A. Paint G. Latex M. Plasticizer B. Vehicle H. Pigment N. Thermoplastic polymer C. Lacquer I. Drier O. Thermosetting polymer D. Varnish J. Extender P. Binder (resin) E. Stain K. Solvent Q. Coating F. Enamel L. Drying Oils R. Additives
	Define in general terms the significance of each of the following paint components in the formation of a paint film: A. Oils C. Solvent E. Resinous Vehicles B. Driers D. Plasticizers F. Extenders G. Pigments
	Relate various types of paints to end-use applications.
	General Background – Discuss the following with Trainer
	How raw materials are acquired and mixed.
	What variations may be present in raw materials
	What variations may exist in binders from different companies
	What a batch of paint is and how large it is
	What quality control procedures are used in the manufacture of paint
	How paint is packaged and distributed
	The application process of OEM finishes to motor vehicles
	Processes used in repainting and repairing vehicles
	Analytical and physical testing methods used by the paint industry
	Describe the basic steps in forensic paint examinations in common terms and explain how these steps are used to identify the components of a paint film
	Microscopical Examination & Characterization
	Describe the random individualizing characteristics which permit a physical fit
	of two paint fragments to be effected and defend why they are conclusive proof of individuality of source
	Binders - Discuss the following with trainer:
	A classification scheme for binders based upon their polymer type (automotive and structural paints)
	The distinguishing characteristics of automotive OEM finishes and after-market refinishes
	The distinguishing characteristics of automotive finishes, architectural and other industrial finishes

	Pigments and Extenders
	Define the following and give examples: A. pigment D. extender F. pearlescent pigment B. interference pigment E. metallic flake G. effect pigment C. anti-corrosive pigment
	Discuss the following MSP topics with trainer:
	The application of UV/VIS MSP techniques to forensic paint examinations. Include the potential use in discriminating UV absorbers in clear coats
	Methods of sample preparation and pros and cons of each (e.g. polishing, edge mounts, thin sections)
	Compare and contrast absorbance and % transmittance formats, emphasizing each format's strengths and weaknesses
	Compare and contrast visible range MSP with extended (UV/VIS) range techniques
	The discrimination potential on a series of similarly colored paints
	Additives
	List and classify commonly used types of additives, i.e. plasticizers, UV absorbers and drier materials
	List additives used for drier materials, plasticizers, hardeners, UV absorbers, coalescing agents, flame retardants, mildew inhibitors, etc.
	Discuss the following with trainer:
	The application of the different methods of analysis to additives. Include a general discussion of limits of detection for each technique
	After-market Treatments, Weathering, Aging and Contaminants
	List commonly used types of after-market treatments and their general composition
	List some commonly observed types of contaminants
	Discuss the following with trainer:
	Factors that contribute to weathering and aging
	The extent to which after-market treatments, weathering, aging and contaminants may affect analyses
	Significance and Interpretation - Discuss the following with trainer:
	The potential and significance of finding paint on clothing
	The correlation between the morphology (e.g. smears, drops or chips) of the paint as it relates to transfer mechanisms (e.g. passive or forceful)

	Given scenarios, discuss the type, morphology and possible method of transfer of the paint evidence, as well as the significance of the findings
	How paint appearance and composition might be used to indicate the method of application or end use of the coating.
	How the morphology, layer structure and composition may be used to assess the forensic significance of paint evidence (e.g. how many potential sources of this type of paint are there and what are they?)
	The information provided by each step in the student's "paint examination protocol" and its contribution to the protocol's overall power of discrimination
	Potential variations in paints which are the result of manufacturing processes (e.g. batch variations, trial runs, substitute suppliers) and the ability of these variations to be detected by the examination protocol
	The added significance of multi-layered transfers versus single layered transfers.
	The added significance of cross transfers
	The added significance of transfers of multiple types of multiple-layered systems.
	The approach and difficulties in the comparison of a liquid paint sample to a dried paint sample.
	Discuss make and model determinations in terms of potential investigative information and limitations of reference collections
	Relate how the examiner would conduct a vehicle make model search on a "no suspect" automotive paint case

Practical Exercises

Date	Tasks
	Microscopical Examination & Characterization
Prepare microscope slides of individual layers within a chip for all below	
Utilizing samples below, characterize at least thirty paint samples in terms of color, layer structure sequence, layer thickness, gloss, and texture. • Include a variety of paint systems including automotive, bicycle, & structural/architectural and maintenance coatings. • Include automotive systems with color coordinated primers, tri-coat systems, variety of effect pigments, etc.	
	Manually prepare thin cross-sections of at least thirty paint chips
	Expose layers of at least ten paint chips using bevel cuts
	Expose layers of at least ten paint chips using the stair-step configuration

	Prepare thin cross-sections of at least three paint chips using a microtome
	Observe & document the fracture and surface characteristics of at least five paint samples
	Compare ten sets of samples provided by the trainer and determine whether any items within a set can be distinguished from the others, based on microscopic characteristics (layers: order, thickness, color)
	Binders - FTIR
	Prepare and analyze ten single layer paint samples using one or more of the techniques: the beam condenser, diamond cell, ATR (attenuated total reflectance), and the IR microscope
	Prepare and analyze individual layers of ten multiple-layered paint chips using one or more of the techniques: the beam condenser, diamond cell, ATR (attenuated total reflectance), and the IR microscope
	Prepare and analyze a series of paints having similar binder types (structural and automotive)
	Prepare and analyze a series of paints having similar colors (structural and automotive)
	Perform spectral subtraction using a single layered paint smeared on top of a painted substrate on five samples
	Perform binder classification from the spectra of ten unknowns
	Demonstrate the effects of varying instrumental parameters including, but not limited to, number of scans, microscope aperture sizes, apodization, gain, and wavenumber resolution
	Pigments and Extenders – Light Microscopy
	Mount reference standards of five different pigments/extenders and characterize their physical and optical properties by reflected and transmitted light microscopy in polarized light microscopy
	Determine the number of pigment/extender types that are present in a prepared sample containing at least two pigments/extenders, using polarized light microscopy
	Characterize as many pigments/extenders as possible in ten paint samples, using polarized light microscopy
	Pigments and Extenders – Infrared Spectroscopy
	Interpret IR spectra and recognize pigment(s) and extender(s) in ten multi-layered paint samples
	Prepare and analyze at least five single layer paint samples using IR
	Prepare and analyze a series of paints having similar colors (architectural and automotive) using IR
	Perform spectral subtraction using a single layered paint smeared on top of a painted substrate

	Pigments and Extenders – SEM /Energy Dispersive X-ray Spectrometry
	Acquire secondary and backscattered images of a paint sample & store and print images on available media
	Prepare and analyze embedded and non-embedded microtomed and hand-sectioned multi-layered paint chips, 2 each then: vary the excitation voltage and describe the effects on the detection of various elements
	Correctly identify the elements present in ten paint layers
	Identify associated elements in extender pigment grains in situ for three different types of extender pigments utilizing spot mode analysis
	Pigments and Extenders-UV/VIS MSP
	Prepare ten paints for analysis. This should include at least one nonmetallic, one metallic, and one effect (e.g. pearlescent or other non-metal flake) paint sample Acquire several spectra from various locations within each layer of the samples. Use transmission and/or reflection as appropriate. Compare results using one or more techniques (e.g. direct comparison, spectral averaging, normalization, standard deviation plots etc.). Note the degree of variation within a single layer of a sample.
	Prepare edge mounts (cross-sectional view) of two pairs of multiple layered paint samples for analysis Acquire several spectra from various locations within each layer of the samples. Use transmission and/or reflection as appropriate. Compare results using one or more techniques (e.g. direct comparison, spectral averaging, normalization, standard deviation plots etc.). Note the degree of variation within a single layer of a sample.
	Prepare thin cross-sections of five multiple layered paint samples for analysis. Acquire several spectra from various locations within each layer of the samples. Use transmission and/or reflection as appropriate. Compare results using one or more techniques (e.g. direct comparison, spectral averaging, normalization, standard deviation plots etc.). Note the degree of variation within a single layer of a sample.
	Acquire several spectra from a single location on an architectural paint sample using different system parameters (e.g. user dependent variables, spectrophotometer and microscope settings). Note the degree of variation
	Acquire transmission spectra from thick and thin areas of a single sample. Present spectra in both absorbance and % transmittance formats. Note the degree of discrimination provided by each format.
	Acquire the spectra of several similarly colored paints. Evaluate the discrimination potential of the method.
	After-market Treatments, Weathering, Aging and Contaminants
	Perform analyses and/or observe data collected from paint that is treated and untreated (e.g. waxed, silicone treated, Teflon treated)

	Perform analyses and/or observe data collected from paint that is weathered/aged
	Perform analyses and/or observe data collected from paint that is contaminated and uncontaminated
	Significance and Interpretation
	Perform comparisons on ten simulated case samples and explain the rationale for choosing the analytical scheme; formulate conclusions and assess the significance of the findings
	Perform a comparison of a liquid paint sample to a previously cured and aged sample of the same paint. A variety of conditions may be explored, including degree of mixing, method of application, conditions of cure, environmental conditions, etc.
	Use available resources (e.g. Paint Data Query, National Automotive Paint File, spectral IR libraries, Collaborative Testing Services Paint Collection, automotive refinish books) to provide potential make and model information on five automotive samples

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Paint Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Review of practical exercises and reports prepared correctly with accurate results
	Analyze 25 samples and record all results; document using Form1 or equivalent
	Analyze 5 practice cases and record all results; document using LabDev
	Successfully completed requirements for the following instrumentation:
	Microscopy
	FTIR
	MSP
	SEM

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Ignitable Liquid Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of samples suspected to contain an ignitable liquid. The trainee must have completed the training module on GC/MS or be working on concurrently with this training.

Goals

- To become familiar with the chemistry and physics of fire, the process of refining products from crude oil, the properties of ignitable liquids, the effects of fire, terminology related to forensic ignitable liquid analysis
- To become familiar with the analysis of ignitable liquids
- To become skilled at determining if an ignitable liquid is present in evidence submitted to the laboratory.

Literature Reading

Date	Literature
	Introduction
	IAAI Forensic Science Committee, **Glossary of Terms Related to Chemical and Instrumental Analysis of Fire Debris**. 1989
	OSBI TR-100 Analysis of Ignitable Liquids
	OSBI TR-101 Ignitable Liquid Sampling Apparatus
	Stauffer, E., Dolan, J.A., and Newman, R., **Introduction**. Fire Debris Analysis. 2008, Chapter 1, pages 1-16
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	OK Statute, Title 21. Crimes and Punishments Section 1402. Arson in the second degree. http://www.oklegislature.gov/osstatuestitle.html (updated 10-04-24)
	OK Statute, Title 21. Crimes and Punishments Section 1403. Arson in the third degree. http://www.oklegislature.gov/osstatuestitle.html (updated 10-04-24)
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	OK Statute, Title 21. Crimes and Punishments Section 1405. Endangering or causing personal injury to human life during commission of arson. http://www.oklegislature.gov/osstatuestitle.html (updated 10-04-24)
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	http://www.interfire.org/features/ourchangingworld.asp (PDF downloaded 06-09-14)
	DeHaan, J.D., **Our Changing World, Part 2: Ignitable Liquids: Petroleum Distillates, Petroleum Products, and Other Stuff**. http://www.interfire.org/features/ourchangingworld2.asp (PDF downloaded 06-09-14)
	Baron, M. Arson Residues. Encyclopedia of Analytical Science, 2nd Ed., 2005, pages 365-372
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	DeHaan, J.D., Kirk's Fire Investigation. 7th Edition, 2012, Chapters 1 through 5
	O'Connor, J.J., **Chemistry and Behavior of Fire**. Practical Fire and Arson Investigation. 1987, Chapter 4, pages 55-79
	Stauffer, E., Dolan, J.A., and Newman, R., **Chemistry and Physics of Fire and Liquid Fuels**. Fire Debris Analysis. 2008, Chapter 4, pages 85-126
	Refining Process
	Stauffer, E., Dolan, J.A., and Newman, R., **Flammable and Combustible Liquids**. Fire Debris Analysis. 2008, Chapter 7, pages 199-231
	Thornton, J. I. and Fukayama, B., **The Implications of Refining Operations to the Characterization and Analysis of Arson Accelerants, Part I. Physical Separation, Part II. Chemical Conversions, Treating Processes, and Subsidiary Processes.** *Arson Analysis Newsletter.* Part I: May 1-16, 1979, Part II: August 1-16, 1979
	Leffler, William L., Petroleum Refining in Non-Technical Language. 4th Ed., 2008
	Effects of Fire
	Lentini, J.J., **Thirty Fire Scenarios**. Scientific Protocols for Fire Investigation, 2nd Ed, 2013, Chapter 7, pages 297-468
	Stauffer, Eric, **Concept of Pyrolysis for Fire Debris Analysts**. *Science & Justice*. 2003, Vol. 43, No. 1, pages 29-40
	Investigative Process
	DeHaan, J.D., Kirk's Fire Investigation. 7th Edition, 2012, Chapters 7, 8 and 9
	O'Connor, J.J., **Determining Cause and Origin**. Practical Fire and Arson Investigation. 1987, Chapter 5, pages 81-104
	Hine, G.A., **Fire Scene Investigation: An Introduction for Chemists**. Analysis and Interpretation of Fire Scene Evidence. Almirall, J.R.and Furton, K.G., Eds., 2004, Chapter 2, pages 31-76
	Lentini, J.J., **Fire Investigation Procedures**. Scientific Protocols for Fire Investigation, 2nd Ed, 2013, Chapters 4, pages 125-162
	Evidence Collection
	Dietz, W.R., **Physical Evidence of Arson: Its Recognition, Collection, and Packaging**. *Fire and Arson Investigator*. 1991, Vol. 41, No. 4, pages 33-39
	Herrera, F., **Evidence Collection, Preservation & Disposal**. *Fire and Arson Investigator.* 1999, Vol. 49, No. 4, pages 31-32

	Stauffer, E., Dolan, J.A., and Newman, R., **Sample Collection**. Fire Debris Analysis. 2008, Chapter 6, pages 163-195
	Williams, M.R. and Sigman, M.S., **Performance Testing of Commercial Containers for Collection and Storage of Fire Debris Evidence**. *Journal of Forensic Science*. 2007, Vol. 52 No. 3, pages 579-585
	Recovery and Separation of Ignitable Liquids
	DeHaan, J.D., Kirk's Fire Investigation. 7th Edition, 2012, Chapter 14
	ASTM International, E1412-19, **Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal**.
	ASTM International, E1386-23, **Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction**. pages 1-2
	ASTM International, E2451-21, **Standard Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples**. pages 1-2
	Newman, R.T., Dietz, W.R., Lothridge, K., **The Use of Activated Charcoal Strips for Fire Debris Extractions by Passive Diffusion. Part 1: The Effects of Time, Temperature, Strip Size, and Sample Concentration**. *Journal of Forensic Sciences*. 1996, Vol. 41, No. 3, pages 361-370
	Sandercock, P. M. L., **Retention of Gasoline and Diesel Fuel Samples on Charcoal: Evaluation of Long Term Preservation of Petroleum Residues**. *Canadian Society of Forensic Science Journal*. 1997, Vol. 30, No. 4, pages 219-224
	Ren, Q., Bertsch, W., **A Comprehensive Sample Preparation Scheme for Accelerants in Suspect Arson Cases**. *Journal of Forensic Sciences.* 1999, Vol. 44, No. 3, pages 504-515
	Stauffer, E., Dolan, J.A., and Newman, R., **Extraction of Ignitable Liquid Residues from Fire Debris**. Fire Debris Analysis. 2008, Chapter 11, pages 377-437
	Gas Chromatography/Mass Spectrometry
	Kelly, R. and Martz, R., **Accelerant Identification in Fire Debris by Gas Chromatography/Mass Spectrometry Techniques**. *Journal of Forensic Sciences.* 1984, Vol. 29, No. 3, pages 714-727
	Wallace, J.R., **GC/MS Data from Fire Debris Samples: Interpretation and Applications**. *Journal of Forensic Sciences*. 1999, Vol. 44, No. 5, pages 996-1012
	Nowicki, F.F., **An Accelerant Classification Scheme Based on Analysis by Gas Chromatography/Mass Spectrometry (GC-MS)**. *Journal of Forensic Sciences.* 1990, Vol. 35, No. 5, pages 1064-1086
	Lennard, C.J., Rochaix, V.T., Margot, P., **A GC-MS Database of Target Compound Chromatograms for the Identification of Arson Accelerants**. *Science and Justice*, Vol. 35 (1), pages 19-30, 1995.

	ASTM International, E1618-19, **Standard Test Method for Identification of Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry**.
	Classification, Interpretation, and Identification
	Bertsch, W., "Analysis of Accelerants in Fire Debris – Data Interpretation," Forensic Science Review, Vol. 9, No. 1, June 1997, pp. 1-8
	Newman, R., Gilbert, M., Lothridge, K., GC-MS Guide to Ignitable Liquids. 1998
	Keto, R.O., **GC/MS Data Interpretation for Petroleum Distillate Identification in Contaminated Arson Debris**. *Journal of Forensic Sciences*, 1995, Vol. 40, No. 3, pages 412-423
	Chasteen, C.E., **Essential Tools for the Analytical Laboratory: Facilities, Equipment, and Standard Operating Procedures**. Analysis and Interpretation of Fire Scene Evidence. Almirall, J.R.and Furton, K.G., Eds., 2004, Chapter 4, pages 99-132
	Dolan, J.A., **Analytical Methods for the Detection and Characterization of Ignitable Liquid Residues from Fire Debris**. Analysis and Interpretation of Fire Scene Evidence. Almirall, J.R.and Furton, K.G., Eds., 2004, Chapter 5, pages 133-158
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	Stauffer, E., Dolan, J.A., and Newman, R**., Interpretation of Data Obtained from Neat Ignitable Liquids**. Fire Debris Analysis. 2008, Chapter 9, pages 295-351
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	Kirkbride, K.P., **Microbial Degradation of Petroleum Hydrocarbons: Implications for Arson Residue Analysis**. *Journal of Forensic Sciences.* 1992, Vol. 37, No. 6, pages 1585-1599
	Trimpe, M.A., **Turpentine in Arson Analysis**. *Journal of Forensic Sciences.* 1991, Vol. 36, No. 4, pages 1059-1073
	Lentini, J.J., Dolan, J.A. and Cherry, C., **The Petroleum-Laced Background**. *Journal of Forensic Sciences.* 2000, 45 (5), pages 968-989

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Articulate and/or Provide Written Answers

Date	Tasks
	Chemistry and Physics of Fire
	Define the following: a. Fire b. Fire triangle/fire tetrahedron c. Fire tetrahedron d. Ignitable liquids vs. accelerants e. Ignition temperature f. Flash point g. Wick effect h. Flammable range i. Ignition Source j. The crime of arson k. Combustible l. Flammable liquid vs combustible liquid
	Petroleum Products and the Refining Process
	Discuss the following with the Trainer
	How petroleum crude oil is formed
	Crude Oil Sources and Composition a. Domestic b. Foreign c. Miscellaneous sources, e.g. shale, tar sands, recycled d. Varied amounts of hydrocarbons, sulfur, nitrogen, oxygen, trace metal e. “Sweet” vs “Sour”

	Refining Processes a. Various physical separations processes b. Distillation fractions c. Physical and chemical properties of distillation fractions d. Various chemical conversion processes e. Blending
	Relating Refinery Fractions to Commercially Available Products
	What the octane level of gasoline refers to
	Is it possible to determine the brand name of a gasoline sample? Or that two samples come from a common source?
	What are pristane and phytane? What petroleum products can they be found in?
	Define paraffinic
	Effect of Fire
	Define the following: a. Melting b. Evaporation c. Distillation d. Pyrolysis e. Combustion f. Thermal degradation g. Weathering
	Effects of Fire--Discuss the following with the Trainer
	How evaporation and combustion of ignitable liquids effects a. Single component liquids b. Multi-component liquids c. Immiscible liquids
	Pyrolysis products
	Thermal degradation products
	Methods of Fire Suppression and their effects on analysis of ignitable liquids a. Foams b. Positive pressure ventilation c. Dry chemicals d. Remediation agents e. Equipment

	Investigative Process
	Define the following: a. Point of origin b. V pattern c. Pour pattern
	Investigative Process—Discuss the following with the Trainer
	Scene preservation and contamination a. Securing the scene b. Awareness of sources of contamination c. Prevention of contamination
	Contributions of ordinary combustibles to fires a. Melting point of materials b. Heat release rates c. Pyrolysis products d. Physical residues e. Ignitability
	The benefit of understanding some of the role of the fire investigator, even though you may never participate in field investigation of an arson.
	Evidence Collection & Preservation—Discuss the following with the Trainer
	Packaging of Evidence-- Containers a. Metal cans (unlined and lined) b. Vapor-tight bags (nylon, Fire DebrisPAK, others) c. Glass jars d. Vials e. Unacceptable containers
	Where samples should be taken from if there is a pour pattern.
	Preservation of Evidence a. Refrigeration b. Freezing c. Microbial degradation d. Protection i. Sunlight ii. Heat iii. Breakage (glass containers) e. Time i. Shelf life ii. Visual inspection—rust on cans

	Recovery and Separation of Ignitable Liquids
	Discuss each of the following techniques, including their advantages and disadvantages, with the Trainer Headspace Steam Distillation Solvent Extraction Passive Headspace (charcoal sampling) Dynamic Headspace Solid Phase Micro Extraction (SPME)
	Discuss with the Trainer, the QA/QC that is performed on solvents and supplies used in ignitable liquids
	Classification, Interpretation, and Identification
	Discuss the following with the Trainer
	Data Analysis: GC/MS a. The IL20 method and the macros used in ignitable liquids b. TIC vs EIC c. Type of column used in the GC/MS d. QA/QC procedures for GC/MS e. Data management on the instrument computer
	What is the carbon range to classify something as light, medium or heavy?
	Ignitable Liquid Classification Scheme—Discuss each class and the characteristics necessary for identification. Also give examples of products from the light, medium, and heavy range of each class. a. Petroleum Distillates b. Isoparaffinic c. Aromatic d. Naphthenic-Paraffinic e. Normal-Alkanes f. Oxygenated g. Others-Miscellaneous
	Interference from Substrate Materials a. Carpet and carpet padding b. Wood c. Paper products d. Shoes and clothing e. Polymers f. Condensates g. Vehicle fires h. Others
	Is carpet or concrete a better sample? Why?

Tasks

Date	Tasks
	Observe an experienced ignitable liquid analyst prepare, label, sample, and analyze five ignitable liquid cases.
	Prepare carbon strip sampling devices
	Perform a wash tests using a microsampling syringe in the laboratory. Create a series of 10 vials, each containing ~1 mL of Carbon Disulfide. In the first vial, place 1µL of gasoline. Place the syringe into the second vial, draw up carbon disulfide to wash the syringe and push it back into the vial. Repeat for the remaining vials. Inject these vials on the GC/MS and analyze/interpret the results.
	Sensitivity of carbon strips—place a carbon strip 3 inches above liquid gasoline for a certain amount of time and then analyze the carbon strip. Do this at the following time intervals 5 seconds, 10 seconds, 30 seconds, and 60 seconds.

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Ignitable Liquid Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Analyze by liquid injection and headspace analysis at least 30 different samples to include: a. Two types of camping fuels b. Two types of lighter fluid c. Gasoline—at least five samples representing different weathering (ex. Fresh, 25%, 50%, 75%, 95%) d. Two types of charcoal started e. Two types of lubricants f. Two types of paint thinner g. Kerosene h. Diesel Interpret the data and classify each sample using the ASTM Guidelines. Document each on Form 1 or equivalent
	Pyrolysis products—Perform analysis on items that are commonly found in fire scenes (i.e. carpet, carpet padding, tile, vinyl flooring, wood, clothing, plastic, etc.). A minimum of 10 samples will be chosen and analyzed under the following conditions (NOTE: The burning will take place under the supervision of the Trainer) a. No burning, no ignitable liquids b. Approximately 30 seconds of burning, without ignitable liquids c. At least 1 minute of burning, without ignitable liquids d. Approximately 30 seconds burning, with ignitable liquids e. At least 1 minute of burning, with ignitable liquids
	Sensitivity of the GC/MS--Perform a serial dilution study with gasoline to determine the “low-end” sensitivity of the instrument
	Analyze 10 blind samples provided by the Trainer and discuss the results
	Interpret data from 30 cases completed by other analysts; document on Form 1 or equivalent
	Successfully completed requirements for the following instrumentation:
	GC/MS

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Gunshot Residue Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of samples for gunshot residue (GSR). The trainee must have completed the training section on SEM/EDS, or working on it concurrently with this training.

Goals

- To become familiar with theory, methodologies, and instrumentation utilized in gunshot residue analysis.
- To become familiar with the analysis of gunshot residue
- To become skilled at collecting GSR evidence and analyzing evidence for the presence or absence of gunshot residue.

Literature Reading

Date	Literature
	OSBI TR-110 Primer Gunshot Residue Analysis by SEM/EDS
	ASTM International, E1588-25 **Standard Guide for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy**. pages 1-5
	Wolten, et.al., **Final Report on Particle Analysis for Gunshot Residue Detection**. The Aerospace Corporation, September 1977, Aerospace Report#: ATR-77(7915)-3
	Schwoeble, A.J., Exline, D.L., Current Methods in Forensic Gunshot Residue Analysis. 2000
	Wright, D., and Trimpe, M., **Summary of the FBI Laboratory 's Gunshot Residue Symposium May 31- June 3, 2005**. *Forensic Science Communications.* July 2006, Vol. 8, No. 3
	Trimpe, M., **The Current Status of GSR Examinations**. *FBI Law Enforcement Bulletin*. May 2011, pages 24-32
	Berk, R.E., **Automated SEM/EDS Analysis of Airbag Residue I: Particle Identification**. *Journal of Forensic Sciences.* January 2009, Vol.54, No. 1, pages 60-68
	Berk, R.E., **Automated SEM/EDS Analysis of Airbag Residue II: Airbag Residue as a Source of Percussion Primer Residue Particles**. *Journal of Forensic Sciences.* January 2009, Vol. 54, No. 1, pages 69-76

	Wolten, G., M., et al. **Particle Analysis for the Detection of Gunshot Residue II: Occupational and Environmental Particles**. *Journal of Forensic Sciences.* April 1979, Vol. 24, No. 2, pages 423-430
	Gialamas, D.M., Rhodes, E.F., Sugarman, L.A., **Officers, Their Weapons and Their Hands: An Empirical Study of GSR on the Hands of Non-Shooting Police Officers**. *Journal of Forensic Sciences.* November 1995, Vol. 40, No.6, pages 1086-1089
	Bergman, P., Enzel, P., Springer, E., **The Detection of Gunshot Residue (GSR) Particles on the Bottom of Discharged Bullets**. *Journal of Forensic Sciences.* July 1988, Vol. 33, No. 4, pages 960-968
	Gunaratnam, L., Himberg, K., **The Identification of Gunshot Residue Particles from Lead-Free Sintox Ammunition**. *Journal of Forensic Sciences*. March 1994, Vol. 39, No. 2, pages 532-536
	DeGaetano, D., Siegel, J.A., **Survey of Gunshot Residue Analysis in Forensic Science Laboratories**. *Journal of Forensic Sciences*. September 1990, Vol. 35, No. 5, pages 1087-1095
	Wallace, et al. **Discharge Residues from Cartridge-Operated Industrial Tools**. *Journal of the Forensic Science Society*. 1984, Vol. 24, No. 5, pages 495-508
	Torre, C., Mattutino, G., Vasino, V. Robino, C., **Brake Linings: A Source of Non-GSR Particles Containing Lead, Barium and Antimony**. *Journal of Forensic Sciences.* 2002, Vol. 47, No. 3, pages 494-504
	Kosanske, K.L., Dujay, R.C., Kosanke, B.J., **Pyrotechnic Reaction Residue Particle Analysis**. *Journal of Forensic Science.* March 2006, Vol. 51, No. 2, pages 296-302
	Harris, A., **Analysis of Primer Residue from CCI Blazer© Lead Free Ammunition by Scanning Electron Microscopy/Energy Dispersive X-Ray**. *Journal of Forensic Sciences*. January 1995, Vol. 40, No. 1, pages 27-30
	Scientific Working Group for Gunshot Residue, **Guide to Primer Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry**. November 29, 2011, http://swggsr.org, pages 1-100 (PDF: SWGGSR Guide for Primer…)
	Kilty, J. W. **Activity After Shooting and Its Effect on the Retention of Primer Residue**. *Journal of Forensic Sciences.* April 1975, Vol. 20, No. 2, pages 219-230

Optional Reading

	Aaron, R., **Gunshot Primer Residue: The Invisible Clue**. *FBI Law Enforcement Bulletin*. 1991
	Andrasko, J., Maehly, A. C., **Detection of Gunshot Residues on Hands by Scanning Electron Microscopy**. *Journal of Forensic Sciences*, April 1977, Vol. 22, No. 2, pages 279-287

	Mosher, et al. **Gunshot Residue-Similar Particles Produced by Fireworks***, Canadian Society of Forensic Science Journal*. 1998, Vol. 31, No. 3, pages 157-168
	Garofano, L., Capra, M., et al. **Gunshot residue, Further studies on particles of Environmental and Occupational Origin**. *Forensic Science International.* 1999, Vol. 103, pages 1-21
	Zeichner, A., Levin, N., Dvorachek, M., **Gunshot Residue Particles Formed by Using Ammunitions That Have Mercury Fulminate Based Primers**. *Journal of Forensic Sciences.* November 1992, Vol. 37, No. 6, pages 1567-1573
	Feeney, W., Vander Pyl, C., Bell, S., Trejos, T., **Trends in Composition, Collection, Persistence, and Analysis of IGSR and OGSR: A Review.** *Forensic Chemistry.* May 2020, https://doi.org10.1016/j.forc.2020.100250.

Articulate and/or Provide Written Answers

Date	Tasks
	Analytical Methods
	Define the following: a. Techniques Paraffin test b. NAA c. AA d. ICP e. SEM/EDS
	Why the SEM/EDS is the preferred method of gunshot primer residue analysis
	Origin and formation of GSR
	Describe/define the following a. The role of the firearm b. Creation of GSR c. Deposition of GSR d. Distribution of GSR
	Describe the components of the ammunition, including purpose and function a. Bullet b. Cartridge case c. Propellant d. Primer
	Collection of GSR
	Describe the impact of collection conditions, such as: a. Moisture b. Blood c. Soil d. Weather

	Describe how to collect from common surfaces a. Hands b. Face c. Clothes
	Describe/list sources of contamination
	Describe how to sample other inanimate objects, such as vehicle surface and upholstery
	Describe the improper way to sample items (i.e. Rubbing rather than dobbing)
	SEM/EDS
	Automated analysis
	Relocation and manual confirmation
	Quality Assurance/ Quality Control
	Blank (also known as environmental blank)
	The appropriate positive controls/references on the SEM/EDS
	The QA/QC procedure for the carbon cohesive and the GSR Kits
	Report Writing
	What constitutes a positive result and what it means? Primary transfer a. Fired a weapon b. Was in close proximity to a firearm when discharged Secondary, tertiary, etc. transfer a. Handled a weapon b. Contacted a surface bearing GSR

Tasks

Date	Tasks
	Observe a trained analyst opening, labeling, and analyzing at least 5 cases
	Observe a trained analyst collecting GSR from items submitted in 2 different cases
	Under the guidance of the Trainer, collect GSR from 3 different items
	Analyze GSR Stubs from at least five different caliber firearms (at least one being .22 caliber). Document results on Form 1 or equivalent
	Time Study—Collect and analyze GSR Stubs from both hands and face of a shooter 0.5, 1, 4, 6, and 8 hours after firing the weapon. Document your results

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Gunshot Residue Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Analyze 20 samples containing GSR and 20 samples containing non-GSR particles to include brake pads and fireworks. Document results on Form 1 or equivalent
	Analyze 10 blind samples, record all results; document using Form1 or equivalent
	Interpret data from 30 cases completed by other analysts; document on Form 1 or equivalent
	Successfully completed requirements for the following instrumentation:
	SEM/EDS

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Physical Fit Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of samples suspected to have at one time been a part of the same unit. The trainee must have completed the training in Trace Evidence Searching, Collecting & Documenting or Microscopy. The trainee may train on these concurrently.

Goals

- To become familiar with the difference between class and individual characteristics
- To understand how a fracture match may be made and why it is considered conclusive that two objects were at one-time part of the same unit
- To know how to document a positive or negative fracture match

Literature Reading

Date	Literature
	OSBI TR-130 Physical Fit Comparison
	Koons, R.D., Buscaglia, J., Bottrell, M., Miller, E.T., **The Mechanical Fit**. Forensic Science Handbook. Saferstein, R., Ed., Vol. 1, 2nd Ed., 2002, pages 179-180
	Saferstein, R., **The Significance of Physical Evidence**. Criminalistics: An Introduction to Forensic Science, 10th ed., 2011, pages 61-67.
	Van Hoven, H.A. and H. D. Fraysier, **The Matching of Automotive Paint Chips by Surface Striation Alignment**. *Journal of Forensic Sciences.* 1983, Vol. 28, No. 2, pages 463-67
	Von Bremen, U. G. and Blunt, L., **Physical Comparison of Plastic Garbage Bags and Sandwich Bags**. *Journal of Forensic Sciences.* 1983, Vol. 28, No. 3, pages 644-654
	Zugibe, F and J. Costello, **The Jigsaw Puzzle Identification of a Hit and Run Automobile**. *Journal of Forensic Sciences.* 1986, Vol. 31, No.1, pages 329-32
	Bradley, MJ, Keagy RL, Lowe PC, Rickenbach MP, Wright DM, and LeBeau MA. **A Validation Study for Duct Tape End Matches**. *Journal of Forensic Sciences*. 2006, Vol. 51, No. 3, pages 504-508
	Christensen, A.M., Sylvester, A.D., **Physical Matches of Bone, Shell and Tooth Fragments: A Validation Study**. *Journal of Forensic Sciences.* 2008, Vol. 53, No. 3, pages 694-698
	Bradley, M.J. et al., **A Validation Study for Vinyl Electrical Tape End Matches**. *Journal of Forensic Sciences.* 2011, Vol. 56, No. 3, pages 606-611

	Laux, D. L., **Identification of a Rope by Means of a Physical Match Between the Cut Ends**. *Journal of Forensic Sciences.* 1984, Vol. 29, No. 4, pages 1246-1248
	Monahan, D., Harding, H., **Damage to Clothing – Cuts and Tears**. *Journal of Forensic Sciences.* 1990, Vol. 35, No. 4, pages 901-912
	Ogle, R., Mitosinka, G., **The Identification of Cut Multi-Stranded Wires**. *Journal of Forensic Sciences.* 1974, Vol. 19, No. 4, pages 865-867

Articulate and/or Provide Written Answers

Date	Tasks
	Define a class characteristic
	Define an individual characteristic
	Is a physical fit considered to be a conclusive identification
	What materials are suitable for physical comparison examination?
	List the essential information for documenting a physical fit
	What is the significance of a physical fit
	What is stretching, how can it complicate a comparison and result in an inconclusive match

Practical Exercises

Date	Tasks
	The trainer will demonstrate a physical fit of a plastic automotive lens to include viewing "on edge"
	The trainer will demonstrate a physical fit of a tape
	The trainer will review and discuss report wording for physical fit cases
	Prepare twenty practice physical comparisons using different techniques (ex. cutting with scissors and a scalpel, ripping, breaking) and using different materials such as duct tape, cloth, plastic, and paint. Document how each technique affects the different materials.
	Perform a physical fit comparison on the twenty prepared items, demonstrating proper documentation.

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Physical Fit Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Physical fit the pieces of at least two plastic automotive lens or similar plastic items, if possible; document using Form 1 or equivalent
	Physical fit the pieces of at least five test samples of paint fragments, if possible; document using Form 1 or equivalent
	Physical fit the pieces of at least five different tape samples, if possible, include at least 3 different types of tapes; document using Form 1 or equivalent
	Successfully completed requirements for one of the following:
	Trace Evidence Searching, Collecting & Documenting
	Microscopy

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Pressure Tape & Adhesive Analysis

This guide is intended to provide a trainee with the necessary skills to perform laboratory analysis of samples suspected to have at one time been a part of the same unit. The trainee must have completed the training module on Microscopy, FTIR, MSP, SEM and Physical Fit Analysis or be working on them concurrently with this training.

Goals

- To become familiar with the history and uses of tapes and adhesives
- To become familiar with tapes and adhesives terminology
- To become familiar with manufacturing processes for tapes and adhesives
- To become familiar with chemical formulations and compositions of various tapes and adhesives

Literature Reading

Date	Literature
	TR-150 Pressure Sensitive Tape Analysis
	Blackledge, R.D., **Tapes with Adhesive Backings: Their Characterization in the Forensic Science Laboratory**. *Conference Proceedings, International Symposium on Polymer Analysis and Characterization.* 1987, pages 413-421
	Maynard, P., Gates, K., Roux, C., Lennard, C., **Adhesive Tape Analysis: Establishing the Evidential Value of Specific Techniques**. *Journal of Forensic Sciences.* March 2001, Vol. 46, No. 2, pages 280-287
	Scientific Working Group for Materials Analysis, **Daubert Admissibility Package for Tape Evidence**. Tape Subgroup, April 2012, http://www.swgmat.org/Tape%20Admissibility%20Package.pdf (PDF downloaded 7-10-14)
	ASTM E3260-21. Standard Guide for Forensic Examination and Comparison of Pressure Sensitive Tapes
	Smith, J., **Forensic Examination of Pressure Sensitive Tape**. Forensic Analysis on the Cutting Edge. Blackledge, R.D., Editor, 2207, Chapter 12, pages 291-332
	Tsach,T., Wiesner, S., Shor, Y., **Empirical Proof of Physical Match: Systemic Research with Tensile Machine**. *Forensic Science International.* 2007, Vol. 166, pages 77-83

	Instrumentation
	Scientific Working Group for Materials Analysis, **Guideline for Assessing Physical Characteristics in Forensic Tape Examinations**. October 2013 Revision, http://www.swgmat.org/Assessing%20Physical%20characteristics.pdf (PDF downloaded 07-10-14)
	Scientific Working Group for Materials Analysis, **Guideline for Using Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations**. http://www.swgmat.org/Tape%20FTIR%20guideline.pdf (PDF downloaded 07-10-14)
	Scientific Working Group for Materials Analysis, **Guideline for Using Light Microscopy in Forensic Examinations of Tape Components.** http://www.swgmat.org/Tape%20Light%20Microcscopy%20guideline.pdf (PDF downloaded 07-10-14)
	Scientific Working Group for Materials Analysis, **Guideline for Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy in Forensic Tape Examinations**. http://www.swgmat.org/Tape%20SEM%20guideline.pdf (PDF downloaded 07-10-14)
	Smith, J.M., Weaver, R., **PLM Examinations of Clear Polymer Films: Identification of Monoaxial and Biaxial Orientation and Other Observations**. *The Microscope.* 2004, Vol 52, No. 3/4, pages 113-118
	Duct Tape
	Snodgrass, H., **Duct Tape Analysis as Trace Evidence**. *Proceedings of the International Symposium on Trace Evidence*. FBI Academy, June 1991, pages 69-73
	Smith, J., **The Forensic Value of Duct Tape Comparisons**. Midwestern Association of Forensic Scientists Newsletter. January 1998, Vol. 27, No. 1, pages 28-33
	Bradley, M.J., Keagy, R.L., et. al, **A Validation Study for Duct Tape End Matches**. *Journal of Forensic Sciences*. May 2006, Vol. 51, No. 3, pages 504-508
	Hobbs, A.L., Gauntt J, Keagy R, Lowe P. and Ward D., **A New Approach for the Analysis of Duct Tape Backings**. *Forensic Science Communications*. January 2007, Vol. 9, No. 1 (PDF: Tape)
	Jenkins, Jr, T.L., **Elemental Examination of Silver Duct Tape Using Energy Dispersive X-ray Spectrometry**. *Proceedings of the International Symposium on the Analysis and Identification of Polymers.* FBI Academy, July 31-Aug 2, 1984, Pages 147-149
	Courtney, M., **Evidential Examinations of Duct Tape**. *SWAFS Journal.* April 1994, Vol. 16, No. 1, pages 10-16
	Electrical Tape/PVC Tape
	Goodpaster, J.V., Sturdevant, A.B., Andrews, K.L., Brun-Conti, L., **Identification and Comparison of Electrical Tapes Using Instrumental and Statistical Techniques: I. Microscopic Surface Texture and Elemental Composition**. *Journal of Forensic Sciences*. 2007, Vol. 52, No. 3, pages 610-629
	Goodpaster, J.V., Sturdevant, A.B., Andrews, K.L., Briley, E.M., Brun-Conti, L., **Identification and Comparison of Electrical Tapes Using Instrumental and Statistical Techniques: II. Organic Composition of the Tape Backing and Adhesive**. *Journal of Forensic Sciences.* March 2009, Vol. 54, No. 2, pages 328-338

	Mehltretter, A.H., Bradley, M.J., Wright, D.M., **Analysis and Discrimination of Electrical Tapes: Part I. Adhesives**. *Journal of Forensic Sciences.* January 2011, Vol. 56, No. 1, pages 82-94
	Mehltretter, A.H., Bradley, M.J., Wright, D.M., **Analysis and Discrimination of Electrical Tapes: Part II. Backings**. *Journal of Forensic Sciences.* November 2011, Vol. 56, No. 6, pages 1493-1504
	Polypropylene Tape
	Sakayanagi, M., Konda, Y., Watanabe, K., Harigaya, Y., **Identification of Pressure-Sensitive Adhesive Polypropylene Tape**. *Journal of Forensic Sciences.* January 2003, Vol. 48, No. 1, pages 1-9
	Handling of Samples
	Choudhry, M.Y., Whritenour, M.P, Whritenour, R.D., **A New Approach to Unraveling Tangled Adhesive Tape for Potential Detection of Latent Prints and Recovery of Trace Evidence**. *Journal of Forensic Sciences.* November 1990, Vol. 35, No. 6, pages 1373-1383
	Teetsov, A., Stellmack, M.L., **Hand Sectioning and Identification of Pressure Sensitive Tapes**. *Proceedings of the Pressure Sensitive Tape Council TECH XXVII*. Orlando, FL, May 12-14, 2004

Articulate and/or Provide Written Answers

Date	Tasks
	General
	What is a polymer
	What is a pressure sensitive adhesive
	What is a pressure sensitive tape
	Briefly describe the differences between warp/machine, and weft/fill. When are these terms used
	Describe the different types of tape and how likely they are to be encountered in case work, include: a. Duct tape b. Electrical/vinyl tape c. Filament/strapping tape d. Packaging tape e. Paper tape f. Masking/acetate tapes
	Why are physical and microscopic comparisons so important in tape examinations
	What instrumental analysis can be used in tape comparison
	Explain why a physical fit is more probative than other conclusions
	Discuss the factors that affect the significance of pressure sensitive adhesive tape examinations.
	What conclusions can be drawn after a tape comparison

	What other types of evidence can be encountered on pressure sensitive adhesive tape
	Discuss, in general, pressure sensitive tape manufacturing process
	What is a tape additive and list at least 3
	What role does scrim fabric play in tape
	Describe the layers found in duct tape and how they can be used to differentiate samples
	Name the 2 most common scrim patterns and draw them
	What is a plasticizer and when might this be found
	What is a pigment and when might they be found
	What is an elastomer and list at least 5
	Collecting Tape Evidence
	Describe how to package tape evidence
	What might cause phthalate contamination in tape components
	Describe different ways of unraveling a wad of tape or removing it from a surface, and list advantages and disadvantages of each
	Stereomicroscopic Evaluation
	What are calendaring marks
	What are manufacturing marks and what influence do they have at this point in the examination
	What characteristics can be observed from a microscopic examination of tapes and adhesives
	How does one compare the colors of known and questioned tapes or adhesives under the stereomicroscope
	What influence does width have at this point in the examination
	How does one ensure the tape or adhesive samples will not be contaminated
	What characteristics cause tapes or adhesives to be eliminated at this stage of the examination
	PLM Evaluation
	What is the difference between monoaxial and biaxial
	How can PLM be helpful in the application to tape analysis
	FTIR Evaluation
	Describe sample preparation for tape and adhesive samples on the FTIR
	Are additives likely to be present in the FTIR spectrum
	What are the advantages and disadvantages of the FTIR analysis of tapes and adhesives

	SEM/EDS Evaluation
	Explain how to prepare samples for analysis on the SEM/EDS
	What is the smallest percentage of an element that can be generally detected by SEM/EDS

Tasks

Date	Tasks
	Collecting Tape Evidence
	Explain to the trainer the information given to an officer over the phone if asked how tape evidence should be collected and packaged in a home invasion case where the victim's hands and feet were bound with tape
	Explain to the trainer the information given to an officer over the phone if asked how tape evidence should be collected from the home invasion suspect's residence.
	Practice using various methods (i.e. methanol, chloroform, heptane, hexane, toluene, inverted Dust-Off, liquid nitrogen, a heat gun, freezer) on different classes of tapes (i.e. duct tape, electrical tape, packaging tape, masking tape, office tape) that have been stuck together. Record observations and results.
	Stereomicroscopic Evaluation
	At the stereomicroscope, the trainer will demonstrate/discuss color, width, thickness, and any other applicable observed characteristics of different tape samples. Demonstration by the trainer will include manipulation of tapes to make cross-sections. The Guideline for the Forensic Examination of Pressure-Sensitive Tapes will serve as a guide for this demonstration/discussion.
	The trainer will provide several tape samples to allow the trainee to familiarize themselves with the manipulation of tapes using the stereomicroscope. The trainee will use these tapes to record physical observations and measurements; a minimum of 5 samples
	The trainee will practice making cross-sections of several tape backings.
	The trainee will practice removing adhesive from tape
	The trainer will discuss removal/recovery of trace evidence from tape or adhesive.
	The trainee will be given tape test samples and they will be asked to fracture match the pieces, if possible. A minimum of 5 comparisons.

	PLM Evaluation
	Record physical observations, observations made on the PLM, and observations when visualized between crossed polarizing sheets to determine if samples match, a minimum of 5 samples
	FTIR Evaluation
	Obtain and compare FTIR spectra from the components of 5 samples
	SEM/EDS Evaluation
	Analyze set of: one known and one questioned tape and/or adhesive samples

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Requirements Prior to Pressure Tape & Adhesive Analysis

Sample Analysis & Instrumentation

Date	Tasks
	Obtain several types and brands of adhesive tape, document physical characteristics, microscopic characteristics, obtain and compare infrared spectra of tape components, and obtain and compare SEM/EDS data. The types of tape should include, at a minimum, five samples of each of the following types of tape: a. duct tape b. electrical tape c. filament tape d. packaging tape e. masking tape f. paper tape g. acetate tape Document using Form 1 or equivalent.
	Analyze 5 practice cases and record all results; document using LabDev .
	Successfully completed requirements for the following instrumentation:
	Microscopy
	FTIR
	MSP
	SEM/EDS

Evaluation of Training

Date	Tasks
	Complete and review a competency test, with accurate results
	Complete a technical questions session with a minimum score of 80%, with Technical Manager or Appointee Average Score _____
	Complete a mock trial session, with approval from Technical Manager (*not required, if previously successfully passed mock trial in another area*)

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Administrative and Technical Reviewing of Casework

Goals:

- To provide the trainee with knowledge and skills necessary to perform Administrative and Technical Reviews on another Analyst's casework

Each case type (i.e. Ignitable Liquids, Fibers, Paint, etc.) requires an authorization to perform Technical Reviews for the specific case type. Requirements for performing case reviews (AR & TR) will follow QP 31. Training will consist of working with trainer to observe reviews (minimum of 2 cases) and then the trainer will observe the trainee performing Administrative and Technical Reviews of a minimum of 5 cases.

Literature Reading

Date	Literature
	Read OSBI QP 31, Reviews
	Read Trace Quality Manual Section 10- Case Reviews

Tasks

Date	Tasks
	Discuss with Trainer how to perform a Technical Review of a Trace Case
	Observe Analysts perform Admin/Tech Reviews (5 cases each Analyst)
	Name of Analyst 1:
	Name of Analyst 2:
	Name of Analyst 3:

Upon signing the approval, the trainee and trainer will review the above information and ensure the trainee has demonstrated knowledge and understanding of the above topics.

Approval

Trainee _____ Date _____

Trainer/
Supervisor _____ Date _____

Comments _____

Mock Trial Evaluation Form

Analyst _____ Score _____

Reviewer _____ Date _____

Please rate the trainee's performance during the Mock Trial:

	Excellent (3)	Good (2)	Fair (1)	Poor (0)
Courtroom demeanor and appearance	☐	☐	☐	☐
Ability to convey information in an understandable manner	☐	☐	☐	☐
Poise and professionalism during direct examination	☐	☐	☐	☐
Poise and professionalism during cross examination	☐	☐	☐	☐
Use of court exhibits/visual aids (if applicable)	☐	☐	☐	☐
Testimony based upon scientific principles	☐	☐	☐	☐
Exhibition of knowledge of OSBI testing procedures	☐	☐	☐	☐
Explanation of results	☐	☐	☐	☐

Remarks/Comments/Suggestions/Explanation for Poor Ratings: _____

_____

_____

_____

Appendix IV – Practical Exercises

Record of Practical Exercises

(note: some numbers have been omitted)

Exercise #	Section	Satisfactory Practical Exercise Completion	Trainee Initials and Date	Trainer Initials and Date
1	Evidence	Fiber Transfer and Persistence		
2	Evidence	Collecting Fibers on Tape		
3	Microscopy	Familiarization with the Stereomicroscope		
4	Microscopy	Familiarization with the Compound Light Microscope		
5	Microscopy	Familiarization with the Polarized Light Microscope		
6	Microscopy	Fiber Manipulations		
7	Microscopy	Observing Effects of Mounting Media		
8	Microscopy	Observing Fiber Shape, Surface and Internal Structure		
9	Microscopy	Observing Color and Pleochroism		
10	Fibers	Distinguishing Natural and Manufactured Fiber Classes		
12	Fibers	Determining Natural Fiber Twist		
16	Microscopy	Determining the Sign of Elongation		
17	Microscopy	Measuring Fiber Birefringence		
18	Microscopy	Measuring Fiber Refractive Indices by the Immersion Method		
19	Microscopy	Cross-sectioning Fibers and Interpretation of Cross Sections		
22	Fibers	Sample Preparation for FTIR-Microscopy		
24	Fibers	Interpretation of Fiber FTIR Spectra		
25	Fibers	Using the Comparison Microscope with Brightfield Illumination		

29	MSP	Microspectrophotometer Setup and Operation		
30	MSP	Acquiring Spectra from Single Fibers		
31	MSP	Acquiring Known Spectral Sets and Comparing Spectral Curves		
32	MSP	Examining Metameric Fibers		
33	Fibers	Examining Fabric Damage		
34	Fibers	Environ., Chemical and Mechanical Effects on Fabrics		
35	Fibers	Composition and Physical Construction of Natural and Manufactured Fiber Cordage		
36	Fibers	Environ., Chemical and Mechanical Effects on Natural and Manufactured Fiber Cordage		

Practical Exercise 1

Subject: Fiber Transfer and Persistence
Time: 4 hours
Objective: To learn some of the factors which contribute to fiber transfers and recovery

Theory:
Forensic fiber examinations are conducted to determine if potential associations exist among people, places or things. Many factors affect whether there is a fiber transfer and whether there is a successful recovery of the fibers. The amount of friction between donor and recipient surfaces, the tenacity with which a donor substrate holds its fibers and the physical characteristics of the individual fibers are some of the factors which determine whether a transfer occurs.

The presence of microscopic niches or binding fibers in a recipient substrate (to hold the foreign fibers), the presence of adhesive materials (blood, semen, saliva, gum, etc.) on donor or recipient materials, and the activity of the recipient after transfer are some of the factors which influence the successful recovery of a transferred fiber. It is possible to stage a few elements of an abduction scenario in order to learn some of the dynamics at work in fiber transfer and recovery.

Preparation:
You will need an extra set(s) of clothing for the day you perform this exercise.
Materials:
- vehicle with carpeted flooring or floor mats
- clean white T-shirt, clean pair of white athletic socks, a pair of shoes with an irregular sole pattern (athletic) and any style pants
- change of clothes to wear before/after phases of this exercise
- solid colored blanket made of acrylic, polyester or wool fibers
- stereomicroscope and compound light microscope
- glass microscope slides, cover slips, fine forceps
- Permount or other mounting media
- xylene substitute or water
- at least 5 paper bags large enough to hold each clothing article, blanket, and drop-paper as individually packaged items
- coin envelopes
- suitable adhesive tape and taping supplies, and Post-Its
- clean white butcher paper to cover examination tables

Directions:
Dress in the white T-shirt, socks, and shoes. Spread the blanket entirely over a passenger seat in the vehicle (=suspect vehicle). Sit on the blanket and squirm in the car seat (i.e. scrape the T-shirt back against the blanket). Remove the shoes and rub your socked feet against the vehicle's carpeted floor. Visually examine the shoe soles for any loose fibers. Put the shoes back on and scuff the shoes against the vehicle carpeted floor. Again take a moment to inspect the shoe soles while still in the vehicle seat to look for possible fibers from the floor that may be on the shoes. Do not collect any observed fibers at this time.

Exit the vehicle and enter an appropriate dressing area. Remove the T-shirt, shoes, and socks over a clean sheet of paper and package each clothing item separately in paper bags. Likewise, package the drop-sheet of paper.

Dress in your spare clothes after removing the evidence clothing.

Simulate the recovery of evidence from the suspect vehicle. Return to the vehicle with your evidence collection supplies including paper bag(s), coin envelopes, forceps, and adhesive taping supplies.

Properly document, collect and package:
- the blanket in the paper bag
- sample of the vehicle floor carpet in the coin envelope
- perform tapings of the car seat on which you sat

Return to the laboratory being careful to keep the known vehicle carpet sample well away from the evidence clothing articles and the blanket.
Hold all evidence for use in Practical Exercises 2 and 7-1.

Observation:
It is generally expected in this simulation that vehicle carpet fibers will be found on the socks due to the relative protection in the shoe after contact, and that blanket fibers will be found on the T-shirt. Any sticky material on the shoe soles or cracks in the sole material may hold carpet fibers that persist through the stages of this simulation. It is also expected in this simulation that extraneous fibers will be found on the socks and T-shirt that do not appear to have originated from any of the known fiber sources (carpet or blanket).

Discussion:
A very important factor in fiber persistence is the wearing time of a garment after fibers have been transferred onto it. Because the time from the contact in the car to the recovery of the evidence clothing and blanket is kept to a minimum in this exercise, fiber persistence should be high.

The trainee may want to perform simulation variations in which there are some additional activities between time of contact in the car and evidence collections. Also, alternative methods for evidence collection (e.g. fiber picking and vacuuming as opposed to tapings) may be used.

Practical Exercise 2

Subject: Collecting Fibers on Tape
Time: 3 hours
Objective: To learn a rapid and effective fiber collection method by tape-lifting

Theory:
Textile fibers are easily transferred when contact occurs between two individuals or between an individual and a crime scene, making fibers an important source of evidential material in crimes of violence and theft. The importance of establishing association depends on the circumstances of the case and is determined through discussion with the investigator. If association is admitted, or if two individuals normally would have been together due to circumstances unrelated to the crime, then there is generally no need to apply the taping procedure.

In attempting to establish whether or not transfer of fibers is likely to have occurred, the following criteria should be considered:
• the "sheddability" of the source and donor items
• the types of fibers composing the source and donor items
• whether the items are damaged, thus exposing fibers for transfer

Evaluate and first examine those garments and fibers which are likely to be of most value. Garments composed of highly sheddable fabrics and fibers should be considered first as a source of donor fibers, and garments of low sheddability should be examined first as a source of target fibers. In general, colored garments, garments composed of less common fiber types, and garments having a coarse texture, yield fibers with a greater number of comparable characteristics. Damaged garments are a good source of donor fibers. Fibers which fluoresce under ultraviolet light make good suspect fibers when the garment to be taped does not fluoresce or fluoresces to a lesser extent.

In addition to garments, other objects such as vehicle parts in hit and run accidents, window ledges in breaking and entering cases, and unclothed bodies in homicide cases can also be taped for fibers.

Preparation:
Separate the items to be taped by source i.e. victim, suspect and scene. Ensure that each item you have been given is properly sealed in its own container. Items from different sources should be taped in different rooms to ensure that airborne transfer of fibers between sources does not occur. A different laboratory coat must be worn for taping items from different sources. Clean the examination tables in each room thoroughly.

Materials:
- three clean laboratory coats
- disposable gloves
- several acetate document protectors, or equivalent
- adhesive tape
- permanent marker
- brush
- two clothing items reportedly from the victim
- two clothing items reportedly from the accused
- one bed sheet from the scene
- the packaged evidence items you collected during Practical Exercise 1
- evidence packaging material

Safety:
Disposable gloves must be worn to avoid possible contact with body fluids present on the clothing items to be examined.

Directions:
Collect control tapings of the table surface prior to opening any of the items to be taped. Label an acetate sheet with the proper markings and discard the exposed portion of tape from the tape dispenser. Remove approximately eight inches of tape, holding each end between index finger and thumb. Press the tape firmly against the table surface in several areas until the tape has lost most its stickiness. Press the tape onto the interior portion of the acetate sheet. Repeat the procedure until all areas of the table have been sampled.

Brush off the outside of the item container to remove adhering contaminant fibers prior to placing it on the clean examination table. Remove the item from its packaging and spread it out on the table. Prepare acetate sheets with the appropriate markings for each area to be taped. Systematically tape the entire garment by area following the basic tape handling procedure as described above. Once taping is complete, sample a portion of the garment for use as a known sample by cutting out a square piece of fabric and retain it in a properly sealed and labeled container.

Return the item to its packaging ensuring that it is properly resealed. Clean the examination table thoroughly, change gloves, and repeat the procedure for the other item from the same source. Remove your laboratory coat when completed and leave it in the examination room.

Repeat steps 1 through 3 to examine and tape the items from the other sources. Each source should be examined in a separate room, using new and clean laboratory coats for each room.

Prepare acetate sheets for comparison purposes containing fibers on tape taken from the known samples.

Examine your tape-lifts visually and with a magnifying glass. Do you see any evidence of sample contamination?

Once you are confident that you can perform the tape-lifting technique without contamination and loss, repeat this exercise using the questioned and known samples that you collected while performing Practical Exercise 5-1. Examine your tape-lifts visually and with a magnifying glass. Do you see preliminary evidence of possible fiber transfers?

Save all of your tape-lift samples for use in Practical Exercise 6.

Observations:
If the examination table surface was properly cleaned before starting, then the control tapes should be almost completely fiber free. If a paper towel or cotton rag was used to wipe the table surface, then a small amount of fibers corresponding to the towel or rag will be observed on the control tapes.

Observing the tapings from the clothing and blanket should reveal a medium to high number of fibers adhering to the tapings. The bulk of these fibers will be from the fabric that composes these articles, with transferred fibers being present in much smaller quantities. A thorough examination utilizing a stereomicroscope will reveal the transferred or target fibers, which can then be removed from the tapes for analyses.

If you performed Practical Exercise 1 and attempted to collect fibers by methods such as picking and vacuuming, then generally compare the efficiency and discrimination capabilities of the picking, taping and vacuuming recovery methods.

Discussion:
Tape-lifting is generally a rapid and efficient method for recovering transferred fibers. Single fibers may appear colorless when viewed under a stereomicroscope. Different colored backgrounds (usually black and white) and different illuminating conditions should be used to make the target fibers more visible.

Other fiber recovery methods can be used and the choice of method is based, in part, on the circumstances of the investigation. Picking single fibers during a visual search is highly discriminatory but can be time consuming. Vacuuming is rapid but so indiscriminate that use of this technique is often discouraged.

Practical Exercise 3

Subject: Familiarization with the Stereomicroscope
Time: 4 hours
Objective: To learn the basic components, limitations and value of the stereomicroscope

Theory:
An initial step in the preliminary examination of evidence should include an overall viewing of the items in question. This can be accomplished through stereomicroscopical examination. In contrast to the restricted stage-objective distance with compound microscopes, the open stage area of the stereomicroscope allows greater flexibility for viewing large samples. Evidence can be freely rotated and moved to obtain information from all angles. The lower magnification of a stereomicroscope also provides a greater viewing area of the evidence offering an opportunity to put the damaged or altered area in perspective with the item as a whole. Objects viewed through the stereomicroscope are not reversed as with the compound microscope. Viewed items will retain their three-dimensional characteristics under stereomicroscopical examination due to separate optical systems for each eye. These qualities in conjunction with the reflected light source will allow viewing of the evidence in its naturally perceived state, varied only by magnification. The low level of magnification still allows fiber characterizations, such as reflected light color, crimp, length, relative diameter and damage. In addition, it allows the discrimination of natural, synthetic or inorganic fibers. Yarn and fabrics can also be examined for basic construction.

Preparation:
Take one (1) five-inch square of the knit fabric and snugly fit it over the head of a hammer. Secure the fabric by twisting a rubber band several times around the hammer neck. With the fabric secured, forcefully hit the fabric-covered hammerhead onto a painted surface at an angle of approximately 45 degrees. Repeat striking, if necessary, until an impression is visible. Repeat the procedure with woven fabric sections, to include plain and twill.

Materials:
- Stereomicroscope
- Blunt tip forceps
- Dissecting needles or sharp tip forceps
- Rubber bands
- One (1) five inch square section of dyed plain woven fabric sample
- One (1) five inch square section of dyed woven twill fabric sample
- One (1) five inch square section of knit fabric sample
- Painted surface (section of car, bicycle or wood)
- Hammer

Safety:
Use standard laboratory safety procedures according to the rules prescribed by your laboratory. Use caution when wielding the hammer; make sure your hands and other people are not within striking range.

Directions:
If there is a need to move the stereomicroscope for use, then carry the microscope with one hand supporting the base and the other hand holding the arm.

Place the hammer head on the microscope stage and view for possible fiber transfer. If the light source is free standing, then the angle of the light path may be changed to provide better visualization.

Remove the hammer and place one of the fabric sections onto the stage area inside the circle of light. Higher quality stereomicroscopes are equipped with a zoom lens system or a rotating drum containing multiple objectives that are used to increase and decrease overall magnification. Select an objective magnification. Your selection will depend upon the detail necessary for the examination.

Note the construction of the fabric. Move the fabric around on the stage until all areas have been viewed. Flip the fabric over and repeat your observations.

Using blunt tweezers to hold the fabric down, take the dissecting needles or sharp tip forceps and push apart the yarns at the cut edge. Note the direction of twist for the yarn. Note whether all fibers appear similar within the yarn.

Separating out a single fiber, note the color and compare this to your color interpretation for the complete piece of fabric.

Move the fabric section around on the stage to view the damaged area. While changing the magnification, observe and record the damage to yarns/fibers and the impression made. Take measurements to compare with the measurement of the hammer head. Note the degree and form of paint transfer.

Repeat with the remaining fabric sections.

Observations:
The color hue of an individual fiber may appear the same as the color for the whole fabric sample when using stereomicroscopic magnification. But, this is not necessarily true in all instances, especially with lightly colored fabrics. Fibers from fabrics that were dyed or printed after construction will show “white” areas where dye was blocked due to the overlapping of another

fiber. Fibers that were dyed prior to construction will show a continuance of color over the length of the strand. If the fabric was printed, then there will be a multitude of color changes along the fiber length.

Woven fabric damage occurs through all yarns. Did the fabric damage created by the hammer head result in a crescent shaped separation? The yarn tips appear frayed and uneven, an example of blunt damage. The damage with knit fabrics results in “holes” or “thinning” due to the reinforced layering of yarns from the knitting stitch. In part, the amount of force used will determine whether sufficient detail is present for comparison between the damaged area and the hammer.

Paint transfer does occur, and can be viewed on the individual fabric surface yarns or embedded in the fabric.

Discussion:
The stage area of the stereomicroscope provides an excellent tool for gross observations. Large items are easily handled, and collected debris samples from clothing scrapings or crime scene recovery can be readily searched. At this point any noted debris attached to the fabric, yarn or fiber can be used to determine the direction of the examination. Minute traces of paint, grease, glass, soil or other debris may provide invaluable associative information overlooked by the investigating agency. This debris may also be used in conjunction with the fiber analysis to associate a questioned sample to a known source.

At this level of stereomicroscopic examination, it may be determined that the evidentiary fiber exhibits characteristics (such as the color, diameter, crimp or adhering debris) that are significantly different from the known source and, thus, will eliminate the need for further examination.

Fiber color represents a readily apparent trait allowing easy fiber recognition and recovery. The eye naturally is attracted to bright or different color hues and because of this, manufacturers provide considerable variability in fiber colors.

Fabric construction (woven, knit or pressed) can be compared to evidentiary fabric pattern transfers as may occur in hit-and-run cases, or in cases involving contact transfer with wet fabric. The stereomicroscope is an invaluable resource when conducting a physical fit. A questioned section of fabric or cordage is aligned with the known fabric or cordage through systematic demonstration of yarn absence or extension from one piece to another.

The fiber examiner should recognize the importance of basic microscopical examination. While increased magnification is important for fiber examination, many informative characteristics can

be obtained from stereomicroscopic examination, including characteristics that would be missed if fibers were to be immediately prepared for instrumental analysis.

Practical Exercise 4

Subject: Familiarization with the Compound Light Microscope
Time: 6 hours
Objective: To learn the proper procedures for correct illumination of a compound transmitted light microscope and calibrating the ocular micrometer

Theory:

In order to obtain the best resolving power and specimen contrast that your microscope will allow, proper illumination of that instrument must be obtained. The most readily accepted technique to obtain this is Köhler Illumination. This technique is based on positioning and alignment of the various optical elements of your microscope (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 or an equivalent in place). In the orthoscopic view with good Köhler Illumination, the field diaphragm, specimen and ocular front focal plane (cross hairs or micrometer) are simultaneously in good focus on the retina and centered on the microscope axis. In the conoscopic view with good Köhler Illumination, the lamp filament, substage aperture diaphragm, objective back focal plane and ocular back focal plane are simultaneously in good focus on the retina and centered on the microscope axis.

Many modern microscopes are now equipped with a ground glass diffuser in the illumination system so that the lamp filaments cannot be observed and are usually not adjustable. Therefore, true Köhler illumination cannot be obtained.

The measurement of particle size is an important tool for microscopists. Measurements of fiber diameter can be used, in part, to determine fiber generic class and end uses, and is a physical feature used in fiber comparisons. Fibers and other particles can be accurately measured by using the linear scales (micrometer) in the microscope ocular. However, the scales must be calibrated before use.

Preparation:

Obtain the following premounted fiber samples from your trainer:

- Colorless, round, moderately delustered polyester
- Dyed, round, moderately delustered polyester
- Colorless trilobal nylon
- Dyed trilobal nylon

Materials:

- Compound transmitted light or polarized light microscope
- Microscope objectives of various magnification (e.g. 4x,10x, 20x, 40x and 100x)
- A focusing eyepiece with micrometer scale
- A stage micrometer (2mm long with intervals = 0.01mm)
- Mounted fiber samples

Safety:

Use standard laboratory safety procedures according to the rules prescribed by your laboratory. Be cautious of the microscope level of illumination to avoid eye damage until you are familiar with the variable light intensities.

Directions:

Part 1--Köhler Illumination

Place a mounted fiber sample onto the microscope stage and focus on the sample with a 10X objective. Set the light intensity at the suggested operating voltage. Depending on the features of the instrument you are using, the microscope may have a centerable rotating stage and/or centerable objectives. If the microscope has a centerable stage, then it should be centered at this time. If the microscope has centerable objectives, then they should be centered at this time. Objectives held in a rotating nosepiece should be moved into the viewing position by grasping the ring of the rotating nosepiece and not the objectives themselves. Why?

Using the 10X objective, close the field diaphragm and focus on diaphragm edges by adjusting the substage condenser focus control knob.

Center the field diaphragm image by centering the condenser with its centering screws. Open the field diaphragm to just beyond the field of view.

Remove the fiber from the optical path.

If you have a Bertrand lens, then insert it. If you do not have a Bertrand lens, then you can simply remove one ocular to see the objective back focal plane.

Focus the image of the lamp filament by moving the lamp fixture back or forth along the lamp axis relative to the lamp condenser. The lamp filament should also be centered by using the adjustment knobs on the lamp housing if so equipped, or by manually adjusting the lamp housing. If your microscope is equipped with a ground glass diffuser, then you will not be able to focus on the lamp filament. Some diffusers may be removable.

Remove the Bertrand lens (or replace the ocular) and again focus on your fiber sample. Use the substage aperture diaphragm to adjust the contrast and resolution to optimal appearance. Do not use this diaphragm to adjust light intensity. Adjusting the light intensity should be done with the light intensity rheostat, or use a neutral density filter.

Record your observations about the fiber including color, structure, and clarity of the detail.

Change your magnification. Adjustments, by the opening or closing of the field diaphragm and the substage diaphragm, should be performed as needed when changing to a different magnification objective. Again examine and record your observations about the fiber including color, structure, and clarity of the detail.

Repeat this process at the various magnifications available on your microscope, and repeat this procedure with the other mounted fibers.

Rack down the condenser and fully open the substage iris diaphragm. Repeat your observations of the mounted fibers and compare the visible detail to that seen under the proper lighting conditions.

Part 1 - **Observations:**
Record and diagram your detailed observations of each fiber type, at various magnifications, with the microscope illumination adjusted properly and with the microscope illumination adjusted improperly. There will be a significant difference in what you are able to discern.

Part 2- Basic Micrometry and Calibrating an Ocular Micrometer
Remember to return your microscope to proper Köhler illumination. Calibration requires comparison of the unknown ocular scale with a stage micrometer having known dimensions.

Line up the stage micrometer and ocular scales. It may be easier to read the scales if they are not directly overlapping, but slightly offset. Focus.

Using as much of each scale as possible in order to increase accuracy:

- Count the number of large divisions of the stage scale (ssd).
- Count how many ocular scale divisions (osd) equals the above stage scale divisions (ssd).

Knowing that each stage scale has divisions 100 micrometers (0.1 mm) apart, calculate the ocular scale divisions for that objective by using the following formula:

Observe # osd = # ssd x 100 µm

or

1 osd = #ssd x 100 µm / # osd

The number generated from this equation will be the calibrated measurement for each division of the ocular micrometer for that specific objective. Record your calculated result.

Repeat steps 1 through 3 for each microscope objective. You should repeat this entire procedure for each microscope you may use. For convenience, keep the calibration values posted nearby each microscope.

Take each of the 4 mounted fiber slides in turn, and measure the fiber diameter using the calibrated ocular micrometer at each available magnification. Properly document each measurement including the magnification used, the observed number of ocular scale divisions and calculation/conversions to microns. Evaluate the data by comparing diameter measurements taken at different magnifications.

Part 2 - **Observations:**

Microscope Identification: _____

Objective	#osd	#ssd	#ssd x 100 µm/ #osd	1 osd (µm)
4X				
10X				
20X				
40X				
100X				

Properly document each fiber diameter measurement including the magnification used, the observed number of ocular scale divisions and calculation/conversions to microns. Did you get the same diameter value at each magnification for a given fiber? Why? How did you measure the diameter of the trilobal fiber?

Discussion:

The resolution of detail observed in a microscopical image depends on the illuminating conditions of the microscope, as well as the condition of the microscope optics, the specimen contrast, and the quality of the human eye.

The calibration of the eyepiece micrometer remains the same unless there is a change in the objectives, tube length, or other part of the microscope that would result in a magnification change. Generally, this calibration procedure needs to be done only once for each microscope. However, your laboratory's quality assurance policies and procedures may require periodic calibration checks. Calibration or calibration checks may be necessary more often particularly if you are not the sole user of a particular microscope and/or objectives are moved among microscopes in your laboratory. The accuracy and precision of your fiber diameter measurements depend on a number of factors including proper microscope illumination for the best resolution and fiber contrast, the magnification at which you are taking measurements, your ability to focus in the proper plane for taking the measurement, and your ability to perceive the cross-sectional shape and points of maximum or minimum diameter from the longitudinal view.

Practice measuring "thickness" in fibers that are not of round cross-sectional shape such as flattened (ribbon-like), kidney-bean shaped, and irregular. Fiber cross-sectional shape and thickness are important discriminating factors in and of themselves. Additionally, fiber thickness influences other optical properties used in comparisons.

Practical Exercise 5

Subject: Familiarization with the Polarized Light Microscope
Time: 6 hours
Objective: To become familiar with the basic components of a polarized light microscope, and to make preliminary observations of Becke lines, interference colors and extinction points

Theory:

In the ordinary polarizing method, the microscope system is quite simple. A “polarizer” is placed below the sample, usually in a rotatable carrier just beneath the condenser. An “analyzer” is placed above the specimen, typically at the back of the objective lens. There is no difference between the polarizing elements of the polarizer and the analyzer. They are only given different names to distinguish their location in the microscope. They are most frequently used in a crossed position (“crossed polars”) with their vibration directions perpendicular to one another so that the field of view, when no sample is present, appears dark. However, when a transparent sample such as a fiber is present in the field of view and the microscope’s polarizer and analyzer are crossed, the fiber may interact with the beam and appear bright with colors on a dark background, or cannot be seen at all (the field remains dark) when viewed in all orientations of polarized light.

Fibers (and other transparent substances) that interact and appear colored at some orientation between crossed polars are termed “anisotropic”. Anisotropic substances have two or more “refractive indices”. Synthetic fibers typically have two refractive indices. Polarized light allows two views of a fiber: (1) where the optical axis of the fiber is parallel to the orientation of the polarizing material, and (2) where the optical axis of the fiber is perpendicular to the orientation of the polarizing material. This creates two different refractive indices termed “n-parallel” and “n-perpendicular”. The refractive indices of the axes are helpful in identifying the generic classes of fibers. Fibers which are aligned with either filter’s orientation appear black. This is because the light passing through them is aligned with either of the filters. These positions are called “extinction positions”.

Some substances have no effect on a polarized light beam, regardless of their orientation, and will remain dark between crossed polars. These substances are called “isotropic”. Isotropic substances have only one refractive index, meaning that light travels through the material at the same speed in all directions.

It is important to remember that interference colors are intrinsic to anisotropic samples. This is how samples can be characterized using polarized light. The interference colors are determined by both the thickness of the sample and the degree of anisotropism or “birefringence”.

For a particular transparent medium, the ratio of the speed of light in a vacuum to the speed of light in that medium is termed its “refractive index”. Fibers vary in shape, but are almost always thicker in the center than near the edges. As a result, they act as crude lenses, either concentrating or dispersing the light that passes through them. If a fiber has a higher index than the mounting medium, then it acts as a converging lens and concentrates light within the fiber image. If the fiber has a lower index than the mounting medium, then it acts as a diverging lens and the light rays diverge from the fiber.

In most fibers, the light rays only slightly converge or diverge and thus appear as a thin bright line called the “Becke line” at the interface between the fiber and the mounting medium. The Becke line resembles a halo and is seen when the microscope is focused through best focus. The Becke line is observed with only the polarizer in place (the analyzer is not in the light path) which allows it to be illuminated with plane-polarized light. To examine the refractive index parallel to the length of the fiber (n-parallel), rotate the fiber until its length is parallel to the vibration direction of the polarizer. To examine the refractive index perpendicular to the length of the fiber (n-perpendicular), rotate the fiber until its length is perpendicular to the vibration direction of the polarizer. If the fiber is mounted in a liquid that shares the same refractive index as the fiber, then no Becke line will be visible and the fiber edges will become “invisible”.

In summary, if the fiber has the higher refractive index, then the Becke line moves toward the fiber as the working distance is increased. If the mounting medium has the higher index, then the Becke line moves toward the medium (away from the fiber) as the working distance is increased. The Becke line moves toward the medium of higher refractive index as the working distance is increased. Therefore, using the mounting medium as a reference, fibers can be classified as having a refractive index of greater than or less than the medium in which they are mounted by observing the Becke line behavior.

Preparation:

It is necessary for polarized light microscopes to have accessories that are not standard on ordinary transmitted light microscopes. For this practical exercise, the important features are two removable polarizing filters and a rotatable stage. The polarizer is located between the light source and the sample, usually just beneath the condenser lens. The analyzer is above the sample, somewhere between the sample and the eye. At least one of the polarizing filters should be capable of being rotated so that the crossed polar position can be set at extinction (dark field of view). The rotatable stage is necessary so the specimen can be oriented for maximum brightness between the crossed polars. Typically, fibers are examined in the diagonal position

between the perpendicular polarizing filters, at a 45-degree angle to both filters. For later practical exercises, it will be necessary to locate additional features such as an accessory slot placed at a 45-degree angle to the plane of polarization for both the polarizer and the analyzer above the objective lens, and a filter or slot for a filter located in the light path before the condenser lens to allow for the production of monochromatic light.

Locate the following components on your polarized light microscope: light source, filter, polarizer, condenser lens, rotatable stage, objective lens, accessory slot, analyzer, eyepiece. Discuss the function of each of these components with your trainer.

Materials:

- microscope with polarized light capabilities
- the image capture system used in your laboratory for taking photomicrographs
- mounted samples of undyed nylon, polyester, olefin, rayon, acrylic, acetate, cotton, silk

Directions:

Examine any four of the manufactured (synthetic) fiber samples at 200X under the following conditions and note your observations:

- with both the polarizer and analyzer removed
- with the polarizer in place and the analyzer removed
- with the polarizer removed and the analyzer in place
- with the polarizer and analyzer in the crossed position
- with the polarizer and analyzer parallel to each other

Examine the nylon and cotton fibers. While viewing each sample, rotate the movable polarizing filter 360 degrees slowly and note your observations.

Perform the following tasks for each part and record your observations on the chart provided in the Observations section of this exercise. Take photomicrographs to document your observations.

Part 1 - Refractive Index by Becke Line Observations
Determine the orientation of the polarizer on your microscope.

Using the mounted nylon fiber, place the fiber parallel to the orientation of the polarizer (analyzer should be out).

Increase the working distance between the stage and the objective lens (focus up). Observe if the Becke line moves towards the fiber or away from the fiber.

Record your observation in the n-parallel column as follows:

- If the Becke line moves AWAY from the fiber, then record observation as "<".
- If the Becke line moves TOWARD the fiber, then record observation as ">".
- If there is no Becke line, then record as "=".

Rotate the fiber perpendicular to the orientation of the polarizer and record the refractive index (as <, >, or =) again in the n-perpendicular column=.

Repeat this procedure with the remaining fibers and record your observations.

Part 2 - Observation of Extinction Positions and Interference Colors in Crossed Polars
Using the mounted nylon fiber, align the fiber with the orientation of the polarizer.

Place the analyzer in position (the field of view, including the fiber, should appear black).

Rotate the fiber 90 degrees and view again (the field of view should still be black).

- Does the fiber go to extinction?
- Record "Yes" or "No" in the extinction positions column.

Place the fiber at a 45-degree angle (in the diagonal position between the crossed polars). The fiber should appear to have colors or be light gray, the fiber should not be completely dark. Describe and record the appearance of the colors (their brightness as dull to vivid, location, etc.) you observe in the fiber in the interference colors column.

Repeat these steps and record your observations of the remaining fibers.

Observations:

Fiber Type	n-Parallel	n-Perpendicular	Extinction Positions	Interference Colors
Nylon				
Polyester				
Olefin				
Rayon				
Acrylic				
Acetate				
Cotton				
Silk				

Discussion:

Because a polarizing filter only allows light to travel through it in a single plane, polarizing filters that are placed at right angles to each other do not permit any light to pass and the field of view will appear black. The lenses of Polaroid sunglasses have this effect. You can demonstrate for yourself that this is true with two pairs of Polaroid sunglasses or two polarizing lenses from your polarizing microscope. If the two lenses are placed one on top of the other so that the axes of polarization coincide, then light passes through both normally. If one lens is rotated 90 degrees with respect to the other, then no light passes through (field of view goes dark). This is the same concept that makes polarized light microscopy useful in forensic laboratories.

You should be able to answer the following questions:

- Is human vision capable of differentiating between ordinary light and polarized light?
- What effect does crossing the orientation of two polarizing filters have on the field of view through the filters?
- What are the necessary components of a polarized light microscope?
- How would you differentiate between isotropic and anisotropic transparent samples?
- What is observed when an anisotropic substance is placed between crossed polars and rotated? Why?

- What is observed when an isotropic substance is place between crossed polars and rotated? Why?
- What is the Becke line method?

Practical Exercise 6

Subject: Fiber Manipulations: Removing Fibers from Tape and Mounting
Time: 5 hours
Objective: To learn best practices for selecting and removing target fibers from tape, and to become familiar with microscope slide preparation techniques

Theory:

Fibers may be transferred when individuals come in contact with each other and with crime scenes. Fibers are an important source of associative physical evidence. This is especially true if a number of similar fibers or a number of different fiber types and colors are identified in a one-way transfer, or if two-way transfers of fibers are established. Target fibers can be easily identified on tapings using stereomicroscopy, and these can easily be retrieved from the tapings for subsequent analysis. Once retrieved from tapings, fibers are mounted on microscope slides for examination and comparison. Details of fiber morphology, color and fluorescence can be accurately visualized when fibers are examined in an appropriate mounting medium. A variety of mounting media are available for these purposes. Fibers may be preserved and stored for a long period of time when mounted in the appropriate medium.

Materials:

- Stereomicroscope
- Compound light microscope
- Scalpel
- Dissecting needles and fine tip forceps
- Slides and cover slips (25 mm x 40mm suggested)
- Mounting medium (use one of the media typically used in your laboratory)
- Spot plates
- Xylene or equivalent
- The tape-lifts that you made in practical exercise 2

Safety:

Know the hazards associated with the mounting media and chemicals used, and handle them according to the rules prescribed in your laboratory. Always clean your forceps with disinfectant and change your scalpel blade prior to beginning any procedure.

Directions:

Examine the known sample tapings collected in Practical Exercise 5-2 under the stereomicroscope. Familiarize yourself with the color, shape and size of the known fibers.

Scan each questioned fiber taping collected in Practical Exercise 5-2 for fibers from the other two sources. Search for one fiber type at a time and use the known sample tapings as a backdrop for comparison purposes. For optimum efficiency and good discrimination, adjust the stereomicroscope magnification so that the width of the tape occupies the entire field of view (provided that you aren't using wide tape). Select fibers that are close in color, diameter and morphology to the source fibers for which you are looking.

Identify the selected fibers with a unique identifier for each type of fiber. This can be done by circling each fiber type with different colored ink markers, or by using different shapes (i.e. circle, triangle, square, etc.) for each type. Keep a record of assigned shapes or colors. These markings are best made on the outside of the document protector to avoid running of the ink later when removing fibers with xylene.

Once all target fibers have been identified, remove them from the tapes one fiber type at a time. With a scalpel, make a V-shaped incision through the tape at the fiber site and place a drop of xylene along the incision. Raise the V-shaped flap to expose the fiber and remove it with fine forceps. Place the retrieved fibers in a spot plate depression containing a few drops of xylene. Use a different spot plate depression for each taping and each type of fiber. Keep a record of the source for each.

Allow the xylene in the spot plates containing the retrieved fibers to evaporate close to dryness.

Mount the fibers on microscope slides by placing fibers from different sources on different slides, with a maximum of ten fibers per slide. Place a large drop of mounting medium in the center of the slide. Retrieve the fibers from the spot plate with forceps and place them in the center of the mounting medium. Holding the cover slip tilted at a 45-degree angle, make contact with the outer edge of the mounting medium drop. Gently lower the rest of the cover slip so that no air inclusions are formed.

Allow the preparation to settle for a few minutes. If areas of the preparation are not infiltrated by the mounting medium, then you may add a small drop of mounting medium at the edge of the cover slip and allow it to infiltrate by capillary action.

Thoroughly clean the forceps with xylene between uses. Repeat the procedure until all fibers have been mounted on slides. Ensure that all slides are properly labeled as to source, and place the slides in slide trays for drying.

Prepare microscope slides of each of the known fiber samples taken from the source items in Practical Exercise 2. Remove one warp yarn and one weft yarn from the fabric sample and place these on a microscope slide. Tease each yarn apart using a probe and forceps. Select several fibers from each and mount them on slides. Return the unmounted fiber portions to the sample container.

After the prepared slides have dried for the recommended amount of time based on the mounting medium that you used, examine your prepared slides with the compound light microscope and evaluate the quality of your preparation.

Observations:

What is the quality of your mounts? Are there air bubbles and/or foreign debris present? Was too much or too little mountant used? Did the fibers remain where you placed them on the slide? Are the fibers placed in a good viewing position, or are they right at the edge of or hanging out from under the cover slips? How skilled were you at handling the fibers? Is there any evidence that you squashed the fibers with too much pressure from the forceps?

Discussion:

It is important that the supply of xylene and mountant be, and remain, clean and free of fibers to avoid contamination.

A variety of adhesive tapes are suitable for tape-lifting procedures. The choice of tape used will depend, in part, on the properties desired for the recovery and subsequent examination, and personal preference. For example, some examiners prefer clear backed tapes and others prefer frosted backed tapes; some taping tasks may be more easily handled with a wide tape or a narrow tape. Some suitable tapes have adhesives that are less sticky than others. Using tape with a less sticky adhesive may enable you to remove fibers from the tape without the use of xylenes.

When mounting questioned fibers in casework, it is beneficial to mount the fibers in such a way that they can be easily retrieved for further testing. The number of fibers mounted per slide may vary by case circumstances, the number and types of fibers recovered, laboratory protocols, and examiner preference.

The choice of mounting medium used will depend on the properties desired for the examination being conducted. For example, in fluorescence comparison microscopy it is important to select a mounting medium with low background fluorescence. A water based mountant is most suitable for temporary mounts such as those used for microspectrophotometry in the UV range. The refractive index of the mounting medium should also be considered for grouping fibers on the

basis of their optical properties. Other factors to consider are ease of handling, drying properties, color and lack of interference with fiber dyes. It is not always possible to conduct all fiber examinations satisfactorily with a single mountant as no single mountant currently available possesses the ideal properties for all of the fiber examinations routinely conducted. The techniques and solvents you may use to retrieve mounted fibers will depend on the mountant used. Practice demounting and retrieving your mounted fibers.

Practical Exercise 7

Subject: Observing Effects of Mounting Media
Time: 4 hours
Objective: To observe the visible differences in fibers due to the refractive indices of the mounting media

Theory:

In order to microscopically study the internal structure of fibers it is necessary that the sample be transparent. The refractive index (n) of a substance is a measure of the extent that light is slowed down as it passes through that substance. If a fiber is examined in air (n = 1.00), then a large difference will exist between the refractive index of the fiber and the medium and there will be total reflection of light at the air-fiber interface. Transparency is improved by placing the fibers in mounting media (other than air) with refractive indices closer to that of the fiber. If there is a large difference between the refractive index of the mounting medium and fiber, then there will be greater contrast at the boundary between the mounting medium and the edge of the fiber. Excessive contrast at the external boundary may obscure observation of internal fiber features. Different aspects of the fibers' (and hairs') external and internal features can be best examined by manipulating the contrast through selection of various mounting media.

Preparation:

Calibrate the ocular micrometer for the various objectives on the microscope you will be using if this has not been done by you previously. Adjust the microscope for proper Köhler illumination.

Materials:

- Polarized light microscope with several different objectives (e.g. 4x, 10x, 20x, 40x), and a focusing eyepiece with micrometer scale
- Stage micrometer
- Dissecting needles and fine tip forceps
- Slides and cover slips (25 mm x 40mm suggested)
- Fiber samples of
- Dyed and undyed cotton
- Round moderately delustered polyester
- Acrylic
- Wool
- Different mounting media including water, glycerol, and a permanent mounting medium with a refractive index in the range of 1.52 - 1.54

Directions:

Place a cotton fiber without a liquid mounting medium between a glass slide and a cover slip. Mount another cotton fiber in water (n=1.33), another in glycerol (n=1.46), and another in a permanent mounting medium with a refractive index in the range of 1.52 - 1.54 such as DPX or Permount. [Note that it is preferable that four concurrent preparations be used so that you can go back and forth and compare observations. If you are trying to use the same fiber for the different mounting media preparations, then the fiber must be cleaned and dried between preparations.]

Compare and contrast the image for each mountant at a variety of magnifications. Rotate the stage and examine in plane polarized light in the N-S and E-W orientation. Does the medium fiber boundary change in contrast? How easily can you observe the Becke line? Measure and record the fiber diameter for each fiber type in each mountant. Does the color and clarity of mountant affect your observation of the fiber color and structure?

Repeat the above steps for each of the remaining fiber types.

Observations:

Record and/or diagram your observations of external and internal fiber structures. Also, note the color and contrast differences among the mounting media for each fiber type.

Discussion:

If there is a large difference between the fiber's and mountant's refractive indices, then the fiber will have a lower internal transparency and surface detail will be more easily observed. Conversely, if a small difference in refractive indices exists, then the fiber will be transparent and surface detail will not be readily observed. Some published refractive indices for the fiber types are:

Fiber Type	n-Parallel	n-Perpendicular
Acrylic	1.511	1.514
Cotton	1.557	1.529
Polyester	1.706	1.546
Wool	1.557	1.547

For cotton you should have noted a dark band at the interface between fiber and air. The internal structure cannot be seen. As you increase the refractive index, from water to glycerol to permanent media, transparency will increase and contrast at the interface will decrease. Cross-markings are more easily seen in the higher refractive index liquids. With plane polarized light it

is difficult to see the difference between n-parallel and n-perpendicular when the sample is mounted in low refractive index liquids such as water and glycerol. The difference in the fiber refractive indices should be easily observed when in a higher refractive index medium such as 1.52.

For wool you should have observed that there is less contrast at the medium-fiber interface as the refractive index of the mountant is increased. Scales are easily seen in the water mount. The refractive index of glycerol (n~1.46) is too low compared to wool (n~1.55) to allow for an examination of the interior of the hair, and the outside portion of the hair (scales) is more readily observed. Differences in n-parallel and n-perpendicular of the wool fiber can be discerned at refractive indices close to that of the wool.

For the manufactured fibers you should have also observed changes in boundary contrast and fiber transparency based on the relative difference in refractive index. If the fibers are mounted in a medium with the same refractive index as the fiber, then the boundary contrast becomes zero and the fiber seems to disappear. As transparency of the fiber is improved the internal structures such as delusterant should have become more apparent. In exceedingly high boundary contrast situations, it may be difficult to see the Becke line and measure fiber diameter. A mountant with n~1.52 -1.54 should result in a very high contrast for the polyester fiber in the n-parallel position and less contrast in the n-perpendicular position. With some practice, the amount of boundary contrast can be used to suggest how different the refractive index of the medium is from those of the fiber. This becomes useful when trying to identify a fiber generic class and selecting mounting media to determine the fiber refractive indices.

Practical Exercise 8

Subject: Observing Fiber Shape, Surface and Internal Structure
Time: 4 hours
Objective: To learn and practice optical sectioning used to differentiate internal fiber structure, surface pigments or debris, and discern fiber shape

Theory:

The fiber cross-sectional shape and internal structure can be examined through use of a transmitted light microscope. Features within the fiber can be differentiated from materials lying on the surface by careful observation while focusing through the fiber. Likewise, the fiber cross-sectional shape can be discerned from the longitudinal view. To observe the internal structure of a fiber, it should be mounted in a medium with a refractive index similar to one of the fiber's indices. The inclusions can be seen more clearly because the fiber appears to "disappear". Making thin cross-sections is another method through which fiber shape and pigments, delustering agents, air pockets or other particles can be observed.

Preparation:

The trainer should provide the trainee with a variety of manufactured (nylon, polyester, acrylic, rayon, acetate and olefin) and natural (animal and vegetable) fiber types to include:

- A range of colorless, lightly colored and deeply colored fibers, both dyed and pigmented
- Bright and delustered fibers
- Bi-component fibers
- Carpet fibers in a variety of shapes and luster

Materials:

- Polarized light microscope with objectives of various magnifications
- (e.g. 10x, 20x, 40x), and a focusing ocular micrometer
- Microscope slides and cover slips
- Suitable mounting medium
- Dissecting needles and fine tip forceps
- Fiber samples obtained from your trainer

Directions:

Ensure that the ocular micrometer is properly calibrated for use at low, medium and high magnifications, and that the microscope is adjusted for Köhler illumination. Mount the fibers using Permount or other suitable mounting medium with a known refractive index of 1.50 + .05.

Begin by observing the mounted fiber under low magnification. Slowly focus up and down through the fiber in both parallel and perpendicular directions. Carefully observe the structural detail in each focal plane while focusing from the top surface of the fiber through to the bottom surface of the fiber. Record your observations. In a like manner, observe the mounted fiber under medium and high magnifications.

Repeat step 2 for all the fiber types.

Observations:

Record and diagram your observations of each fiber type. Observations to make include notation of:

- Any surface debris, pigment particles, fiber surface textures or other treatments
- Internal inclusions, voids, draw marks and striations
- The amount, size, shape and distribution of each observed feature
- Mentally assemble the series of 2-dimensional focal plane images of the fiber surface into a 3-dimensional cross-sectional shape

If you are unsure of what you are seeing, then try this as a starting point. Olefin fibers usually can't be dyed so if they are colored, then pigment is generally added to the polymer mixture during fiber production making these fibers likely candidates to observe pigment within the fibers. Olefin would also be a good synthetic fiber to observe air pockets and any draw marks spreading from those voids.

Discussion:

The fiber cross-sectional shape and the amount, size, shape and distribution of the internal structures or inclusions are important features used to identify and discriminate fibers. During manufactured fiber production, pigments and delustrants may be added to the polymer solution. Internally pigmented fibers differ greatly from dyed and surface pigmented fibers. Voids and delustrants may be confused at low power, but the properties of each can be discriminated and compared at higher magnifications with careful focusing and observation. Draw marks and striations are often easily apparent as part of the surface structure of the fiber. Fabric production and finishing processes may result in visible fiber surface texture, pigmentation and other additives.

The drawing of the polymer may produce internal structures and striations which can be seen under the microscope as markings within the fiber. Pigment granules added for coloring the fiber may have air pockets surrounding them in the polymer solution. During drawing, air pockets may become elongated to appear like cones on either side of a pigment granule or agglomerate. These inclusions are in the fiber itself and are easily differentiated from outside debris and pigment lying on the surface.

Voids are collapsed air spaces formed inside of the fiber during spinning. Draw marks, similar to those which may appear from the air pocket surrounding pigment granules, may be formed from voids. These draw marks appear as "<0>" in melt spun fibers, sometimes called a fish eye because of the shape.

Many organic polymer manufactured fibers crystallize as spherulites. The crystals' spherical symmetry elongates along the fiber axis when the fiber is drawn. Some spherulites, particularly in large denier nylon carpet fibers, may be large enough to be seen with the light microscope. They will appear as elongated streaks inside the fiber when viewed with the fiber in a longitudinal mount. If you have found any fibers with possible spherulites while performing this exercise, then you should retain them for further examination in physical cross section Practical Exercise 19.

Inclusions are relatively large objects added to the polymer mix before spinning. Pigments and delustrants are the most prevalent forms of inclusions found in fibers. The most common delustrant is rutile titanium dioxide while a less common form is anatase titanium dioxide. Pigment observation and comparison includes the color of the pigment, which is a highly discriminating characteristic, and the size and shape of the granule or aggregate.

Become familiar with the longitudinal appearance of various cross-sectional shapes. These mounted and unmounted fiber samples should be retained and used in a self-study adjunct to Practical Exercise 19

Practical Exercise 9

Subject: Observing Color and Pleochroism
Time: 3 hours
Objective: To observe dyed color and pleochroism in fibers

Theory:

Textile fibers may be dyed or pigmented. Dyes are soluble materials that are incorporated into fibers by chemical reaction, absorption or dispersion. Pigments are insoluble, finely ground materials incorporated into the fiber polymer, or adhering to fibers' exterior surfaces. About 8,000 dyes and pigments are available to the textile industry. Any one color is nearly always derived from more than one dye. The range of colors possible in the textile industry is almost infinite, making color an important characteristic for discriminating between fibers of the same generic class. Textiles are colored in batches and the variation between batches adds to the potential significance of a fiber comparison.

Pleochroism is the phenomenon of an object displaying different colors depending upon its orientation when viewed in polarized light. Of the manufactured fibers, only those which are highly oriented have the capacity to display pleochroism. The dye molecules become aligned with the fibers' micelles and respond differentially to the vibrations of light in the two directions of light. Pleochroism may be called dichroism when referring to fibers because they have two optical axes and are, therefore, only capable of displaying two colors. The range of pleochroism may be anywhere from a slight change of color shade to completely different colors. Rayon, particularly, may exhibit almost no color in n-perpendicular and a deep color in n-parallel. It is important to observe and record pleochroism in fiber examinations and comparisons because not all fibers are pleochroic, and of those that are, not all are pleochroic to the same degree.

Preparation:

Obtain samples of colored natural and manufactured fibers to include as many generic types as possible. Dyed fibers, pigmented fibers and printed textiles should be examined. Mount the fibers using a suitable mounting medium.

Materials:

- polarized light microscope
- microscope slides and cover slips
- suitable mounting medium
- dissecting needles and fine tip forceps
- fiber samples

Directions:

Ensure that the microscope is properly set-up and adjusted for Köhler illumination.

Observe each fiber sample in whole mount under (non-polarized) transmitted light noting the following:

- Color of the fiber
- Uniformity of the color (even, cloudy, patchy, streaky, blotchy, etc.)
- Whether the fiber appears dyed, pigmented, or printed
- Whether variations exist between the colors of the fibers in the same sample

View the same samples under polarized light (analyzer out) in the perpendicular and parallel orientations noting the following:

- The color of the fiber in each of the two orientations
- The intensity of the color(s) in each of the two orientations
- If any of the natural fibers exhibit pleochroism
- If any differences in pleochroism exist between fibers of the same generic type
- And color, or between differently colored fibers of the same generic type

Observations:

Record your observations of each fiber type noting in particular the items listed in the Directions.

Discussion:

Not all fibers accept the same dye(s) uniformly and the quality of the final textile product is dependent on hundreds of variables. This variability works in the fiber examiner's favor for distinguishing between fibers that would otherwise appear similar. The goal of textile producers is to have uniform products that fall within predetermined standards. Dyeing is one of the most variable processes in textile production. Natural fibers, in particular, exhibit variation in the uptake of certain dyes and this can lead to undesirable final products which are off-shade or even

the wrong color. The main focus of the dyeing industry is to limit this variation as much as possible to repeatedly yield a uniform product.

In general, manufactured fibers will take dyes more uniformly than natural fibers. This generalization was one of the motivating factors in the development of manufactured fibers.

Although natural fibers generally do not exhibit pleochroism, some dyed cottons and bast fibers do. Not all manufactured fibers exhibit pleochroism. In manufactured fibers, pleochroism is seen most often in highly oriented fibers such as rayon, nylon and polyester.

Practical Exercise 10

Subject: Distinguishing Natural and Manufactured Fiber Classes
Time: 3 hours
Objective: To observe, compare and distinguish among the natural and manufactured fibers by basic morphological features

Theory:

Natural fibers come from organic renewable resources and can be broadly classified as animal, vegetable, or mineral in origin. Manufactured fibers are produced by extensive chemical and manufacturing processes. Any of these fiber types may be found in textile products of forensic interest. One of the first steps in a forensic fiber examination is for the fiber examiner to be able to distinguish between natural and manufactured fibers when they are viewed under the microscope.

Fibers of animal origin include hairs, silk, leather and spider silk. The most commonly encountered animal fibers of forensic interest are hairs. Hairs are recognized by the presence of surface scales, internal medullation, and the overall shape. Vegetable fibers can originate from any part of a plant including the stem or bast (e.g. flax, ramie, jute, hemp), leaf (e.g. sisal, manila hemp [= abaca, *Musa textilis*]), fruit (e.g. coir, kapok), and seed (e.g. cotton, akund). Many of the vegetable fibers that are too coarse to be used in textiles are used in different types of cordage or consumer products such as doormats (coir) and various paper products. Vegetable fibers can be recognized by the presence of cellulosic walls, features of the cell wall (dislocations, pits and spiral thickenings), various crystals, and the central cavity called a lumen. Compared to natural fibers, manufactured fibers are fairly uniform and regular in their microscopic appearance as a result of the materials and processes used in manufacturing. Useful clues that a fiber is manufactured include: the absence of features typically associated with animal and vegetable fibers (i.e. no scales, no cell walls), its cross-sectional shape and usually uniform shape, diameter and thickness along the fiber length, the presence of interference color bands parallel to the long axis that only vary when the fiber is stretched or bent, and the presence of delustrant.

Preparation:

Obtain authenticated and commercial samples of natural and manufactured fibers to include: cotton (mercerized and unmercerized), flax, hemp, sisal, wool, silk, mohair, rabbit, suede, fiber glass, rayon, nylon and polyester. There should be colorless, dyed and/or pigmented representatives of each fiber type. The manufactured fibers should include bright and delustered representatives of each.

Materials:

- Stereomicroscope and polarized light microscope
- Microscope slides and cover slips
- Suitable mounting media
- Dissecting needles and fine tip forceps
- Fiber samples

Directions:

Ensure that the polarized light microscope is properly set-up and adjusted for Köhler illumination.

Place small quantities of one fiber type on a clean glass slide. Examine the fiber(s) under the stereomicroscope. Tease the fibers apart if necessary to obtain a clear view of their morphological features at this level of magnification. Record your observations.

Mount the fibers using a suitable mounting medium. Observe each fiber sample with plane polarized transmitted light and with crossed polars. As a minimum, you should examine each fiber sample at 100X and 200X. Note the fiber's gross morphological features as well as the internal and external structures used to characterize the natural (animal and vegetable) versus manufactured general categories. Record your observations.

Repeat steps 2 and 3 for each fiber sample.

Observations:

Make a chart of the various features you can use to distinguish animal, vegetable, mineral and manufactured fibers. Record your observations relative to these features for each fiber sample you examined. Sketch the appearance of the fibers and features observed.

Discussion:

Fibers can be screened and class categorized as natural (animal, vegetable, mineral) versus manufactured by microscopical examination of the longitudinal view alone with time and practice. Differentiating among these groups is fairly straightforward if you are familiar with the various microscopic characteristics each class of fiber possesses. It is the initial evaluation and grouping of fibrous material in casework that helps determine which items are probative, and which analytical schemes to pursue.

Caution should be used when examining fibers that have been heavily dyed or processed, such as mercerization, which could alter or obscure characteristic morphological features. Also, consider the level of magnification necessary to ensure you were clearly seeing the features necessary to categorize the fiber. How many of the characteristic morphological features can you clearly see and describe when using the stereomicroscope? Can you categorize any of the fibers you examined based on the stereomicroscopic examination or is higher magnification necessary to confirm your initial observations? Is that true for all the fiber types you examined?

Silk is one fiber type that may present some observational confusion. Silk, a fiber of animal origin that is not a hair, will have a smooth appearance usually without visible internal structures, and has interference color bands parallel to the long axis. Silk may exhibit “cross-over marks” that are oblique flattened areas along the fiber, or may exhibit internal striated or granular appearance depending on the type of silk you examined. Were there other fibers or fiber features that presented observational confusion?

In this particular exercise you may have started with fiber samples that contained multiple fibers or tufts of material. In casework material you cannot assume or rush to judge all fibers in the sample to be similar without the appropriate level of examination.

Practical Exercise 12

Subject: Determining Natural Fiber Twist
Time: 3 hours
Objective: To learn the Herzog effect and the drying twist test

Theory:

Vegetable fiber cell walls are composed of parallel microfibrils running in a longitudinal spiral. These fibers can be categorized according to the direction of fibril twist. Fibers having a left-hand twist (e.g. flax and ramie) are designated as having an “S-twist”. Conversely, fibers having a right-hand twist (e.g. hemp, jute and sisal) are referred to as having a “Z-twist”. The direction of twist can be ascertained through the use of polarized light microscopy or by the drying twist test.

Identification of vegetable fibers is accomplished primarily through the recognition of distinguishing botanical characteristics that are unique to a particular type of vegetable fiber. Unfortunately, some vegetable fiber types exhibit similar gross botanical characteristics thereby making their identification more difficult. Differentiation of vegetable fibers which have similar gross botanical features may be accomplished by observing the effect the fiber has on polarized light, referred to as the Herzog effect, as a result of the differences in their microfibril orientation. Interference colors are observed when vegetable fibers are examined between crossed polars. These colors result from the destructive interference of light waves of certain wavelengths and their consequent removal from white light. When a first order red compensator (~530 nm) is inserted into the microscope between the fiber sample and the analyzer, the resultant change in the interference colors displayed by the fiber will be additive or subtractive because of the microfibril orientation.

Preparation:

You should use remaining unmounted fiber samples and longitudinal mounts of authenticated fiber samples of cotton, coir, flax, hemp, ramie, jute, sisal, henequen, and manila hemp.

Materials:

- Stereomicroscope (for initial observation and mounting slides)
- Polarized light microscope with various objectives (e.g. 4x,10x, 20x, 40x and
- 100x)
- First order red plate (530-570 nm compensator)
- Microscope slides and cover slips (if new slide mounts are made)
- Suitable mounting medium (if new slide mounts are made)
- Dissecting needles and fine tip forceps
- Hot plate and beaker of warmed water
- Authenticated fiber samples, mounted and unmounted

Directions:

Part 1 - The Herzog Effect

Place the mounted fiber sample on the microscope stage between crossed polars.

Orient the longitudinal axis of the fiber ultimate parallel to the vibration direction of the analyzer.

Insert the compensator plate into the microscope between the sample and the analyzer.

Observe and record the resultant color of the fiber ultimate. It should be either orange or blue.

Repeat steps first 4 steps for each of the mounted fiber samples.

Part 2 - The Drying Twist Test

Take a sample of unmounted fiber, large enough to be seen visually, and place it in the beaker of warm water. Note that too large of a fiber bundle can result in equivocal results. Some experimentation may be needed to determine optimal sample size.

Remove the fiber from the water bath by holding one end with forceps, directing the other end toward you as an axial view, over and near the hot plate heated surface.

As the fiber dries note whether it rotates clockwise, counterclockwise or has some other response.

Record the drying rotation observation and relate it to the observed Herzog effect.

Observations:

The resultant color of the fiber should be either orange or blue. The S-twist fibers will demonstrate an additive effect, having a second order blue appearance. The Z-twist fibers will demonstrate a subtractive effect, having a first order orange appearance. Record the axial view rotation direction for each fiber during the drying twist test. Compare and contrast the drying twist test and Herzog effect observations.

Discussion:

Vegetable fibers are identified primarily through a microscopical examination of morphological characteristics. Some vegetable fibers have distinctive botanical features that can be considered unique thus leading to discrimination and identification of the fiber type. Other vegetable fibers have observable features that are less discriminating. The twist of these natural fibers is another characteristic which can be used to characterize and/or identify the type or origin. The microfibril structure and direction of twist is not directly observable by transmitted light microscopy. The Herzog effect observed with polarized light and the drying twist tests are methods by which the microfibril twist can be indirectly observed and deduced. In some instances, this allows the analyst to differentiate between two vegetable fibers with similar microscopical appearances but different botanical origins.

The Herzog effect and drying twist test are simple, inexpensive and nondestructive methods that can be used in conjunction with other botanical identification characteristics for differentiating between similar types of vegetable fibers. Neither test should be considered definitive for identification purposes. Some authors have warned that the drying twist test observed results can be influenced by fiber finishing processes, and observation of the Herzog effect works best with undyed fibers. The literature reports the following fiber twists:

S Twist Fibers	Z Twist Fibers
Flax	Hemp
Ramie	Jute
Coir	Sisal
	Manila hemp
	Henequen

Were your observations in both sets consistent with these reported data? Note that cotton exhibits both S and Z twist characteristics.

Practical Exercise 16

Subject: Determining the Sign of Elongation
Time: 4 hours (Part 1 ~2 hrs, Part 2 ~2 hrs)
Objective: To learn simple techniques for determining the sign of elongation of a manufactured fiber using a first order red plate or a quartz wedge

Theory:

Polarized light is limited to a single vibration direction for any position of the polarizer. A crystal can be oriented so that a particular crystallographic direction is parallel to the vibration direction of the polarized light. The elongation of a manufactured fiber depends on the orientation of the molecules along its length. If the refractive index for light polarized parallel to the fiber length exceeds that for light polarized perpendicular to the fiber length, then the elongation is said to be positive. If the reverse is true, then the fiber is said to have negative elongation. If the two refractive indices are determined by an immersion technique using liquids of known refractive index, then the sign can be determined by subtraction (n-parallel minus n-perpendicular). The sign of elongation can also be determined by inserting a compensator into the optical path of a polarized light microscope between the specimen and the analyzer. Determining the sign of elongation is the first step toward establishing fiber identity.

Compensators are made from anisotropic crystalline material. That is to say, they have different refractive indices for light passing through them parallel and perpendicular to the crystal axes. The slow direction will be that with the greater refractive index, and is usually marked as the “z” ray on the body of the compensator. The compensators are inserted in the microscope tube at an angle of 45 degrees from the vibration direction of the polarizer and analyzer. The interference colors produced will be at maximum brightness in this position. The colors produced will depend on the refractive indices of the substance as it is oriented and its thickness. If the thickness is variable, then several colors may be observed.

The first order red compensator (or λ plate) is a layer of selenite or quartz of a thickness which will produce a retardation of ~530 to 570 nm (first order - magenta). It is useful for determining the sign of elongation of fibers having low birefringence (exhibit a grey-white color under crossed polars).

As its name implies, a quartz wedge is made from a quartz crystal which gradually increases in thickness. It can, therefore, produce variable retardation resulting in different interference colors in sequence known as Newton’s series. The further the wedge is pushed in, the higher the order of interference colors exhibited. The higher the order, the paler the colors become. The color

series is represented on the Michel-Lévy chart. Wedges can be purchased that exhibit different numbers of orders. The least expensive will cover four orders.

Quartz wedges are used to determine the sign of elongation of fibers with a range of birefringence from about 0.015 - 0.100. The wedge is used to compensate the retardation produced by the fiber. Under these circumstances, the center of the fiber will appear black. Retardation can only be achieved when the slow vibration direction of the wedge lies perpendicular to the slow vibration direction of the fiber. By measuring the fiber thickness and using the Michel-Lévy chart to estimate retardation, the birefringence can be calculated. Tables of optical values for the commonly encountered types of manufactured fibers can be found in Palenik, 1999.

Preparation:

Make permanent microscope preparations of undyed fibers from authenticated samples as directed below. They can be retained and used for reference purposes and will last you for years if you look after them. You will need these same slides to complete Practical Exercise 17.

Select a single acetate fiber and fasten one end to a slide with adhesive tape. Draw the fiber taut and fasten down the other end. Add a small drop of mounting medium and holding the edge of a cover slip in the medium with the forceps lower it gently to make a permanent mount without air bubbles in it. Select a single acrylic fiber and repeat the process with a new slide and cover slip. Make a third preparation, mounting an acrylic fiber and an acetate fiber parallel to each other and as close together as possible on a single slide so that their behavior under polarized light can be observed simultaneously. These prepared slides will be used in Part 1 below.

Make four permanent microscope slide preparations as you did above, one each of authenticated, undyed samples of nylon, polyester, viscose rayon and polypropylene. Make a fifth preparation, mounting these four fiber types side by side and as close together as possible on a single slide so that their behavior under polarized light can be observed simultaneously. These prepared slides will be used in Part 2 below.

Materials:

- Polarized light microscope with rotatable stage and 10X, 20X and 40X objectives
- First order compensator
- Quartz wedge compensator
- Michel-Lévy chart
- Slides, cover slips, and adhesive tape
- Fine forceps and fine scissors
- Suitable fiber mounting medium (Permount, XAM, etc.)
- Authenticated samples of undyed acetate, acrylic, nylon, polyester, viscose rayon and polypropylene fibers

Directions:

Part 1 - Determining the Sign of Elongation Using the First Order Red Compensator

Make sure the microscope is correctly set up for Köhler illumination. Insert the polarizer, and note the vibration direction. Align it in the E-W or N-S position, so that it is oriented at 90 degrees from the analyzer's vibrational direction. [Note that currently manufacturers are consistently orienting the polarizer in the E-W vibrational direction. This has not always been the case and some microscopes have N-S oriented polarizers. Consult your instrument's user's manual to determine your microscope's polarizing filters' configurations.]

Place the mounted acetate fiber slide on the stage so that the fiber lies at 45 degrees to the polarizer's vibrational direction. Insert the analyzer. Observe the fiber under crossed polars (black background). Note the color of the fiber. Rotate the stage through 45 degrees so that the fiber is in one of the extinction positions. Note what happens to the fiber color.

Rotate the fiber through 45 degrees back to its original position. Insert the first order red compensator into the compensator slot. Note the background color which should be an intense reddish purple. Locate this color on your Michel-Lévy chart. It appears with a path difference of 530-570 nm and lies in the first order. Note that the colors on the chart on either side of this are orange and blue.

Note the interference color of the acetate fiber.

Rotate the fiber through 90 degrees. Note the color of the fiber. Make a diagram illustrating your observations.

Place the mounted acrylic fiber slide on the stage and repeat steps 2-5. Note the color of the fiber in the various positions, making diagrams to illustrate your observations.

Place the slide with both the acetate and acrylic fibers on the stage. Repeat the procedure while observing both fiber types simultaneously and confirm your observations.

Part 2 - Determination of Sign of Elongation Using the Quartz Wedge

Make sure the microscope is correctly set up for Köhler illumination. Insert the polarizer, and note the vibration direction. Align it in the E-W or N-S position, so that it is oriented at 90 degrees from the analyzer's vibrational direction.

Place the slide with the four fiber types (viscose rayon, polypropylene, nylon, polyester) on the stage so that the fibers' lengths lie at 45 degrees to polarizer's vibrational direction. Insert the analyzer. Observe the interference colors produced under crossed polars in each of the four fiber types. Observe how the interference colors produced under crossed polars differ among each other in four fiber types and how these differ from those you observed with the acrylic and acetate fibers.

Remove this slide and place the slide with the mounted viscose rayon fiber on the stage so that the fiber lies at 45 degrees to the polarizer's vibrational direction with the center of the fiber beneath the cross hairs. Insert the analyzer. Note the interference colors produced.

Insert the quartz wedge into the compensator slot between the polarizer and analyzer. Observe Newton's series of colored bands produced by the wedge which appear behind the fiber. As you slowly push the wedge in, observe how these colors successively follow the colors through the orders on the Michel -Lévy chart. Does the center of the fiber become black?

Rotate the stage through 90 degrees. What is the color of the center of the fiber? Note the position of the wedge at which the center of the fiber becomes black. This position is known as "compensation". Compensation can only occur when the slow directions of the fiber and the wedge are perpendicular to each other. Because the slow direction of the wedge is known, by observing whether compensation occurs perpendicular to the fiber length or parallel to the fiber length, the fast and slow directions of the fiber can be determined. Thus, the elongation can be determined (negative when n-perpendicular > n-parallel, and vice versa for positive). Determine and record the sign of elongation for the fiber type you are examining.

Repeat 3rd-5th steps for each of the other mounted fiber types (polypropylene, nylon, polyester).

Observations:

Record your observations as requested in the directions. Produce appropriate colored illustrations to document your observations.

Discussion:

When two anisotropic substances are superimposed (fiber and compensator) addition or subtraction of their retardations can occur. If both substances are placed in the brightness position (45 degrees away from the vibration direction of the polarizer and analyzer) and their slower components are parallel, then the two retardations are added. The resulting interference color is higher than that of either substance alone and is the numerical sum of the retardations. If the slow components are perpendicular to each other, then subtraction of the retardations will occur. The resulting interference color will be of a lower order than that of either component and numerically equal to the difference in retardation of the two substances.

In completing these exercises the trainee should see clearly that the directions of the fast and slow rays differ for acrylic and acetate fibers. Compensators have their slow direction indicated on the holder. The compensator is inserted into the body of the microscope at an angle of 45 degrees to the vibration direction of polarizer and analyzer. By rotating the stage and fiber, the fiber's slow direction can be successively placed parallel and perpendicular to those of the compensator.
In an orientation where slow directions of fiber and red plate are parallel to one another, addition will occur and a second order blue color will result. In an orientation where the slow directions of the fiber and red plate are perpendicular to each other, subtraction will occur and a first order orange/yellow color will result. The slide with both the acrylic and acetate fibers on it allows simultaneous viewing of both situations. For the acrylic fiber n-perpendicular exceeds n-parallel (the slow vibration direction will be perpendicular to the fiber length), and it has a negative sign of elongation. For the acetate fiber n-parallel exceeds n-perpendicular (the slow vibration direction will be parallel to the fiber length) and it has a positive sign of elongation.

The trainee should be capable of determining the sign of elongation of all fibers which are weakly birefringent (i.e. exhibit a grey-whitish appearance when viewed by themselves under crossed polars) after completing Part 1 of this exercise. This will include acrylics, modacrylics, acetates, and chlorofibers (vinyon). All manufactured fiber types with a birefringence over 0.015 exhibit bright interference colors under crossed polars and all have positive sign of elongation. This should be determined using the quartz wedge.

As time permits, obtain and examine as many authenticated reference samples of modified acrylic, cellulose diacetate and triacetate as possible. The elongation within these generic types

can be positive, negative or zero (fiber exhibits same color as background). Also practice determining the sign of elongation on dyed samples of the appropriate fiber types.

Practical Exercise 17

Subject: Measuring Fiber Birefringence
Time: 2 hours
Objective: To learn one of the techniques for determining fiber birefringence

Theory:

Birefringence is the numerical difference between the refractive indices of a fiber. Retardation of a light beam increases linearly with sample thickness and with the birefringence. These optical parameters are related by the formula:

r = 1000 t X B where r = retardation in nm;
t = thickness of fiber in μm;
B= the numerical difference in refractive indices.

If any two of the formula parameters are known, then the third can be calculated. All of these parameters are used to examine, characterize, identify and compare fibers in forensic casework.

In polarized light microscopy, the quartz wedge is used to produce variable retardation resulting in different interference colors which occur in a sequence known as Newton´s series. The further the wedge is pushed in (increasing the thickness of quartz in the optical path), the higher the order of the interference colors that will be exhibited and the paler the colors will become. Quartz wedges are used to determine the sign of elongation of fibers with a range of birefringence from about 0.015 - 0.100. The wedge is used to compensate the retardation produced by the fiber. Compensation can only occur when the slow rays of the fiber and wedge are perpendicular to one another. Compensators have their slow direction marked on the holder. The compensator is inserted into the body of the microscope between the polarizer and analyzer and at an angle of 45 degrees to their vibration directions. By rotating the stage and the fiber, the slow directions can be successively placed parallel and perpendicular to those of the compensator. When compensation is achieved the center of the fiber will appear black. Birefringence can be determined by estimating the retardation, from the corresponding interference colors, using the Michel-Lévy chart and by measuring the fiber thickness. If the cross-sectional shape of the fiber is not round, then care must be taken to estimate the actual thickness of the fiber. Exactly how this is done will depend upon its shape.

Preparation:

You should still have the microscope slides that you prepared from authenticated fiber samples in Practical Exercise 16. If for some reason they are no longer available or could be improved, then repeat the preparation. Make four permanent microscope slides, one each of authenticated undyed samples of nylon, polyester, viscose rayon and polypropylene using the instructions in Practical Exercise 16.

Materials:

- Polarized light microscope with rotatable stage and 10X, 20X and 40X objectives
- Quartz wedge compensator covering 4 orders
- Michel-Lévy chart
- Previously prepared mounted slide of authenticated nylon, polyester, viscose
- Rayon and polypropylene, or if preparing new slides
- Slides, cover slips and adhesive tape
- Fine forceps and fine scissors
- Suitable fiber mounting medium (Permount, XAM, etc.)
- Authenticated samples of nylon, polyester, viscose rayon and polypropylene fiber

Directions:

Make sure the microscope is correctly set up for Köhler illumination. Insert the polarizer, and note the vibration direction. Align it in the E-W or N-S position, so that it is oriented at 90 degrees from the analyzer's vibrational direction.

Place the slide with the viscose rayon fiber on the stage so that the fiber's length lies at 45 degrees to polarizer's vibrational direction. Insert the analyzer. Observe the interference colors produced under crossed polars.

Insert the quartz wedge into the compensator slot between the polarizer and analyzer. Observe Newton's series of colored bands produced by the wedge which appear behind the fiber. As you slowly push the wedge in, observe how these colors successively follow the colors through the orders on the Michel-Lévy chart.

Note the position of the wedge at which the center of the fiber becomes black. This position is known as compensation. Rotate the stage through 90 degrees and observe what happens to the black color.

Determine and record the sign of elongation.

Look at the background color immediately behind the fiber at the point where compensation has occurred. Locate the color on the Michel-Lévy chart and determine the value for the path difference in nm on the abscissa. The order of the color can be noted by observing how many times that background color is passed when the wedge is slowly withdrawn.

Measure the fiber thickness using the calibrated ocular micrometer. Be sure to take into account the appropriate fiber cross-sectional shape before making this measurement.

Determine the birefringence value using the Michel-Lévy chart. Draw a line from the origin through the point coordinate where the thickness and retardation values meet on the chart and project it to transect the ordinate scale on the bottom or right hand side of the chart.

Observations:

Construct a chart showing the sign of elongation and the birefringence values that you have obtained from your observations of the four fiber samples.

Discussion:

After completing this exercise, the trainee should be able to use the quartz wedge to determine the birefringence of any manufactured fiber exhibiting birefringence between 0.015 and 0.100. The interference colors seen under crossed polars are characteristic for particular fiber generic types. If as many samples as possible from a reference collection are examined, then the trainee will begin to recognize these characteristics and this will make the identification of manufactured fibers easier in the future.

Examining deeply dyed fibers can be difficult as the dye may mask the interference colors to some extent. It may be possible to improve the situation by looking for an area where the fiber is slightly squashed, increasing the illumination, or by concentrating on the edges of the fiber. The trainee is encouraged to examine as many dyed fibers as possible so that s/he becomes familiar with the difficulties deep dying presents and is able to overcome these difficulties by using the suggested improvement techniques.

It would be particularly useful to repeat this quartz wedge exercise using a slide preparation of a regular tenacity rayon fiber mounted parallel to a high tenacity rayon (polynosic) or lyocell fiber to observe how the interference colors and birefringence differ.

There are other types of compensators which you may encounter:

- The quarter wave plate is made from a thin mica plate of uniform thickness which can be used to produce a retardation of about 125 nm. It can be used for compensation studies for fibers having very low (first order) birefringence.

- The Berek Compensator is a tilting compensator made from a 0.1 mm thick calcite or quartz plate that can be tilted with a micrometer screw so that progressively thicker sections of the plate are placed in the light path. The tilting axis is parallel to the tube slot. The effect is the same as that of the quartz wedge. Compensation must be achieved and the angle of tilt read using the micrometer. There is a mathematical relationship between the angle of tilt and the retardation for which tables are available. The thickness of the fiber must also be measured. The retardation divided by the thickness will give the birefringence. Berek compensators may cover up to ten orders and allow more accurate measurements for fibers with higher birefringence such as polyesters.

- The Ehringhaus Compensator is a tilting compensator containing two quartz or calcite plates of exactly the same thickness that are cemented together in the subtraction position and cut parallel to the crystal axis. The measuring range is 20 orders.

- The Senarmont Compensator is an elliptical compensator based on a quarter wave plate that can be rotated around the microscope axis. The quarter wave plate is adjusted exactly in the extinction position and the object in a diagonal position (SW-NE). Compensation is obtained by rotating the analyzer. Normally this compensator is used for measuring phase differences of up to 1 order. Measurement must be carried out in monochromatic light at a wavelength of 546 nm.

Practical Exercise 18

Subject: Measuring Fiber Refractive Indices by the Immersion Method
Time: 3 hours
Objective: To learn the Becke Line method of measuring fiber refractive indices

Theory:

Manufactured fibers can be differentiated by their refractive indices. The maximum and minimum refractive indices of a fiber can be measured by examining the fiber parallel and perpendicular to the plane of polarization, using a polarized light microscope.

The refractive index is a measure of how much light is slowed down as it passes through a substance. Fibers have two refractive indices for light polarized parallel or perpendicular to the fiber axis. The refractive indices can be determined microscopically by successively immersing the fibers in liquids of known refractive index. The fiber will show minimal contrast or its edges will become invisible in one such liquid when the refractive index of the liquid is close to or equals the refractive indices of the fiber.

The Becke line is a bright line appearing at the boundary of the fiber. It arises from total internal reflection at the boundary of the two media. It moves with respect to that boundary as the microscope is focused up or down. As the working distance of the microscope is increased the Becke line will move toward the medium with the higher refractive index. A false Becke line may sometimes be observed as a second bright line which moves in the opposite direction to the Becke line. This occurs especially when the difference between the refractive index of the specimen and the immersion liquid is low and the true Becke line is faint. This phenomenon of a false Becke line can be weakened by closing down the substage iris diaphragm.

Materials:

- Polarized light microscope with rotatable stage and 10X, 20X and 40X objectives
- Monochromatic light filter - sodium light (589 nm)
- Solid black watch glass (or equivalent for contrast with undyed fibers)
- Ethanol for washing fibers
- Slides and cover slips
- Fine forceps and fine scissors
- Fume hood (portable)
- Cargille refractive index liquids:
- Series A, varying in steps of 0.002 and covering the R.I. range 1.460 - 1.640
- Series B, varying in steps of 0.004 and covering the R.I. range 1.644 - 1.700
- Series C, varying in steps of 0.005 and covering the R.I. range 1.705 - 1.800

- Sample of round polyester fiber - Dacron
- Sample of partially oriented (partially drawn) polyester yarn

Directions:

Use the polarized light microscope in conjunction with the appropriate circular filter to produce a monochromatic light source. Set up the microscope to produce plane polarized light, and close down the condenser's iris diaphragm until only a small aperture remains.

Place a length of Dacron fiber on a clean slide under a cover slip.

Switch on the portable fume hood and leave it on while working with the refractive index liquids.

Choose one of the refractive index liquids expected to correspond approximately with n-parallel for the polyester fiber (e.g. 1.720). Allow a drop of the liquid to flow under the cover slip and immerse the fiber.

Place the slide on the microscope stage with the length of the fiber oriented parallel to the polarizer's vibrational direction. Examine the fiber at high magnification.

Using the fine focus, rack up and down from the position of sharp focus, and observe the edges of the fiber. The Becke line is a bright line which appears inside or outside the edge of the fiber when the microscope is slightly out of focus and when the refractive index differs from that of the medium. The line will move toward the medium with the higher refractive index when the microscope working distance is increased.

Repeat with different refractive index liquids until you find the liquid in which the Becke line becomes invisible. Before trying a new liquid, the fiber must be cleaned in a little ethanol in a solid black watch glass and then blotted dry. Determine which liquid to try next based upon the results. For example, is the refractive index higher or lower than the current Cargille liquid and how strong is the contrast?

Rotate the fiber through 90 degrees so that it lies perpendicular to the polarizer's vibrational direction and repeat the refractive index measurement beginning with a liquid close to the expected value of n-perpendicular (e.g. for polyester 1.540).

Record the refractive indices for the two positions.

Repeat the exercise using the partially oriented polyester yarn (POY).

Hold a length of the POY between finger and thumb. Pull the yarn to stretch or draw it out.

Then repeat the exercise steps 2-9 using the POY you have stretched.

Observations:

Construct a chart showing the refractive indices that you have obtained from your observations of the fiber samples. What was the effect of drawing the POY? Why do you think this happens?

Discussion:

Differentiation by refractive index can be particularly useful with some fiber groups such as the round polyesters in which there may be a very large variance in this optical property. As time permits, practice this technique with other fiber generic types and on dyed fiber samples.

The birefringence of a fiber will depend on the degree of molecular orientation of the microcrystalline structure. As fibers are stretched the degree of orientation will increase. It can quickly be seen that in the polyester yarn that is not fully stretched the n-parallel value is indeed far lower than in normally stretched polyester fibers. Polyesters that are produced for use in tire cords are deliberately produced with a high stretch that in turn will be reflected in a high n-parallel value.

It is possible to make more accurate measurements of fiber refractive indices using interference microscopy, according to the method of Heuse and Adolf (1982) by which the exact position of the fiber on a standard diagram can be determined. New Interference microscopes are not being manufactured at present.

Practical Exercise 19

Subject: Cross-Sectioning Fibers and Interpretation of Cross Sections
Time: 8 hours
Objective: To learn different techniques used to cross section fibers

Theory:

The examination and comparison of the cross-sectional shape of fibers is an integral part of fiber examination. Often times, the cross-sectional shape of a fiber can be determined longitudinally. However, more detail and information can be obtained from sectioning a fiber, and examining the cross section. A fiber cross section may provide information about the manufacturer, spinning process used, end use, fiber quality, and dyeing method and quality. Through time and technology, the variety of cross-sectional shapes has increased. A fiber manufacturer can change the appeal and end use of a fiber simply by modifying the cross section.

Preparation:

Obtain a variety of dyed and undyed fibers (nylon, polyester, acrylic, rayon, acetate and olefin) from different end uses (carpet, clothing, upholstery, automobile interiors) including bi-component fibers.

Materials:

- Stereomicroscope, polarized light microscope and comparison microscope
- Polyethylene sheet
- Razor blade, hot plate, microscope slides
- Jolliff plates, filler yarn, needle threader
- Variety of dyed and undyed fibers

Safety:

Use caution when using razor blades and place used blades in a sharps disposal container.

Directions:

Perform the following steps. After you have obtained satisfactory cross sections of single, multiple and tufts of fibers, practice using case-sized samples of fibers (~ 4 mm).

Mount fibers to examine them longitudinally.

Examine fibers microscopically (longitudinally). Document all information obtained from optical examination of fiber including diameter, delustrant (size, shape, concentration), voids, spherulites, pigment particles (size, shape, concentration), dye penetration, shape, and surface treatment.

Obtain physical cross sections of the fiber examined optically by these methods:

Polyethylene Method

1. Heat hot plate to approximately 150 OC, just enough to melt polyethylene (PE).
2. Cut two small pieces of PE, slightly larger than your fiber sample.
3. Place one piece of PE at one end of a microscope slide.
4. Place the fiber on the PE sheet. Place the second sheet of PE over the first, forming a sandwich with the fiber in the middle.
5. Place a second microscope slide or a small piece of glass over the PE sandwich.
6. Place the slide with the PE sandwich on the hot plate. Let the PE sheets melt together.
7. Press down on the top microscope slide or glass with an eraser tip to allow the PE to melt around the fiber and release all air bubbles.
8. Wait until the PE turns clear, then wait a few additional seconds.
9. Remove the slide with the PE sandwich from the plate. Allow the PE sandwich to cool.
10. Remove the top microscope slide.
11. Trim away the excess PE around the fiber that will be sectioned.
12. Using the stereomicroscope, begin cutting thin sections of the fiber with a sharp razor. The fiber should be cut at a 90-degree angle. The sections should be cut as thin as possible to enable the sections to lie with the fiber cross sections facing up.
13. Mount the cross sections in xylene or Permount and examine under the polarizing scope.

Jolliff Method

1. Slide your finger across the Jolliff plate. The smooth side is considered the front.
2. Push the threader through the back of the slide so it comes out the front.
3. Cut an approximately 5 inches long piece of filler yarn. The color of the filler yarn
4. should contrast the color of the fiber.
5. Pass the required amount of filler yarn through the eye of the threader, centering it. The combined amount of the filler and the sample should be just enough to require some pressure to pull it through the hole.
6. Pull the threader down through the hole leaving a tuft on the upper side of the Jolliff slide.
7. Looking at the front of the Jolliff plate, lay the fiber(s) to be cross sectioned across the valley created by the two free ends of the filler yarn.
8. Pull the filler down, drawing the sample into the hole.

9. Place the long end of the Jolliff plate in contact with the desk, tilt the plate so that it is perpendicular to the desk. Use a sharp razor to slice off fibers and filler yarn off the backside of the plate.
10. Lay the Jolliff plate flat on the desk, with the front side up. Trim off the top.
11. Cut out the sectioned part of the plate. Place the cross sections on a microscope slide, cover with a cover slip and tape it down.

Examine the cross sections microscopically. Document all information obtained from the physical cross section including diameter, delustrant (size, shape, concentration), voids, spherulites, pigment particle (size, shape, concentration), dye penetration, shape, and surface treatment. Draw the shape you observed.

Compare and contrast the types and quality of information obtained from longitudinal examination and physical cross-sectioning.

Observations:

Observe the information obtained from the longitudinal examination of the fiber versus the physical cross sections. Evaluate the relationship among fiber cross-sectional shape, generic class and end usage.

Discussion:

The examination and comparison of fibers is an essential part of fiber examinations. The cross-sectional shape of a fiber often can be determined longitudinally. However, additional information may be obtained by examining the physical cross section of a fiber, including information about possible manufacturers, spinning processes, end use(s), fiber quality and dyeing processes.

It is important to cross section a representative sample of fibers from a known source. For example, carpet fiber tufts may contain several cross-sectional shapes.

Cross sections are produced as a result of the manufacturing process or an engineering process. In the manufacturing process, spinning conditions and extrusion processes affect the cross section of the fiber. In dry spinning, the fibers may have a dog bone shape due to the extruded fiber passing through jets of hot air to evaporate the solvent. Dry spun fibers include acetate and acrylic. In wet spinning, the fibers are extruded through a chemical bath that may alter the cross section. For example, fibers may be extruded through a round hole then placed in an acid bath causing the outer skin to shrink resulting in a crenulated cross section. Wet spun fibers include rayon and acrylic.

Engineering processes are used to develop cross sections for a specific end use. The first fiber to be engineered was a trilobal fiber developed by DuPont in the 1960s. DuPont patented the trilobal cross-sectional shape based on the modification ratio. Since then, there have been many fibers patented based on the cross-sectional shape. The characteristics of a fiber cross section coupled with patented information may enable the identification of a specific manufacturer.

Most manufactured carpet fibers have a trilobal shape which is intended to obscure the appearance of soil. Only a small amount of light is transmitted through a trilobal fiber resulting in the ability to hide particles trapped on the surface of the fiber. On the other hand, round fibers magnify the surface particles due to the large amount of light transmitted through the fiber.

Hollow fibers are often used for insulation. The hollow center holds air, which results in additional thermal insulation. Hollow fibers have a reduced weight due to the hollow center, which gives the textile a loftier appearance.

In the apparel industry, there are a variety of synthetic and regenerated fibers with different cross-sectional shapes. The most common cross-sectional shape is round. Star-shaped or pentalobal cross sections are often used in silk-like fabric. Trilobal cross sections may also be seen in apparel to achieve the appearance and texture of silk.

Practical Exercise 22

Subject: Sample Preparation for FTIR - Microscopy
Time: 4 hours (Part 1 ~2 hrs, Part 2 ~2 hrs)
Objective: To illustrate the effect of fiber thickness on the quality of spectral data and to explore the phenomenon of interference fringing

Theory:

The shape and thickness of fiber samples can affect the data obtained from them. Up to a certain thickness, Beer´s Law is obeyed and there is a linear relationship between specimen thickness and peak absorbance. Beyond a certain thickness this relationship breaks down. The ideal sample for making transmission measurements using infrared microspectroscopy is one which is 5-15 µm thick. Some fibers may be much thicker than this such as nylon, polyester and polypropylene carpet fibers (e.g. 50-70 µm). The absorption bands (particularly the Amide I and II bands in polyamides) will be unresolved and will produce spectra where the relative peak absorbances are distorted in samples of such thickness. This can be overcome by flattening the fibers prior to running spectra. Flattening also reduces refraction effects and inaccuracies due to variable path lengths through the fiber.

If a fiber has a flattened surface (ribbon-like), then internal reflection of the beam may occur. In this situation, some of the beam reaches the detector after having passed through the sample once, but a small fraction may be reflected twice thus reaching the detector after passing through the sample three times. The detector receives the sum of two interferograms, one from the beam that passes through the sample once and one from the beam that has passed through it three times. The centers of the interferograms do not coincide because the doubly reflected beam has a longer path length. Therefore, the total interferogram has two centerbursts, a main one and a subsidiary. The position of the subsidiary centerburst depends upon the thickness and refractive index of the specimen. Upon Fourier transformation of the spectrum, the total interferogram produces the sample spectrum and the subsidiary is transformed into a broad band impurity in the spectrum which is manifested as a sinusoidally modified baseline. The interference fringes can be removed after data acquisition, but this is a difficult process. The simplest remedy is to remove or reduce them by sample preparation involving roughening of the surface of the fiber.

Preparation:

It is assumed the trainee has already been taught the standard operating techniques for producing infrared spectra from fibers using the microscope-FTIR. Consult with your trainer to ensure that the instrument is properly prepared for use at least 20 minutes before you will use it.

The trainer should provide authenticated samples of acrylic fibers and nylon 6,6 fibers. The polymer composition of the acrylic fibers is not very important, but it will be helpful if the fibers are not too fine. A diameter of 20-25 µm should produce good results.

Materials:

- Microscope-FTIR instrument properly cooled and purged
- Stereomicroscope
- Fine scissors, fine forceps and scalpel blades
- Glass slides (smooth and rough)
- Double-sided adhesive tape
- Sample holder/discs (These may be the round metal disks with a central aperture. As normally supplied with the FTIR-microscope or double sided adhesive paper Disks with a punch-out central aperture obtainable from specialty suppliers.)
- Sample roller
- Microcompression cell accessory
- Authenticated fiber samples
- Dried potassium bromide

Directions:

Part 1 - The Effect of Fiber Thickness
Using the nylon 6,6 fiber sample and standard operating procedure, obtain spectra under the following conditions:

1. Without any sample preparation: Position a single nylon 6,6 fiber across the aperture of a sample disk. If necessary, secure the fiber ends with adhesive tape. Acquire the sample and background spectra.

2. Using some sample preparation: Take a single nylon 6,6 fiber and secure the ends with small pieces of adhesive tape taut across the smooth area of glass slide. While viewing with the stereomicroscope, apply gentle pressure using the sample roller to flatten the fiber. Keep the roller flat to produce a uniform ribbon. Remove a portion of the flattened fiber and mount it across the aperture of an appropriate sample disk, using double-sided adhesive tape if necessary. Acquire the sample and background spectra.

3. Using increased pressure: Make sure the transmission windows of your microcompression cell accessory are clean by viewing with the stereomicroscope. Do not touch the transmission windows with your fingers. Place a short length of nylon 6,6 fiber (about 2mm) centrally across the face of one diamond. Assemble the cell. While viewing

with the stereomicroscope, squeeze with very gentle pressure. With an incident light source above the cell you can observe the flattening of the fiber. Separate the windows. Place the window with the fiber under the FTIR microscope. Acquire the sample and background spectra.

4. Compare the spectra you have obtained from these three different preparation conditions and measure the peak ratios for the 2900/1650 cm-1 bands in each case.

Part 1- **Observations:**

If the three experiments have been carried out successfully, then you should observe significant differences in the quality of the spectra.

Part 2 - Reducing the Interference Fringes in Spectra

The spectra generated in this part of the exercise should be retained for future reference and comparison with those obtained in Practical Exercise 15. Using the acrylic fibers and standard operating procedure, obtain spectra under the following conditions:

1. Pressing the sample on a glass slide: Take a single acrylic fiber and secure the ends with small pieces of adhesive tape taut across the smooth area of glass slide. While viewing with the stereomicroscope, apply gentle pressure using the sample roller to flatten the fiber. Keep the roller flat to produce a uniform ribbon. Remove a portion of the flattened fiber and mount it across the aperture of an appropriate sample disk, using double-sided adhesive tape if necessary. Try to avoid twisting the fiber. Acquire the sample and background spectra.

2. Pressing the sample on a roughened surface: Repeat the procedure above to acquire the sample and background spectra, except mount and roll the fiber on the frosted end of a glass microscope slide this time.

3. Use the microcompression cell: Check that the transmission windows of the microcompression cell accessory are clean by viewing with the stereomicroscope. Do not touch the transmission windows with your fingers. Place a short length of acrylic fiber (about 2mm) across the center of one window with a very small crystal of potassium bromide next to it. Assemble the cell and apply very gentle pressure while viewing with the stereomicroscope. The crystal should spread out to a thin film around the fiber. Separate the windows. Place the window with the fiber and KBr under the FTIR microscope. Acquire the sample and background spectra. Note that the background spectrum will include the KBr.

4. Compare the spectra you have obtained from these three different preparation conditions.

Part 2 - **Observations:**

If the three experiments have been carried out successfully, then the trainee should observe significant differences in the quality of the spectra. With some fiber samples, it is practically impossible to totally eliminate all traces of interference fringes just by sample preparation. Their occurrence will depend on fiber thickness, fiber cross section and presentation of the sample to the infrared beam. Variance will occur from sample to sample. Nevertheless, the spectra produced by roughening (2 and 3 above) should be of substantially better spectral quality than that produced by flattening on a smooth slide only.

Discussion:
The trainee must decide what preparation is necessary to obtain satisfactory spectra and whether more preparation is necessary to improve spectral quality. After the completion of these experiments and having read the appropriate literature, the trainee should be able to assess the amount of pressure required for flattening fibers to produce good quality spectra. The trainee should be able to answer the following questions:

- Which of the three preparation methods has produced the best spectral resolution and peak ratios?
- Why is it important to make sure that the pressure used in flattening fibers is kept relatively constant when comparing samples?
- What are the advantages of flattening fiber samples?
- Name three possible disadvantages caused by applying too much pressure to the microcompression cell.
- Why is it advantageous to place only one transmission window in the infrared beam?

Interference fringes can lead to a sinusoidal modification of the baseline, and, if not eliminated, could lead to erroneous conclusions during spectral comparisons. The effect can be reduced by sample preparation. Roughening of the surface of the flattened fiber can help to reduce interference fringes by removing plane, parallel, and smooth surfaces. If the surface is too rough, then the incident beam will be attenuated by reflection and scattering. This may also occur due to the presence of titanium dioxide delustrant particles in fibers. Scattering may result in a sloping baseline, with the higher absorbance (lower transmittance) occurring at higher wavenumbers. The trainee must decide whether more preparation is necessary to improve the quality of the spectra.

Practical Exercise 24

Subject: Interpretation of Fiber FTIR Spectra
Time: 4 hours
Objective: To become familiar with recognition and interpretation of infrared spectra from the most commonly encountered generic types of manufactured fibers

Theory:

Fiber spectra may be identified in one of two ways. The first method is by comparison of spectral data against a data base (computerized or hard copy). The position and intensities of the bands in the spectrum are compared with the spectra of authenticated reference samples. The conditions under which the spectra have been recorded must also be taken into account. The generic class and the sub-generic class of synthetic fibers may be recognized. The second method is by using skilled interpretation of the spectral features in which peaks can be assigned to functional groups using published peak tables or flow charts. Spectra are often characterized by dominant bands, such as the nitrile group at 2245cm-1 or the carbonyl C=O stretch found in fiber spectra between 1750-1650cm-1.

Comparing to spectra in a data base may be done using software supplied with your instrument. The quality of spectral data will depend upon how they were collected. For this reason, the highest level of accuracy will be achieved using an operator-generated data base.

Preparation:

The trainer will provide unlabeled copies of fiber spectra, including, but not limited to, the following generic types:

- Cellulose acetate
- Regenerated cellulose (rayon)
- Polyester
- Polyamide (nylon 6 and 6,6)
- Acrylic (vinyl acetate and methylacrylate co-polymers)
- Modacrylic
- Polypropylene
- Polyethylene
- Chlorofiber

Materials:

- unidentified fiber spectra from the trainer

- fiber identification flow charts A, B and C included in this exercise (provided and adapted by MC Grieve from Robertton and Grieve, 1999)

Directions:

Use the identification flow charts to identify the fiber types from each of the spectra as accurately as possible by assigning functional groups to the most important peaks in each spectrum.

Determine which of the peaks and functional groups are crucial to the identification of each of the various fiber generic classes.

Observations:

Discuss your identifications with the trainer. Once the results of your interpretive exercise have been confirmed as correct, the labeled spectra and charts may be retained for reference purposes.

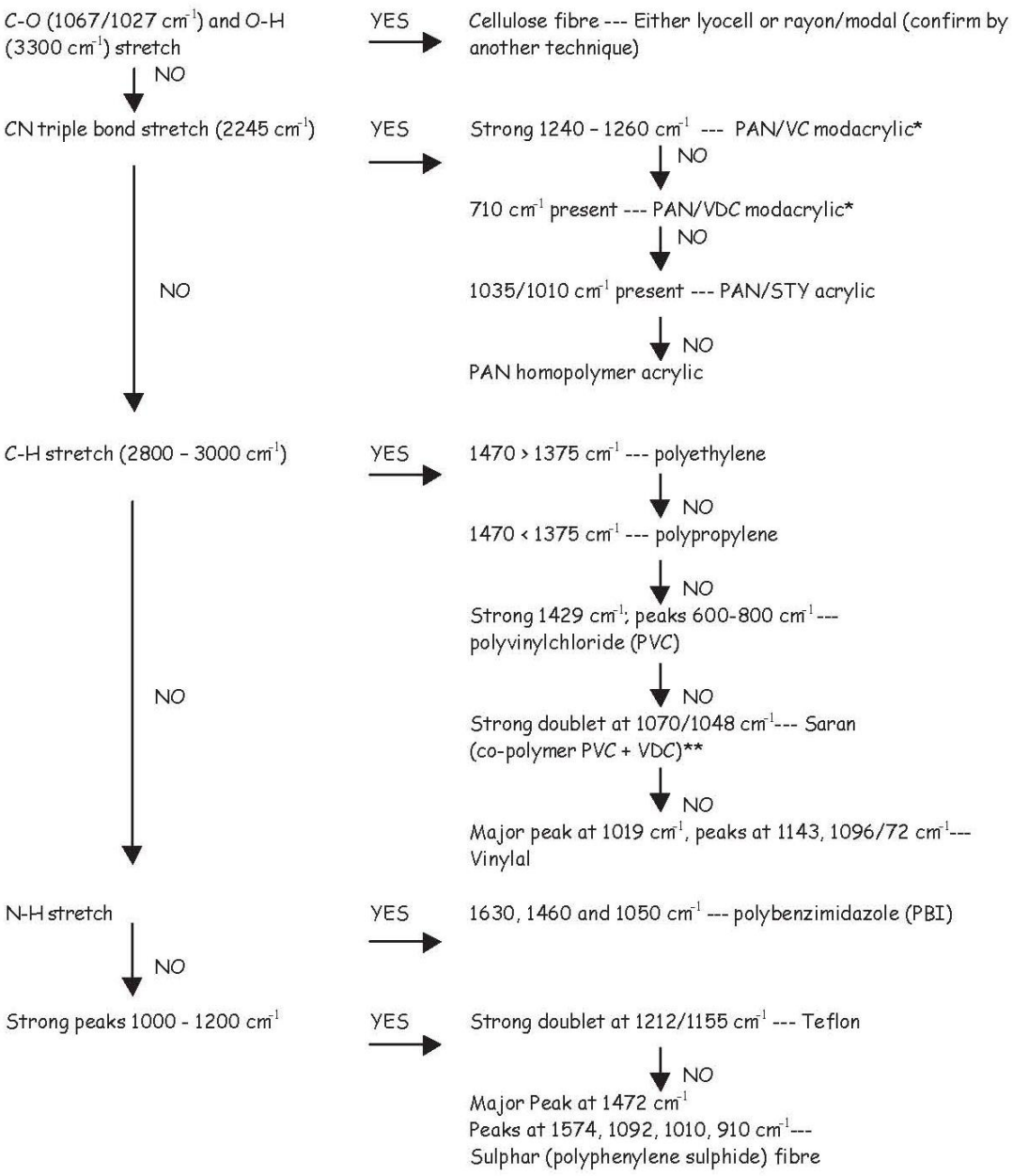
Discussion:

Successful identification is a matter of experience. One must be familiar with the basic spectral patterns that you are likely to encounter, and must also be aware of all of the factors that may cause deterioration of spectral quality. Attempts to assign "trade names" to fibers should be avoided and discouraged. Fibers of the same polymer composition produced in different plants may show the same spectrum. Your identifications should be limited to generic class or sub-class.

Infrared spectra will not permit distinction between natural or man-made cellulosic fibers, or between natural protein fibers (with the exception of silk).

Chart A

STRONG CARBONYL ABSORPTION (1650 – 1750 cm^{-1}) ABSENT



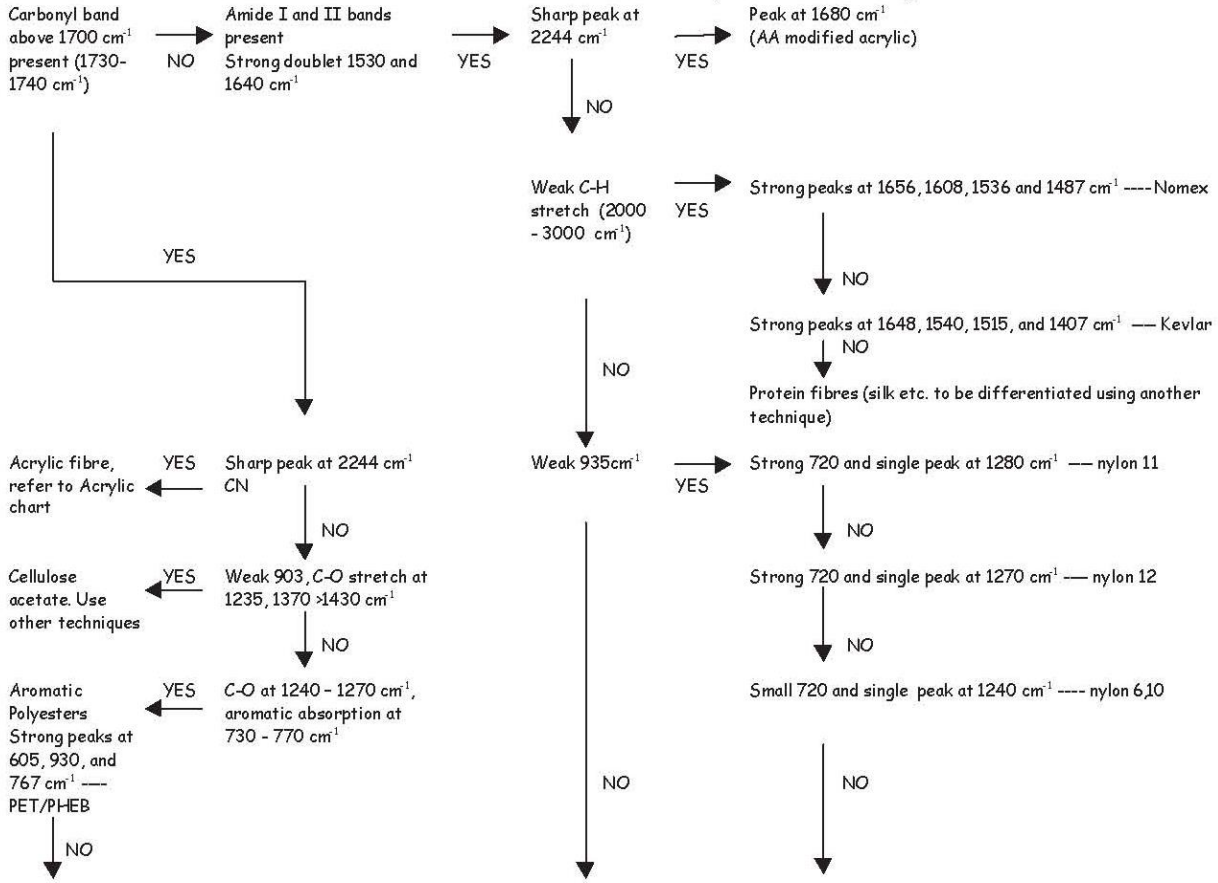
* For further details on modacrylic fibres see :
Grieve MC, Griffin RME. Is it a modacrylic ? Sci Justice. 1999; 39:151-162.

** In some Saran fibres PVC may be co-polymerised with vinyl acetate resulting in a carbonyl peak (of varying size) appearing in the spectrum at c.1730 cm^{-1}

Definitions:
VC = vinyl chloride; VDC = vinylidene chloride ; STY = sodium styrene sulphonate

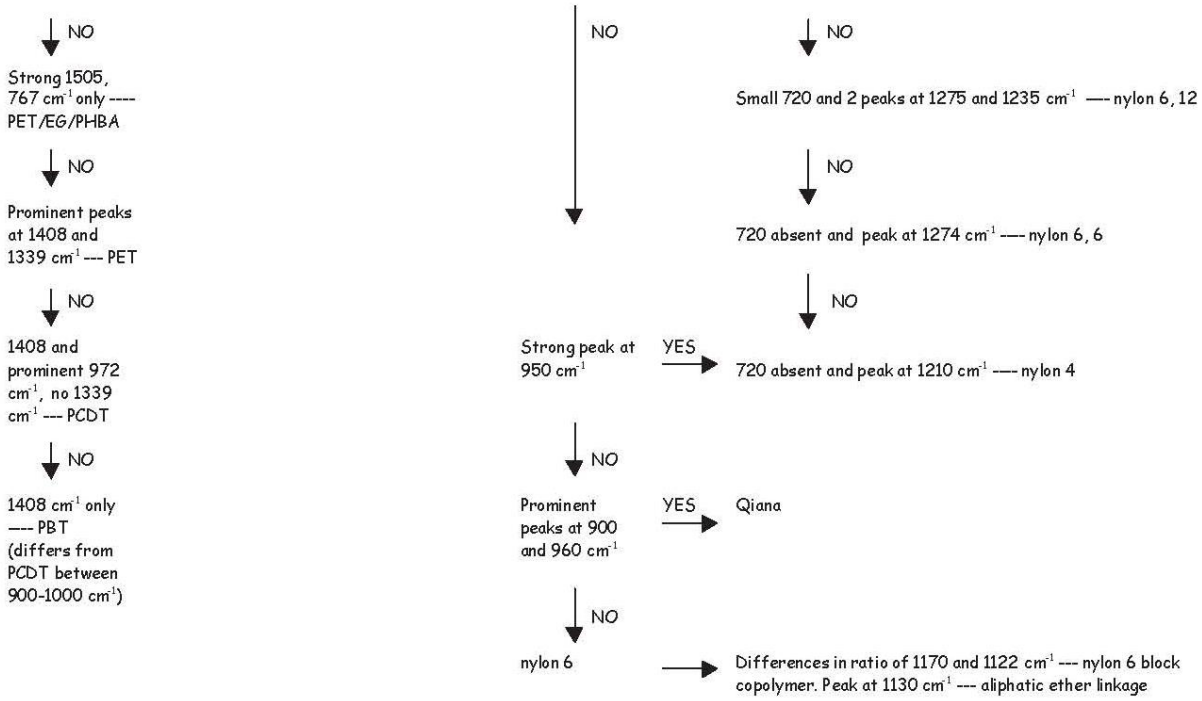
Chart B

STRONG CARBONYL ABSORPTION (1650 – 1750 cm^{-1}) PRESENT



(Chart B continued on next page)

(Chart B continuation from previous page)



Definitions :

AA	acrylamide
PET	poly(ethyleneterephthalate)
PHEB	poly(hydroxyethoxybenzoate)
EG	ethylene glycol
PHBA	*p*-hydroxybenzoicacid
PBT	poly(butyleneterephthalate)
PCDT	poly-1,4-cyclohexylene-dimethyleneterephthalate

Chart C
MOST FREQUENT ACRYLIC FIBRE TYPES

CN triple bond stretch
At 2245 cm^{-1} present

↓ YES

C-O stretch 1220 cm^{-1} and 1130 cm^{-1} present. → YES → PAN/MMA 1

↓ NO

C-O stretch 1170 cm^{-1} and 1204 cm^{-1} present → YES → PAN/MA 1, 2, 3, 4, 5, 6, 8

↓ NO

C-O stretch 1235 cm^{-1}. Minor peak at 940 cm^{-1} → YES → PAN/VA 2, 3, 7

MMA = methyl methacrylate
MA = methylacrylate
VA = vinyl acetate

Additional compounds may appear in acrylic spectra as indicated below.

Solvent residues	1670 cm^{-1}	dimethylformamide	1
	1805, 1785 cm^{-1}	ethylene carbonate	2
	2053 cm^{-1}	Sodium thiocyanide	3
Terpolymers	1580 - 90 cm^{-1}	itaconic acid	4
	1672, 1532 cm^{-1}	aromatic sulphonate	5
	1680, 1611 cm^{-1}	acrylamide	6
	1493 cm^{-1}	methyl vinylpyridine	7
	1042 cm^{-1}	aliphatic sulphonate	8

For further details on acrylic fibre spectra see :

Grieve MC. Another look at the classification of acrylic fibres. Sci Justice 1995; 35:179-190.
Grieve MC, Griffin RME. Is it a modacrylic ? Sci Justice 1999; 39:151-162.

Practical Exercise 25

Subject: Using the Comparison Microscope with Brightfield Illumination
Time: 8 hours
Objective: To compare the color and morphological features of fibers using the comparison microscope

Theory:

A comparison microscope is used to perform side by side comparisons of questioned (recovered) fibers with those from a known (control) source. A comparison microscope consists of two compound transmitted light microscopes connected with an optical bridge so that both fibers can be observed simultaneously in the same field of view. In order to gain optimal resolution, specimen contrast and color balance, it is essential that the optical conditions on both sides of such an instrument be properly balanced. Without the background illumination being properly balanced it would be very risky and dangerous to come to any conclusions. Color perception by humans is subjective and depends upon ambient light, the object being viewed, and the observer. Perceived color impressions can vary not only between individuals, but also by the same observer at different times. For this reason, it is necessary to employ additional objective methods of color examination in forensic examinations.

Preparation:

Before commencing these exercises, it is assumed that the trainee is familiar with the instrument in their laboratory and is able to:

- Set up and operate the microscope
- Color balance the light sources
- Correct any defects in the system

Notes to the trainer for slide preparations:
Color matching: The trainer should prepare a labeled set of ten (10) pairs of slides of different fiber types (e.g. wool, cotton, polyester, nylon, acrylic, viscose rayon, etc.) including four (4) pairs of natural fibers and six (6) pairs of manufactured fibers. The sample pairs should be a mixture of those that match in color (from one source), and those which are of a very similar color but which are dyed with a different dye or a different concentration of the same dye. Each pair should be made from fibers of the same generic type, cross-sectional shape, delustrant status and denier for manufactured fibers. The best source of “matching” fiber samples is to select them from dye manufacturers shade cards, where the dye has always been applied to the same fiber stock. (The trainer should check that this is the case.) The trainee should retain these slides for use in the microspectrophotometry exercises 29-32.

Delustrant matching: The trainer should prepare six (6) fiber slides of authenticated, round, colorless, semi-dull polyester. All of the fibers on the slides should be the same diameter. Four (4) of the slides should be made from fiber samples which originate from different manufacturers (e.g. Du Pont, Hoechst) or are different types from the same manufacturer. Two (2) of the slides should be made from fiber samples which originate from the same source.

Cross-sectional shape matching: The trainer should prepare a set of paired slides that contain fibers with matching or non-matching cross-sectional shapes and without having any other obvious differences in color, diameter, delustrant, etc. Recommended subtle differences could be obtained from mixtures of round acrylic fibers together with those of bean and dog-bone cross sections, nylon carpet fibers which exhibit different modification ratios, and black pigmented fibers showing different cross-sectional shapes, e.g. rayon and modal.

Permanent microscope slide preparations made during this exercise should be labeled carefully.

Materials:

- comparison microscope
- microscope slides and cover slips
- fine forceps and fine scissors
- suitable permanent mounting medium (e.g. Permount, XAM, etc.)
- a set of 10 pairs of slides prepared by the trainer for color matching (The trainee should retain these slides for use in the Microspectrophotometry exercise.)
- a set of 6 slides prepared by the trainer for delustrant matching
- a set of slides prepared by the trainer for cross-sectional shape matching
- a set of slides and a fabric swatch prepared by the trainer for all morphological features matching in manufactured fiber materials
- a set of 3 black cotton fabric swatches and a tuft of black cotton fibers prepared by the trainer for all morphological feature matching in natural fiber materials

Directions:

Part 1 - Making Slides to Assist in Balancing the Illumination

Choose a sample of a manufactured fiber which is uniformly dyed with a good depth of dyeing and preferably delustered (specific color is not important). An interesting cross section or morphological feature is an advantage. Considering the purpose for which this fiber use is intended, it is better that the yarn contains a single fiber type only. Pigment dyed fibers (not black) are quite good for this purpose.

Take a length of yarn (about 1cm) from the sample and cut it in half. Tease some of the fibers from each half out in drops of mountant on two slides, so that you end up with two identical preparations from the same source. Do not overload the preparations with fibers.

Observe these two preparations on the comparison microscope after both sides of the microscope have been set-up with Köhler illumination and the standard procedure for balancing the light sources has been followed. The fibers should appear identical.

If the position of the slides is now reversed so that the slide on the left stage is now put on the right stage, then the fibers should still appear identical. If these conditions are satisfied, then the trainee can be assured that the illumination is correctly balanced.

The trainee should retain these slides for personal use in evaluating the performance of the comparison microscope before casework analysis.

Part 2 - Color and Morphological Feature Comparison

Your trainer should provide you with sets of slides or fabric (which are a mixture of matching and non-matching pairs) for each of the examinations and comparisons specified below.

Examine all 10 pairs of slides made for the purpose of color comparison and decide which pairs are considered to be a match. For those which do not match, the trainee should discuss with the trainer why they have reached this decision.

Examine the set of slides made for the purpose of delustrant comparison and compare all the samples among themselves. Decide which, if any, match.

Examine the set of slides made for the purpose of cross-sectional shape comparison and compare all the samples to each other. Decide which, if any, match.

Use the comparison microscope to examine the 12 fibers for the purpose of morphological feature comparison and decide which, if any, could have originated from the "known" material.

Make the appropriate slide preparations from the black cotton swatches and fiber tuft. Use the comparison microscope to examine and compare your preparations. Try to determine from which of the black cotton fabric swatches the "recovered" fiber sample originated.

Observations:

Observe, compare and document all of the fiber morphological features. Any of these features may be of value in fiber comparisons. Did you observe any general or qualitative differences when comparing manufactured fibers and natural fibers?

Discussion:

In a casework situation, the comparison microscope must be used in a fiber association to ensure that all morphological features are indistinguishable in both "recovered" and "known" samples. Careful and detailed observations must be made of all features. For example, subtle differences in cross-sectional shape may be missed by inattention to detail.

After carrying out these experiments the trainee should be aware that one can expect variation in dye uptake and fiber diameter in natural fiber preparations (i.e. wool and cotton). They will have to decide whether they think that the features in the "recovered" sample fit within the range of those exhibited by the "known" sample.

Also, the trainee should have learned the very important role which delustrant plays in fiber comparisons. Not only the amount of the delustrant is important, but also the size and distribution of the particles.

If the microscope is fitted with a comparison fluorescence capability, then this exercise could be repeated using the different filter combinations available to determine if additional information becomes available which will help to confirm matches or exclude samples.

Practical Exercise 29

Subject: Microspectrophotometer Set-up and Operation
Time: 2 hours
Objective: To familiarize the trainee with the instrument's operational limitations including system stability, wavelength accuracy and resolution, photometric accuracy, linearity and absorbance limits

Theory:

It is important, if not essential, to document instrument performance, accuracy and stability. For this reason, most forensic laboratories have established quality assurance policies and procedures which specify instrument set-up and performance guidelines.

All instrumentation has practical and theoretical limitations. The instrument's operator must understand and document instrument performance, accuracy and stability before case samples are analyzed.

Preparation:

Review the operator's manual for your instrument and your laboratory's standard operating procedures for microspectrophotometry.

Materials:

- UV/VIS microspectrophotometer (MSP) system (UV, if available)
- NIST traceable reference absorbance standards (usually a set of 3 filters with a range from 0.1 to 1.0 absorbance units)
- NIST traceable wavelength calibration standards (e.g. Neodymium, Holmium, Didymium or Erbium oxide glass filters)
- An optical grade, spectral quality quartz reference filter (a clear reference filter usually with the same refractive index as the absorbance filters)
- Any adjustment tools or peripheral instruments required for microspectrophotometer set-up and calibration
- The instrument's historical calibration data

Safety:

Review and adhere to the manufacturer's safety recommendations for your instrument's operation, adjustment and maintenance.

Directions:

Turn on the instrument and light sources, allow them to thermally stabilize. Adjust microscope and spectrophotometer system for Köhler or partial Köhler illumination, and optimize spectrometer and detector optics. (Only partial Köhler illumination may be achievable because it is not possible to see the image of the filament on most modern microscopes.)

Set the instrument scan parameters:

- Step size for spectral scan instruments
- Number of scans for multi-channel instruments
- Desired wavelength range

Set instrument gain and/or measurement acquisition time as specified by the manufacturer. If available, system masking apertures will typically be left fully open for this test unless the MSP illumination is too intense for the detector. Always make sure to use the same objective for standards measurement.

Place the clear reference filter in the light path. Focus on the filter (it will usually have a dot to focus on) and acquire a "background spectra" on a clean area on the filter. This procedure will set the integration time for the instrument.

Perform a "dark scan" after closing off any light to the detector. The scan parameters should not be altered. This will measure the amount of dark current in the instrument.

Restore the light path to the detector and take a reference scan of the reference filter. The scan parameters should not be altered. This measures and compensates for the light absorbing effect of the clear reference filter, the light source, and the optics.

Run and record a standard wavelength calibration filter (or set of filters) spectra in both UV, if possible, and VIS range. Evaluate the wavelength accuracy of the instrument against the reported wavelength absorbance maxima supplied with the filter. Confirm that the MSP wavelength accuracy is within the required resolution window. This value is instrument and filter-set dependent, typically ranging from 1 nm to 10 nm. Note any corrections or correction factors in the instrument's calibration log. Evaluate the instrument's wavelength accuracy against historical performance data.

Run and record a set of standard neutral density filter spectra in both UV, if possible, and VIS range. Evaluate the absorbance accuracy of the instrument against the reported absorbance values at the various wavelengths in the filter manufacturer's certified calibration data. Note that the filter set should have been selected to test and validate both the detector linearity and cut-off wavelengths. Certified calibration values will be available for the near UV region for UV capable instruments.

Observations:

Assess and compare your MSP calibration results to the manufacturer's provided expected results and the instrument's historical record. Is the instrument set up and operating properly? What are the performance limitations of the MSP with respect to absorbance non-linearity, spectral regions of excessive noise, and spectral bandwidth limitations?

Define and record the performance check and calibration results, and any correction factors necessary to meet the laboratory's documentation requirements.

Discussion:

Absorption spectrophotometry is an inherently quantitative procedure that requires appropriate calibration of wavelength and photometric response. Instrumental operating parameters for the calibration should be the same as those used for normal casework. Periodically, the instrument performance should be comprehensively evaluated by using the same wavelength calibration standards with the instrument settings chosen to maximize system accuracy, precision and resolution. If the wavelength and/or absorbance values cannot be brought within the desired ranges by optimizing the microscope system, then the instrument's manufacturer should be contacted.

Practical Exercise 30

Subject: Acquiring Spectra from Single Fibers
Time: 5 hours
Objective: To evaluate dye variations, pleochroism and effects of various instrument operating parameters on single fiber examinations

Theory:

Fibers rarely dye uniformly. A degree of non-uniformity exists within fibers that appear to be uniformly dyed. In addition, other factors such as fiber morphological variations (cross-sectional shapes, inclusions, voids) and the optical phenomenon of pleochroism can affect the spectra obtained. Therefore, a range of absorbance spectra may be obtained if measurements are made along the length of a single fiber.

Preparation:

Perform instrument set-up, performance check and calibration as described in Practical Exercise 29. This should be done as frequently as is specified by your laboratory's operating procedures. Note that fibers used in this exercise will be needed for Practical Exercise 31.

Materials:

- UV/VIS microspectrophotometer (MSP) system (UV, if available)
- non-fluorescent mounting medium (spectral grade glycerin required if UV spectra are to be run)
- slides and cover slips (made of quartz if UV spectra are to be run.)
- forceps
- round, semi-dull, uniformly colored, authenticated polyester, nylon and olefin
- fibers (These fibers should originate from a colored thread or yarn which should be retained for use in Practical Exercise 31.)
- single colored authenticated fibers including an acrylic, rayon and a natural fiber such as cotton, ramie, or silk (These fibers should originate from a colored thread or yarn which should be retained for use in Practical Exercise 31.)

Directions:

Mount the round, semi-dull polyester, nylon and olefin fibers per your laboratory's operating procedure for UV/VIS MSP.

Place the mounted fiber preparation on the MSP stage with the fiber just outside of the optical path, adjust for Köhler or partial Köhler illumination.

Run and record background and fiber absorbance spectra using as many of the following system configurations as are possible:

- no specimen mask(s) in the beam path
- specimen mask(s) set just outside of the fiber edges
- specimen mask(s) set just inside of the fiber edges
- illumination field mask set just outside of the fiber edges and measuring aperture set just inside of the edges

[Note: Older instruments have a variable specimen mask(s), after the specimen and not between the light source and the specimen, to reduce/eliminate stray light. Most modern instruments do not have variable specimen masks and the field diaphragm is used to reduce/eliminate stray light]

Evaluate the resulting spectra and select the operational configuration that yields the spectrum with the highest absorbance values, assuming that the fiber is not too dark for the dynamic range of the MSP. This spectrum should be further evaluated for acceptable noise levels and any signs of exceeding the operational limits of the MSP. If the selected spectrum shows signs of exceeding the operational limits of the instrument, then make such corrective adjustments as are listed in the instrument manual. What are the possible remedies?

Note where the fiber's absorbance maxima are in relation to the maximum system (lamp) energy shown in the background scan. What are the possible ramifications of absorbance maxima that lay in the lower energy areas of the MSP's background/blank spectrum?

Using the selected configuration and system parameters, run and record 5 absorbance spectra from a single location on the sample fiber in both the UV and VIS range. Make no adjustments to the MSP between runs. Compare the spectra for reproducibility.

Place a polarizing filter oriented E-W in the light path, somewhere between the light source and the microscope stage. Using the selected configuration and system parameters, run and record 3 absorbance spectra from a single location on the sample fiber with a sample rotation between each measurement. (Rotate the MSP stage if equipped with a rotatable stage, or rotate your sample by hand on the MSP if equipped with a fixed stage.) Start with the fiber oriented E-W (horizontal), rotate 45 degrees in any direction for the second measurement, and rotate it again so that the fiber orientation is N-S for the last measurement. Do not make any adjustments to

the MSP between runs other than ensuring that the masks are appropriately set inside the fiber edges. Compare the spectra for reproducibility. Use both the UV and VIS range if available.

Remove the polarizing filter. If your instrument has a specimen mask, then using the selected configuration and system parameters, run and record 5 absorbance spectra at the same location on the fiber, resetting the mask(s) size, and refocusing the fiber and condenser between each run. Compare the spectra for reproducibility and note any apparent variations.

Using the selected configuration and system parameters, acquire, store and print absorbance spectra from 5 different regions of the sample fiber. Compare the spectra for dye uniformity.

Repeat this exercise using the mounted acrylic, rayon and natural fibers. In addition to the usual observations and comparisons among the spectra, take extra note of any absorbance spectra effects or variations related to fiber cross-sectional shape, inclusions or voids, diameter and surface variations.

Observations:

Assess and compare spectra as requested in each part of the directions for wavelength and absorbance differences.

Discussion:

Changing instrumental parameters has a notable influence on absorbance spectra. The optimal operational parameter set should be determined to yield the highest absorbance values. This parameter set should not change within a set of analyses. Reducing stray light in the MSP system will yield sample spectra with increased absorbance values and reproducibility.

By obtaining repeated absorbance spectra from a single location on the sample fiber it should be evident that the spectra are reproducible.

If a fiber exhibits pleochroism, then taking measurements as the fiber is rotated on the microscope stage will produce spectra that can have considerable wavelength and absorbance differences. When comparing fibers, it is generally recommended that the same relative fiber orientation is maintained for each sample.

By obtaining absorbance spectra from different regions along a single sample fiber without changing any operating parameters, it should be seen that the absorbance spectra do display variations which correlates with the heterogeneity of the fiber dye even in an apparently uniformly dyed fiber. These measurements will vary depending on the fiber sample. Generally, spectra of fibers from natural sources are more variable than from manufactured fibers. Other

physical (e.g. cross-sectional shape) and chemical variations within and between fibers can influence the spectra obtained. Measuring sites should be chosen to avoid obvious in-homogeneities within the area being measured.

Practical Exercise 31

Subject: Acquiring Known Spectral Sets and Comparing Spectral Curves
Time: 3 hours
Objective: To learn the typical dye uptake variation by fibers within a single yarn and to learn spectrum comparison rules, guidelines and quality criteria

Theory:

Fiber dyeing is generally non-uniform at the microscopic level. Dye concentration variations can, and usually do, exist along single fibers and among fibers dyed in a single thread or yarn. Dye variations occur in fibers that appear to be "uniformly dyed" (such as round cross section nylons or polyesters) and in fibers that appear unevenly dyed (such as cotton).

The meaningful comparison of UV/VIS spectra requires that they be evaluated from absorbance plots to accurately compare the dye concentration differences that exist between different shades of the same color. The color comparison of a questioned fiber to a known thread, yarn or fabric requires a complete analysis of the full range of color variation shown by both items. For this reason, it is important to compare the complete UV/VIS range of the questioned and known fibers.

Preparation:

Perform instrument set-up and calibration as in Practical Exercise 29. This should be done as frequently as is specified by your laboratory's operating procedures. You should have retained a sufficient amount of fiber yarn from Practical Exercise 30 to use in this exercise. If you did not retain such, then it is necessary for you to characterize new material before proceeding.

Materials:

- UV/VIS microspectrophotometer (MSP) system (UV, if available)
- stereomicroscope (for fiber sampling and mounting)
- forceps and teasing needles
- non-fluorescent mounting medium
- (spectral grade glycerin required if UV spectra are to be run)
- slides and cover slips (made of quartz if UV spectra are to be run.)
- a colored thread or yarn (same as used in Practical Exercise 30) used as the "known"
- electronic and/or hard copy plots of the spectra produced in Practical Exercises 30 and the same for any spectra produced in this exercise

Directions:

Part 1 - Acquiring Known Spectral Sets
Select individual fibers from the known thread or yarn to form a group of fibers that represents the complete range of colors and color shades exhibited by the known. Anywhere from 5 to 10 or more fibers may be necessary to represent all the variations in the known sample. These fibers should be selected and not randomly picked.

Mount the individual known fibers per your laboratory's operating procedure for UV/VIS MSP.

Place the mounted fiber preparation on the MSP stage with the fibers just outside of the beam path and, if present, set the instrument mask(s)/aperture(s) to approximate the size and position they will be in for the fiber analysis. Run and record both background and 100% line spectra under the conditions that will be used for the UV/VIS fiber analysis. Evaluate the 100%-line spectrum for indications of absorbance from the mounting medium, slide and cover slip.

Obtain and record one absorbance spectrum for each of the selected known fibers in both the UV and VIS range. Use the same instrument setup and parameters that produced the optimum spectra in Practical Exercise 30.

Randomly pick a group of individual fibers from the known thread or yarn and repeat above.

Part 2 - Comparing Spectral Curves
The "known" fiber spectra obtained from *Part 1* can be printed/plotted on a single graph.

"Questioned" fiber spectra from Practical Exercise 18should each be printed/plotted on a separate sheet of paper for overlay comparison with the knowns. All spectra should have the same units and ranges for the X and Y axes.

Examine the known and questioned fiber spectral data by overlaying them, and evaluate the following spectral features:

- Absorbance wavelength maxima and minima
- Peak slope angles
- Peak slope inflection points
- Overall peak shapes
- Absorbance range(s)
- Convoluted peaks
- The relative peak heights within multiple peak spectra

Determine which of the spectra match. The questioned fiber spectra should fall within the range of the known set and should precisely overlay one or more of the known fiber spectra. Variations

in dye concentration may preclude a precise overlay without spectral normalization and other manipulation

If the questioned sample spectrum is outside the range of the known data set, then select mount-run additional known fiber samples and/or analyze different areas of the previously mounted known fibers to insure that the known material was comprehensively sampled. Re-evaluate the comparison in light of the additional spectra.

Observations:

Spectra should be reviewed and evaluated for any focusing errors or other instrumental errors, and any unacceptable spectra should be rerun. Using the technique and criteria in Part 2 complete the following evaluations:

- Compare spectra from the individually selected fibers in Part 1 to each other.
- Compare the spectral range of variations observed in the group of fibers selected to represent the total range of the known in Part 1, to the range of variations observed in spectra collected from various locations along the length of a single fiber in Practical Exercise 30.
- Compare the spectral range of variations observed in the group of fibers selected to represent the total range of the known in Part 1, to the spectral range of variations observed in the fibers randomly selected from the known in Part 1.
- Compare the spectra from the individual fibers obtained in Practical Exercise 18 (the questioned fibers) to the known spectral set obtained in Part 1.

Review your evaluations of the spectral comparisons with your trainer. Discuss any spectral features or comparison questions you may have. Can you correctly answer the following:

- What are the limits of variation between spectra described as “similar” that are acceptable in your laboratory? Why?
- What are the spectral differences that are considered “exclusionary” in spectral comparisons made by your laboratory? Why?
- What is the acceptable terminology used to describe spectral comparisons? Note in particular which terms are considered unacceptable or misleading, and understand why that is so.

Discussion:
The need for multiple fiber sampling and multiple spectra collections during the evaluation of known fiber or textile samples should have become evident. A range of absorbance spectra should have been obtained from the same thread or yarn. This spectral variation range typically is greater than the spectral variation range obtained from different locations along a single fiber.

Determining how many known fibers to select to be certain that you have a representative range is not an easy task. Known fiber samples used to represent the color range should be critically selected to include the fibers from lightest to darkest and with visible differences in color. The full range of color variations present in a known sample may not be represented in a randomly selected fiber sampling.

The trainee is encouraged to repeat this exercise with fiber types that typically have more inherent dye uptake variation such as cottons. It is the discriminating eye and the experience of the examiner that ensures the detailed and adequate sampling of the knowns. As an approximation, usually 5 to 10 known fibers will be sufficient, with 3 to 5 measurements along the length of each fiber.

If a representative range of the known sample has been established and the questioned fiber spectral features do not occur within this range of the known set, then the questioned fiber cannot be associated with the known fibers/garment.

Practical Exercise 32

Subject: Examining Metameric Fibers
Time: 4 hours
Objective: To learn what metameric fibers are and how to identify them

Theory:

A metameric pair are two colors that appear the same in one set of lighting conditions but appear different in another set of lighting conditions. Metameric textile fibers will therefore appear similar macroscopically and microscopically but can be distinguished by UV/VIS spectroscopy and/or thin layer chromatography.

Materials:

- UV/VIS microspectrophotometer (MSP) system (UV, if available)
- stereomicroscope (for fiber sampling and mounting)
- forceps and teasing needles
- non-fluorescent mounting medium (spectral grade glycerin required if UV spectra are to be run)
- slides and cover slips (made of quartz if UV spectra are to be run.)
- a pair of metameric fibers

Directions:

Examine the fibers microscopically and compare their color. Mount the metameric fiber pair per your laboratory's operating procedure for UV/VIS MSP.

Place the mounted fiber preparation on the MSP stage with the fibers just outside of the beam path and, if present, set the instrument mask(s)/aperture(s) to approximate the size and position they will be in for the fiber analysis. Acquire and record both background and 100% line spectra under the conditions that will be used for the UV/VIS fiber analysis. If your instrument is not UV capable, then perform this step for the VIS range.

Acquire, store and print one absorbance spectrum for each of the fibers. Use the same instrumental set up and parameters that produced the optimum spectra in Practical Exercise 30 using the full UV/VIS range if your instrument is UV capable. If your instrument is not UV capable, then perform this step using the same instrumental setup and parameters that produced the optimum spectra in Practical Exercise 18-2 in the VIS range.

Observations:

Compare spectra from the fibers by examining all of the parameters previously learned as points of evaluation in Practical Exercise 18-3 Part 2. The metameric fibers should match in color by microscopical observation, but have different absorbance spectra in the UV and/or VIS ranges.

Discussion:

Dyes that visually match in color can exhibit different UV and/or VIS spectra. Therefore, a visual or microscopical color comparison is not sufficient when comparing two fibers of visually similar colors. Microspectrophotometry is an essential technique to be used in fiber comparisons, and it is a technique that should be considered complimentary to other methods of color analysis.

Practical Exercise 33

Subject: Examining Fabric Damage
Time: 6 hours
Objective: To understand the theory of how damage is created on fabric and recognize some characteristic patterns of textile damage

Theory:

Although it may not always be possible to identify the weapon causing damage to a fabric, often there are recognizable and characteristic patterns of damage that could be associated to a type of weapon or instrument utilized in creating the damage. There are many variables including, but not limited to, the type of textile, fiber composition and construction of the fabric, type of instrument and motion creating the damage, shape and sharpness of instrument edge(s), and underlying supporting structure which can affect the fabric damage appearance.

The term STAB refers to damage caused by penetration of an instrument through the fabric. The term SLASH refers to damage caused by a sharp instrument along the fabric. The term TEAR refers to damage by physical stress exerted in opposing directions which creates a break through the fabric.

Despite the number of variables that may be involved in creating fabric damage, with some appropriate experience the characteristic patterns of damage should become recognizable and aid in the forming of an opinion as to the general type of weapon used or not used.

Preparation:

There should be a specific time/day determined to perform this exercise in order to accommodate purchase of the simulated skin without decomposition occurring, and to ensure other personnel's presence for safety reasons.

Materials:

- stereomicroscope
- 3 cutting and stabbing instruments such as a kitchen knife, razor blade, scissors, or screwdriver
- at least two types of fabric including woven, knitted or nonwoven composed of different fiber types and blends
- uncooked rolled or flank of pork (described as a close representation of human skin)

Safety:

This exercise requires the unusual and forceful use of sharp “weapons” on atypical substrates. Therefore, it is recommended that the weapon wielder take extra precaution in selecting personal protective equipment which should include heavy duty eye protection and workman type gloves. The weapon wielding should be performed in the presence of other personal who must be outside the range of “weapon” action at all times but are available for immediate assistance should any accidental injuries or events occur.

Directions:

Perform the following steps using the different cutting and stabbing instruments and fabrics constructed with different materials. Repeat these steps with each different instrument.

Place the fabrics over the rolled or flank of pork. It is a good idea to cover the pork with paper to protect the fabric from absorbing the pork oils.

Select one of the instruments to be used in creating fabric damage. Create damage to the fabric by stabbing, stabbing and tearing, or slashing the fabric in the area supported by the pork flank. Create “wounds” at varying angles to the weave or knit pattern. Also, create “wounds” in fabric that is folded or wrinkled. Use a permanent marker to designate the type of instrument and method used. You should create a minimum of eight “wounds” per instrument and “wounding” method.

Repeat for each instrument and each fabric sample.

Examine each of the damaged areas noting similarities or dissimilarities in the characteristic fabric damage as related to the type of instrument used and the fabric composition.

Cut each fabric sample in several directions and compare the appearance of the cuts to the other types of damage. Manually tear parts of the fabric and compare the appearance of the tears to the damage created by stabbing and slashing the fabrics. Attempt to physically match the torn free ends. Have someone else tear several types of fabric into pieces for you, and then attempt to physically match the torn free ends.

Retain the damaged fabric sections for use in Practical Exercise 34 .

Observations:

The trainee should identify and describe in appropriate terms the characteristic similarities and dissimilarities among the types of instruments, acts of force, and fabrics used in this exercise. The

trainee should start by noting the differences among sharp instruments (razor blade) and a dull knife utilizing the stabbing, slashing and tearing methods.

Discussion:

Some of the described characteristic patterns of fabric damage that are typically reported in the literature (see Monahan and Hearle) include:

- Stab damage with a sharp instrument often displays a clean-even edge with a “nick” at one end
- Stab damage with a dull instrument often displays a disarray of fibers along the edge and evidence of stress to the yarns
- Slash damage often displays interrupted damage with yarns partially severed usually found at the ends
- Tear damage displays evidence of stress along the margin and ends

Performing similar tests for an actual case may be necessary to substantiate one’s opinion as to the type of instrument and act used to create the fabric damage. It should be emphasized that when performing these types of tests for an actual case the same type of fabric should be used in the test as is found in the actual case material. Different fabrics may display different characteristics using the same instrument.

Practical Exercise 34

Subject: Environmental, Chemical and Mechanical Effects on Fabrics
Time: Variable
Objective: To explore and examine how different environmental, mechanical and chemical conditions affect fabrics

Theory:

During the course of laboratory examinations or crime scene investigations you will encounter fabrics that have been subjected to environmental, mechanical and chemical damage. The textile material most commonly encountered will be clothing, however, one should not overlook other textile materials.

Some conditions that may have led to observed fiber and fabric damage can be duplicated under laboratory controlled experiments. Some types of observed fiber and fabric damage can be recognized by experience. The ability to recognize some types of damage, and to discriminate the damage from normal wear patterns, can be very important in situations in which the conditions that may have led to the damage are difficult or impossible to duplicate in controlled experiments. For example, some fabric materials melt because of friction that may occur during the mechanics of a hit-and-run. These may be difficult, if not impossible, to duplicate but are important to recognize. This exercise will explore some of the conditions that may be duplicated under controlled circumstances and allow noting the effects on fabrics.

Preparation:

In obtaining the clothing and other textiles for the exercise be sure that there is sufficient fabric of a like type to have comparison swatches and multiple swatches for various exposures. A second hand clothing store may be a useful source of these materials.

Materials:

- Stereomicroscope
- Sharp forceps, fine scissors and razor or scalpel blades
- Various types of clothing composed of different fiber types and weaves to include single fiber compositions and blends
- Various other types of textile materials such as sheets, blankets, carpeting, towels
- Various chemicals and mechanical devices as suggested in the directions section
- Your ingenuity and imagination

Directions:

Cut each of the fabrics into swatches such that a sample of the original material is retained as a comparison sample, and there is sufficient material to be used for experimentation.

Subject the fabric swatches to a variety of environmental, mechanical and chemical conditions. Record the type and duration of exposure. Some suggested types of exposures are:

- Environmental
- Weathering (sun, rain, snow, heat, cold)
- Burial
- Submersion in fresh, salt or pool water
- Mechanical
- Laundering
- Stretching/loading
- Crushing/impact
- Tying
- Abrading by rubbing, dragging, scuffing
- Burning
- Pinching, crimping
- Repeated use over a pulley system
- Chemical
    - Acids like HCl, H2SO4, acetic
    - Bases like NaOH, ammonia compounds (fertilizers)
- Ignitable liquids like gasoline, kerosene
- Bleach, hydrogen peroxide, other household chemicals

Perform some of the environmental testing, particularly burial or burning, on fabric samples from Practical Exercise 33. Examine severed edges from Practical Exercise 33 after the fabric has been soaked with blood.

Compare the exposed fabric to the comparison sample. Note any changes, if any, which have occurred.

Observations:

Examine the test fabrics for damage and compare them to the saved comparison exemplars. Note any changes from the original fabric samples, if any. Look for fading, degradation, and other physical changes. Look for differences in the reactions of the different fiber types in blends. Is one more susceptible to exposure than the other? Accurate notes should be kept as to the type of exposure or action, and the length of time or number of repetitions for the exposures.

Discussion:

Your observations may show a wide range of variation. Some textile compositions may exhibit obvious changes while others exhibit no change. There may be some subtle differences that can only be seen through very careful and thorough observation.

Blends may pose a situation in which one type of fiber may be more susceptible to damage than the other(s) such as bleaching or chemical degradation. It is important to recognize and note the composition of blended materials.

One should also observe the normal and typical wear and tear patterns exhibited on clothing. Look at different wear patterns on your own clothing (e.g. pants, shirts, socks) to recognize some normal wear patterns. For example, it's not unusual for blue jeans to exhibit fading at the knees and fraying around the pocket areas.

The experimental exposures suggested in this exercise are not intended to be comprehensive. You will encounter circumstances related to casework that have not been included in this exercise. You are encouraged to devise your own experiments that will simulate these newly encountered scenarios and conditions, such as insect and animal damage.

Practical Exercise 35

Subject: Composition and Physical Construction of Natural and Manufactured Fiber Cordage
Time: 6 hours
Objective: To learn techniques for assessing the composition and physical construction of cordage

Theory:

Numerous variations in construction, assembly, and fiber content can be found among existing cordage. The fiber content may be of natural or synthetic origin, and be homogeneous or heterogeneous. The fibers may be staple, filament or film. The cordage construction may vary from simple one-ply twisting of fibers to complex multi-component multi-ply twisted or braided arrangements. All of these characteristics can serve as points of comparison between separate pieces of rope.

Preparation:

Note that Practical Exercise 37 will also require the use of the same cordage types examined in this exercise. You will have to have sufficient material of the cordage type used in this exercise, or plan on doing more cordage characterizations in order to complete the next exercise.

Materials:

- Stereomicroscope
- Sharp forceps, fine scissors, razor or scalpel blades
- A dark velvet-covered board (minimum 6 x 6cm)
- Various types of natural and synthetic fiber ropes, twines, and cords to include shoelaces and drawstrings

Directions:

Perform for at least 6 different types of cordage. The variety of cordages should encompass natural and synthetic types, as well as various levels of structural complexities.

Examine the cordage macroscopically and under the stereomicroscope. Determine the general outer structural details to include, where applicable:

- Diameter
- Length
- Type of structure (twisted and/or braided)
- Number of plies/strands
- Twist direction (z or s)
- Type of braiding
- Length of lay (crowns or turns/cm)
- Color
- Internal and external marker yarns

Determine parts/components present (core and/or sheath(s)). This can be done by gently prying apart the outer plies to examine underneath. Also examine a cross-sectional view of a cut end of the cordage.

Separate the outer sheath plies at the end of the piece of cordage. Cut a one cm sample of one of these plies. If other components exist (inner sheath, core), then sample these as well.

Where applicable, determine the following from the cut plies of each component:

- Twist direction of yarns or fibers (Z or S)
- Number of yarns twisted together
- Length of lay of twisted yarns
- Type of twisted fibers (staple, filament or film)
- Type of core (mono- or multi-filament)
- Color

For twisted yarns, determine the following:

- Twist direction of fibers (Z or S)
- Type of fibers (staple, filament)
- Presence of a core filament at center of twisted fibers

Determine the number of filament fibers per yarn or ply. This can be done by cutting a small section of the ply or yarn and spreading the fibers parallel to each other on a velvet-covered board. By displacing one fiber at a time with forceps, the fibers can be counted under a stereomicroscope.

For a complete comparison of each component/part of the cordage the chemical composition and color of the fibers must be analyzed with the appropriate microscopic, chemical or instrumental technique(s), as applicable. This aspect has been dealt with in other sections of this training document.

For each type of cordage, you are examining, cut one piece into two and attempt a physical fit. Disrupt the cut ends to some degree and again attempt a physical fit.

For each type of cordage, you are examining, attempt single and double edged force cuts, with and without tension stress. Attempt a physical fit of the free ends, and examine the free ends macroscopically and microscopically to observe resultant separation characteristics.

Observations:

A checklist should be set up to document the relevant parameters observed by the trainee with each cordage examination. Drawings may be useful in documenting the type of braiding present but the trainee should still be learning to apply the appropriate terminology.

Discussion:

In casework, the free ends of evidentiary cordage should be protected and not sampled to preserve the possibility of a physical fit between a questioned and known item. In the absence of a physical fit, cordage is commonly examined in forensic casework for comparison purposes to determine if two pieces could have originated from the same source. In this instance, all components of the cordage should be examined and evaluated until a significant difference is found. Sometimes this requires full examination to determine if differences are “minor” or “significant”, and to possibly identify a manufacturer. The identification of a manufacturer may be useful for assessing production volume, availability and commonality of some cordage, and for other investigative purposes.

It is important to note that one must be wary of the possibility of a heterogeneous fiber content in cordage. This is particularly true for natural fiber cordage which may, in fact, vary along the length of the rope or twine.

Practical Exercise 36

Subject: Environmental, Chemical and Mechanical Effects on Natural and Manufactured Fiber Cordage
Time: Variable
Objective: To learn how different environmental, mechanical, and chemical conditions affect natural and synthetic fiber cordage

Theory:

Cordage encountered during the course of an investigation or at a crime scene may have been subjected to a number of different environmental, mechanical, and/or chemical conditions depending on the case circumstances. In some case situations the effects of these various conditions on cordage fibers could appear to be significant and unexplainable discrepancies between questioned and known samples, and, yet really be explainable discrepancies due to differential exposures. In other case situations the various exposure effects on cordage fibers could be a significant point of positive comparison between questioned and known samples.

Preparation:

Refer to completed Practical Exercise 36. Make sure you have sufficient cordage from each previously examined item to subject each to the following conditions. If this is not the case, then you should examine and characterize additional cordage with sufficient material at hand to complete this exercise.

Materials:

- Stereomicroscope
- Sharp forceps, fine scissors and razor or scalpel blades
- A dark velvet-covered board (minimum 6 x 6 cm)
- Various types of natural and manufactured fiber ropes, twines, and cords to include shoelaces and drawstrings
- Various chemicals and mechanical devices as suggested in the directions section, and your imagination

Directions:

Subject the cordage previously examined in Practical Exercise 22 to a variety of environmental, mechanical, and chemical conditions. Record the type and duration of exposure. Some suggested types of exposures are:

- Environmental
- Weathering (sun, rain, snow, heat, cold)
- Burial
- Submersion in fresh, salt or pool water
- Mechanical
- Laundering
- Stretching/loading
- Crushing/impact
- Tying
- Abrading by rubbing, dragging, scuffing
- Burning
- Pinching, crimping
- Repeated use over a pulley system
- Chemical
- Acids like HCl, H2SO4, acetic
- Bases like NaOH, ammonia compounds (fertilizers)
- Ignitable liquids like gasoline, kerosene
- Bleach, hydrogen peroxide, other household chemicals

Re-examine the cordage using the same techniques learned from Practical Exercise 36. Note any changes from the original conditions you observed, if any. Examine all fiber types and layers of construction from outside to inside for signs of damage or change. Accurate notes should be kept as to the type of exposure or action, and the duration and number of repetitions.

Observations:

Careful examination and documentation of the rope fibers from the outside to the inside is important. Can you make any generalizations about any of the exposure conditions relative to the fiber or cordage types you examined?

Discussion:

Your observations probably show a wide range of variation among the different cordage types, and among the different conditions to which they were exposed. Some ropes may be very vulnerable to one set of conditions while another may be inert or unaffected. Careful examination of the rope fibers from the outside to the inside is important to determine the depth

and extent of damage, if any. For example, the outer exposed fibers may show sun bleaching and disintegration while internal fibers may be protected and retain their original coloration and viability. A rope composed of different materials may not show any significant changes externally because the outer material is inert to chemical exposure while the internal fibers, with a different composition, may be more susceptible to the chemical exposure.

The experimental exposures suggested in this exercise are not intended to be comprehensive. You will encounter circumstances related to casework that have not been included in this exercise. You are encouraged to devise your own experiments that will simulate these newly encountered scenarios and conditions, such as insect and animal damage.

Form 1

	Sample Type/#	Microscopy	MSP	FTIR	SEM	GC/MS	Notes
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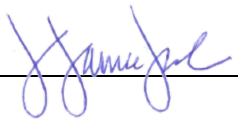
Approval

Technical Manager  Date 11/19/25

Laboratory Director  Date 11/19/2025

Comments

History

Revision	Effective Date	History
0	01-17-2012	Original Issue
1	08-15-2014	Complete rework, including instrumentation and adding specific disciplines
2	12-31-2015	Moved some reading in FTIR, MSP, SEM/EDS, Microscopy and GSR from required to optional. In Fiber Analysis, increased number of samples to be analyzed from 25 to 60. Removed Practical Exercises 11, 13, 14, 15, 20, 23, 26, and 29.
3	12-31-2016	Changed wording in Physical Match training to the trainee must have completed search and collecting trace evidence in the laboratory or Microscopy. Added "Goals" to the "Introduction" section. Added statement regarding the trainee's knowledge and understanding of the Orientation section, prior to signing off on the section.
4	12-01-2017	Annual Review. Changed Table of Contents formatting. Added language to each section in regards to signing off on a section and what it means**.** Added formatting to sections to match the Drug Training Manual (i.e. spacing, bolds, bullets, etc.). **Evaluation of Training -** Added language, the last two paragraphs, to meet ANAB requirements. Added requirement for documentation of training on the QA server. **Orientation to OSBI** – Added bullets to Goals. Changed formatting of tables (to reduce size) and match Drug Training Manual. Added "Testimony" to Presentation of Evidence in Court title. **Trace Evidence Searching & Collecting** - Added lettering to subgroups of Tasks. Added section: Requirements Prior to Evidence Searching… **Trace Analysis by FTIR** – renamed to **Fourier Transform Infrared Spectroscopy (FTIR)** **Microspectrophotometry (MSP) for Trace Analysis** – renamed to **Microspectrophotometry (MSP)** **Instrumentation** & **Trace Analysis –** Moved paragraph/sections to **Introduction** **Fiber Analysis** - Added section: Requirements Prior to… **Elemental Analysis** - Added section: Requirements Prior to… **Paint Analysis** - Added section: Requirements Prior to… **Practical Exercise 19** – Removed **Practical Exercise 29** - Added
5	07-01-2019	Annual Review. Updated Table of Contents to include Microscopy section. **Introduction** – Added goals for the Introduction section.

Trace Evidence Searching, Collecting & Documenting – renumbered sections in Trace QA Manual. Removed the following articles: "Selection of an Adhesive Tape…" and "Paint & Tape: Collection & Storage…", "Fiber Transfer & the Influence of Fabric Softener", "Can your Digital Images Withstand...", "The Admissibility of Digital Photographs…", "SWG Imaging Technology: Section 11 Best Practices… & Section 19", "The Transfer of Textile Fibers During Simulated Contacts", "An Improved Method for Rapid…". In the Casework Section: changed "Assist with" to "Observe the processing of 2 cases…". Removed the requirement to "Search & recover evidence in two separate cases…" In the Processing Outside the Lab section: changed "Assist with" to "Observe the processing of 2 scenes…" and removed the "Process a scene outside the lab…" requirement.

FTIR – Literature Reading, article: Uniting Microscopy and Spectroscopy, changed pages from 41-84 to 41-75. Removed the following reading: "Pavia, Lampman & Kris, Intro to Spectroscopy, Suzuki, Forensic Science Handbook, Skoog, Heller & Nieman, Principles of Instrumental Analysis, Articulate/Microscope: corrected spelling from sign to sine

MSP – Literature Reading: added "(user manual)" to MSP operations manual

SEM/EDS – Changed page #s for Chandler, X-ray Microanalysis in the Electron Microscope to 327-356 & removed 410-411.

Fiber Analysis – Title changed to **Synthetic Fiber Analysis**. Added "synthetic" when referring to fibers in intro & goals. Removed the following readings: Watson, Encyclopedia of Analytical Science, Annis, Bresee & Cooper "Influence of Textile Structure…", Salter, Cook & Jackson "Differential Shedding from Blended Fabrics", Grieve "Influential Factors, Quality Assurance…", Petraco, DeForest & Harris "A New Approach to the Microscopical…", Grieve "Another Look at the Classification…", Tungol, Bartick & Montaser "Analysis of Single Polymer Fibers…". In the Discuss with Trainer section, added "Cotton Analysis." In the Skills Demonstration section added "Detection & id of cotton." In the Sample Analysis & Instrumentation section added "Analyze 10 cotton samples."

Paint Analysis – Updated ASTM E1610-02 to E1610-17. Added ASTM E2937-18 and E2809-13. Added the following readings: Morgans, Outlines in Paint Technology, VanHoven & Fraysier, "The matching of automotive paint chips…", Ryntz, "Automotive Coatings Current Trends for Coating Plastic, Part 1." Removed Nielsen, "Forensic Analysis of Coatings," Duerst, "Depth Profiling & Defect Analysis…" Added Ryland, "Discrimination of 1990s Original…" Removed Stoecklein, "Forensic Analysis of Automotive Paints…" Coatings Encyclopedic Dictionary, Fuller, "Introduction to Coatings Technology," Fuller, "Formation and Structure of Paint Films," Fox, "Oils for Organic Coatings," Morgans, Outlines of Paint Technology. Changed required reading in Automotive Paints and

Coatings. Removed Farkas, "The Industrial Paint Making Process." Added Thornton, "Solubility Characterization of Automotive Paints." Removed Triplett, "Lab Tests: Where the finish starts." Changed heading from **Microscopy & MSP Review** to **Microspectrophotometry**. Removed Palenick, "Microscopy and Microchemistry of Physical Evidence." Added Kopchick "Color Analysis of apparently…" and Stoecklein, "The role of colour…" Removed "Review" from **Infrared Microspectroscopy Review** section name. Added ASTM International E2937-18. Removed Derrick, "Infrared microscopy…" and Teetsov, "Unique preparation techniques…" Added Suzuki, "Forensic science applications…" and Beveridge, "Use of infrared spectroscopy…". Renamed **Microtome** section to **Sample Preparation**. Added McEwen "Infrared microscopic…," Wilkinson, "The examination of paints…," Orzechowski, "An optical microscopy method…," Derrick, "Infrared microspectrocopy…," Teetsov "Unique preparation techniques…" and Groves, "Applications of blue light-curing…" Removed Laing "The examination of paint films…" Removed "Review" from **SEM Review.** Added ASTM International, E2809-13. Renamed section **Microscopical Recognition/End Use Classification** to **Microscopy**. Removed Hudson, "The paint index…" and Norwicki, "Examination of US automotive paints…" Added Palenik, "Microscopy & Microchemistry of Physical Evidence." Removed Orzechowski, "An optical microscopy method…," Williams "Automotive Finishes," & Schoff, "Surface defects…" Removed sections and all readings: **Fracture Characteristics and Comparison, Binder Classification** and **Binder Examination (IR).** Renamed **Pigment and Extender Examinations** section to **Pigment and Extender Pigments**. Added Morgans, Outlines in Paint Technology as a reference. Removed Crown, The Forensic Examination of Paints & Pigments and Coatings Encyclopedic Dictionary. Removed section header **Pigments and Extenders – Light Microscopy**. Removed section header **Pigments and Extenders – Infrared Spectroscopy** and all readings except Buzzini "A market study of green…" and Henson "Scanning electron microscopy…" Changed The Particle Atlas to a reference. Removed section **Pigments and Extenders – UV/VIS MSP** and all readings except Laing, "The discrimination of small fragments…" Removed sections **Additive Examinations** and **Aftermarket Treatments, Weathering, Aging and Contaminants** and all readings. Changed section name from **Significance and Interpretation Presence of Paint** to **Significance and Interpretation**. Removed Pearson "Glass and paint fragments…" Removed section header **Significance and Interpretation Type of Paint (Classification)** and reading Deaken, "Automotive body primers…" Removed section header **Significance and Interpretation Comparison and Discrimination of Paint** and readings: Ryland "The evidential value of automobile paint chips" and "The evidential value of automotive paint Part II…" Removed McDermott "The evidential value of paint…" Removed **Additional Optional Reading** and all readings. In

		Articulate &/or Provide Written Answers, General Background section removed "Give examples of materials used in each component" and "Define the difference between a liquid paint and a dried paint film…" In **Binders – Discuss the following with trainer**, removed "A classification scheme for binders suspended in different solvents…" and "A classification scheme for binders based upon their mode of cure…" In **Practical Exercises**, **Microscopical Examination & Characterization**: added requirement to "Prepare microscope slides of individual layers with a chip for all below," "Utilizing samples below, characterize at least 30 paint samples…" and "Manually prepare thin cross-sections of at least 30 paint chips." Changed sample requirements from 3 to 10. Removed "Prepare microscope slides of individual layers within a chip," "Manually prepare this cross-sections using a scalpel…", "Prepare polished cross-section…," "Prepare pigment dispersions…," "Microscopically characterize at least 30…" Changed section **Binders** to **Binders-FTIR**. Changed the number of samples from 5 to 10 for single layer paint samples. In **Pigments and Extenders – Infrared Spectroscopy**, added "multi-layered" to the description of paint samples for IR spectra interpretation. Removed "Prepare and analyze individual layers of…" In **Pigments and Extenders-UV/VIS MSP**, changed the number of samples from 5 to 10 for analysis and removed the word "surface." Removed "Acquire a spectrum from at least one clear coat…" Removed section **Additives** & requirement. **Requirements Prior to Paint Analysis** – Changed number of samples from 50 to 25 and number of practice cases from 15 to 5. **Ignitable Liquid Analysis** – No changes. **Gunshot Residue Analysis** – No changes. **Physical Match Analysis** – Removed readings: Von Bremen, "Shadowgraphs of bulbs…," Klein, "Physical match of fragmented bullets," Shor, "The Identification of Stolen Paintings…," Shor, "Physical match: insole and shoe," Stowell, "Use of Scanning Electron Microscopy…" **Pressure Tape & Adhesive Analysis** – Removed readings: Developments in Pressure Sensitive Products, Sclademan, "Tackifiers and their effect on adhesive curing," Polymer Chemistry An Introduction, Handbook of Adhesive Technology and Handbook of Pressure Sensitive Adhesive Technology. In **Instrumentation**, removed: Merrill, "Analysis of pressure sensitive adhesive tape…," Rappe, "Measurement of the Principal Refractive…," and Hemsley, The Light Microscopy of Synthetic Polymers. In **Electrical Tape/PVC Tape** removed readings: Bradley "A validation study for vinyl electrical tape…," Kee, "The characterization of PVC adhesive tape," Kero, "Forensic characterization of black polyvinyl…" In **Polypropylene Tape** removed reading: Smith, "Forensic examination of pressure sensitive tape.
6	01-15-2021	Manual was reviewed 11-20-2020 for year 2020.

		Introduction - Added paragraph regarding Training Notebook being scanned & uploaded into folder on QA server. **Orientation to OSBI** – Removed Goals: ethics (Removed entire Ethical Practices in Forensic Science section); intro to OSBI & CSD (Removed entire General OSBI/Laboratory Overview section); OSBI Safety Polices (Removed entire Safety section). General Knowledge of FS & Applicable Criminal & Civil Law & Procedures-removed Saferstein readings. Quality System Overview – removed all but OSBI Trace QAM. **Liquid Nitrogen Safety** – No changes. **Trace Evidence Searching, Collecting and Documenting** – No changes. **FTIR** – No changes. **GC/MS** – No changes. **MSP** – No changes. **SEM/EDS** – No changes. **Microscopy** – No changes. **Fiber Analysis** – No changes. **Elemental Analysis for Trace Analysis** – No changes. **Paint Analysis** – Removed from **Literature Reading** – Stoecklein, "Forensic Science: Paints, varnishes & lacquers," Ryntz "Automotive Coatings: Current Trends for Coating Plastic – Part 1." **Ignitable Liquid Analysis** – No changes. **Gunshot Residue Analysis** – No changes. **Physical Match Analysis** – No changes. **Pressure Tape & Adhesive Analysis** – No changes. **Administrative and Technical Reviewing of Casework** – No changes. **Mock Trial Evaluation Form** – No changes. **Appendix IV Practical Exercises** – No changes.
7	09-30-2021	Annual Review. **Orientation to OSBI** – Removed blank lines in reading sections. **Liquid Nitrogen Safety** – No changes. **Trace Evidence Searching, Collecting and Documenting** – No changes. **FTIR** – Tasks: Removed 2 tasks associated with KBr and demonstrating sample prep using the bench. **GC/MS** – No changes. **MSP** – Removed from Literature Reading: ASTM E2808-19 and Principles of Color Technology book. **SEM/EDS** – No changes. **SEM/EDS Appendix III** – Updated instrument make and model **Microscopy** – No changes. **Fiber Analysis** – Removed articles from Literature Reading. Moved articles to Optional Reading. Added relevant ASTM standards. **Elemental Analysis for Trace Analysis** – Removed articles from Literature Reading. **Paint Analysis** – No changes. **Ignitable Liquid Analysis** – No changes.

		Gunshot Residue Analysis – No changes. **Physical Match Analysis** – No changes. **Pressure Tape & Adhesive Analysis** – Replaced SWGMAT article for ASTM 3260-21 in Literature Review. Changed number of PLM samples to be analyzed from 10 to 5, in Tasks Section. **Administrative and Technical Reviewing of Casework** – No changes. **Mock Trial Evaluation Form** – No changes.
8	01-31-2022	Annual Review. **Orientation to OSBI** – Removed Forensic Chemistry from the Orientation exemption requirement, allowing previous completion exemption from any CSD unit. **Liquid Nitrogen Safety** – No changes. **Trace Evidence Searching, Collecting and Documenting** – Removed Skills Demo by "combing". **FTIR** – Articulate: Removed Control questions regarding polystyrene. **GC/MS** – No changes. **MSP** – Exercises: Added "To be completed during fiber training". **SEM/EDS** – Tasks: Removed checking pump oil and coolant. **Microscopy** – Exercises: Added exemption to Exercises 3 and 4, and 19 optional. **Fiber Analysis** – Removed articles from Literature Reading. Deleted three Exercises (21, 27 and 28). **Elemental Analysis for Trace Analysis** – No changes. **Paint Analysis** – Practical Exercises: Added descriptor to Comparison of 10 samples. Deleted searching binder spectra to library. Pigments & Extenders: removed identification requirement, searching spectra to library, performing peak deconvolution, varying raster sizer. **Ignitable Liquid Analysis** – Updated the ASTM standards. **Gunshot Residue Analysis** – Tasks: changed GSR collection to three different items vs from submitted casework. **Physical Match Analysis** – No changes. **Pressure Tape & Adhesive Analysis** – No changes. **Administrative and Technical Reviewing of Casework** – No changes. **Mock Trial Evaluation Form** – No changes. Added "or equivalent" to all references to Form 1 throughout the document and added "or equivalent, as approved by Technical Manager" in the introduction. **Exercises -** Removed Exercises 21, 27 and 28
9	08-15-2022	**Orientation to OSBI** – Added "or equivalent…" in Evaluation of Training, with regards to Form 1 in paragraph 3. **Liquid Nitrogen Safety** – No changes. **Trace Evidence Searching, Collecting and Documenting** – No changes. **FTIR** – No changes. **GC/MS** – Added information in Gas Chromatograph Appendix I regarding expansion volumes and liners. Changed injection port temperature from

		290 to 300 and oven temp from 190 to 250. Added Restek.com Solvent Expansion Calculator to the References. **MSP** – No changes. **SEM/EDS** – No changes. **Microscopy** – No changes. **Fiber Analysis** – Added "or equivalent" in regards to Form 1 in Requirements Prior to Fiber Analysis section. **Elemental Analysis for Trace Analysis** – Added "or equivalent" in regards to Form 1 in Requirements Prior to Elemental Analysis section. **Paint Analysis** – Added "or equivalent" in regards to Form 1 in Requirements Prior to Paint Analysis section. **Ignitable Liquid Analysis** – Added "or equivalent" in regards to Form 1 in Requirements Prior to Ignitable Liquid Analysis section. **Gunshot Residue Analysis** – Added "or equivalent" in regards to Form 1 in Tasks and Requirements Prior to GSR Analysis sections. **Physical Match Analysis** – Added "or equivalent" in regards to Form 1 in Requirements Prior to Physical Match Analysis section. **Pressure Tape & Adhesive Analysis** – Removed article regarding Masking Tape; provided only what they considered doing and no real information. In Articulate: rearranged words to combine warp/machine and weft/fill and removed redundant "paper" in describing types of tape. Added "or equivalent" in regards to Form 1 in Requirements Prior to Pressure Tape Analysis section. **Administrative and Technical Reviewing of Casework** – No changes. **Mock Trial Evaluation Form** – No changes.
10	12-31-2023	Annual review. **Orientation to OSBI** – Replaced West Virginia University online course with Advanced Expert Witnessing online course. **Liquid Nitrogen Safety** – Added header "**Discuss the following with trainer".** **FTIR** – Added demonstrate analysis of polystyrene standard. **MSP** – Added 2 articles regarding fluorescence to the required literature reading and added 4 articles to the optional literature reading. Section reformatted. Added task of demonstrating acquiring spectra using fluorescence. **SEM/EDS** – Added article and 4 SEM training videos to the required literature reading. Reworded question regarding sum peaks. Reworded question regarding EDS resolution. Removed question regarding offset and gain. Removed task of performing a line scan. **Microscopy** – added article to the required literature reading. Made Practical Exercise 17 optional due to availability of quartz wedge. **Elemental Analysis for Trace Analysis** – Added article to required literature reading. **Ignitable Liquid Analysis** – Updated State Statute and links for Title 21 Sections 1401-1405.

		Gunshot Residue Analysis – Removed 5 articles and added an article to the optional reading section. **Administrative and Technical Reviewing of Casework** – Removed the requirement of "for each case type" when performing reviews. **Exercises –** 29: Corrected spelling of "dependent".
11	10-31-2024	Annual review. **Table of Contents** – Corrected spelling of Microspectrophotometry. Removed "Synthetic" from Synthetic Fiber Analysis. **Fiber Analysis** – Removed "Synthetic" from title. **Microspectrophotometry (MSP)** – Removed "Why or why not?" as it relates to dyes because the spectra can be used for identification. Reworded question regarding characteristics used for MSP comparisons. **Paint Analysis** – Removed discussion topic regarding the use of MSP for pigment identification. Removed discussion topic regarding quantities of additives used in paint.
12	12-31-2025	Annual review. **Table of Contents –** Changed "Physical Match" to Physical Fit" and changed throughout manual. **Introduction –** Added a colon before list of training manual sources. **Instrumentation –** Added commas during list of what training will consist of. **Trace Analysis –** Added Criminalists "in the" Trace Evidence Laboratory, and added commas during list of what training will consist of. **Evaluation of Training –** Changed Forensic Chemistry to Trace Evidence, and added MSP as a required instrument for Pressure Tape & Adhesive Analysis. **Orientation –** Added familiar "with" forensic science…, removed empty bullet in list. Under Miscellaneous, changed "Acceptance" to "Management" for QMA 2, and changed "Guidelines" to "Requirements" for QMA 3. **Trace Evidence Searching, Collecting, and Documenting –** Under Literature Reading, changed "Imaging" to "Photography" for TR-18, changed Section 8 to 9, updated wording for OSBI CSD Quality Manual sections to refer to Physical Evidence QP 1, 2.1, 2.3, and 3, and removed the OSBI Evidence Collection Handbook since it is no longer provided. **Fourier Transform Infrared Spectroscopy (FTIR) –** Under Tasks, edited a box to be empty instead of dark so it could be filled in with a date when completed. Under Articulate and/or Provide Written Answers- corrected spelling of "effect" to "affect". **Microspectrophotometry (MSP) –** Under Literature Reading, added "Microspectrophotometer Analysis" after TR-19. **Gas Chromatograph/Mass Spectrometer (GC/MS) –** edited an article to move two chapters to the optional reading section. Corrected spelling of etc.

Scanning Electron Microscope with Energy Dispersive Spectrometry (SEM/EDS) – Edited formatting to move Optional Reading title above table. Under Literature Reading, added “Carbon Coating of SEM Specimen, Elemental Analysis, and Scanning Electron Microscope with Energy Dispersive Spectrometer (SEM/EDS)” after their respective protocol numbers. Corrected spelling of Principles.

Microscopy – Corrected spelling of Principles.

Fiber Analysis – Edited formatting of reading section and Tasks section. Under Literature Reading, changed “Manufactured Fibers Protocol” to “Fiber Analysis”. Corrected spelling of Chapter.

Elemental Analysis – Under Tasks, changed “Calcium Silicate” to “Calcium Carbonate”, added Silicon Dioxide, and corrected spelling for Talc.

Paint Analysis – Changed the box next to Significance and Interpretation to gray since it is a title. Under Pigments and Extenders- SEM/EDS, changed “embedded and non-embedded” to “microtomed and hand-sectioned”. Removed two blank lines under Tasks. Edited formatting of Literature reading and Practical exercises section. Under Literature Reading, added “and” between Paint and Polymer. Under Articulate and/or Provide Written Answers, “fracture match” was changed to “physical fit”.

Ignitable Liquid Analysis – Updated ASTM 1386-15 to 1386-23 in literature reading. Removed empty row under Tasks. Under Literature Reading, for TR-100, removed “Residues” and changed “liquid” to “Liquids”, and updated all OK Statute date references to the most recent date updated (10-04-24). Corrected spelling of etc.

Gunshot Residue Analysis – Updated ASTM 1588 to 1588-25 in literature reading. Edited formatting of Tasks section and questions under Articulate and/or Provide Written Answers. Under Literature Reading, changed “GSR Analysis by SEM-EDS" to "Primer Gunshot Residue by SEM/EDS".

Physical Fit Analysis – Removed E2288-09 Standard Guide for Physical Match of Paper Cuts, Tears, and Perforations in Forensic Document Examinations from literature reading due to being withdrawn. Edited formatting of Practical Exercises section. Under Requirements prior to Physical Fit Analysis, added comma on third task.

Pressure Tape & Adhesive Analysis – Removed GC/MS as a required instrument as it is not utilized in tape analysis. Added MSP as a required instrument, since it is utilized in tape analysis. Under Literature Reading, added TR-150 Pressure Sensitive Tape Analysis.

Administrative and Technical Reviewing of Casework – Under Literature, added “Reading” to section title, and added "Read Trace Quality Manual Section 10- Case Reviews".

Practical Exercises – Edited titles for Exercises 17, 18, 19, 25, 31, 35, and 36 to reflect full titles of exercises. In Practical Exercise 33, the reference

to Practical Exercise 35 was corrected to 34. In Practical Exercise 34, references to Practical Exercise 34 were corrected to 33. In Practical Exercise 3, under Directions corrected spelling of used. In Practical Exercise 9, under Directions corrected spelling of etc. In Practical Exercise 22, under Theory fixed capitalization of Fourier. In Practical Exercise 30, under Directions corrected spelling of modern.
History – Under Revision 4 notes, corrected spelling of Microspectrophotometry. Under Revision 5 notes, corrected spelling of Encyclopedic.