Scientific Working Group for Materials Analysis (SWGMAT)

Fiber Subgroup

A FORENSIC FIBER EXAMINER TRAINING PROGRAM

Dedicated to the memory of Mike Grieve, a golden thread in the tapestry of our lives.

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A FORENSIC FIBER EXAMINER TRAINING PROGRAM

TABLE OF CONTENTS

TOPIC		PAGE
Participants and Affirmation		**i**
Chapter 1	**Introduction**	**7**
	Scope of Training Program	
	Educational Prerequisites	
	Training Program Objectives	
	Training Steps and Schedule	
	Responsibilities	
	Competency Evaluation	
Chapter 2	**User's Guide**	**11**
	Training Step/Record Keeping Task Descriptions	
	Record of Practical Exercise Completion	
	Record of Quiz Completion	
	Record of Examination Completion	
	Record of Observed Court Testimony	
	Record of Supervised Casework	
	Record of Mock Trial Evaluation	
	Training Program Critique	
	Certification of Competency	
Chapter 3	**Introduction to Fibers and Textiles**	**27**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Competency Evaluation	
Chapter 4	**Fiber Transfer and Persistence**	**28**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Competency Evaluation	
Chapter 5	**Casework Familiarization/Search and Recovery**	**29**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	

TOPIC		PAGE
Chapter 6	**Microscopy Review** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation *Practical Examination A*	**33**
Chapter 7	**Fiber Examinations/Preliminary Observations** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**35**
Chapter 8	**Identification of Vegetable Fibers** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**37**
Chapter 9	**Identification of Animal Textile Fibers** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**39**
Chapter 10	**Identification of Inorganic Fibers** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercise Competency Evaluation	**41**

	TOPIC	PAGE
Chapter 11	**Manufactured Fiber Examination - Optical Properties**	**43**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	
Chapter 12	**Manufactured Fiber Examination - Cross Sections**	**45**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	
Chapter 13	**Manufactured Fiber Examination - Solubility**	**47**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	
Chapter 14	**Manufactured Fiber Examination - Thermal Microscopy**	**48**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	
	Practical Examination B	
Chapter 15	**Manufactured Fiber Examination - Infrared Spectroscopy**	**50**
	General Discussion	
	Objectives	
	Training Steps and Check Lists	
	Reading Assignments	
	Basic Skills	
	Practical Exercises	
	Competency Evaluation	

TOPIC		PAGE
Chapter 16	**Fiber Examination - Comparison Microscopy** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation *Practical Examination C*	**52**
Chapter 17	**Fiber Examination - Fluorescence Microscopy** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**54**
Chapter 18	**Fiber Examination - UV/VIS Microspectrophotometry** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**56**
Chapter 19	**Fiber Examination - Dye Analysis/TLC** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation *Practical Examination D*	**58**
Chapter 20	**Fiber Examination - Other Techniques** General Discussion Objectives Training Steps and Check Lists Reading Assignments Competency Evaluation	**60**

TOPIC		PAGE
Chapter 21	**Textile Examinations** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation	**61**
Chapter 22	**Cordage Examinations** General Discussion Objectives Training Steps and Check Lists Reading Assignments Basic Skills Practical Exercises Competency Evaluation *Practical Examination E*	**63**
Chapter 23	**Interpretation and Presentation** General Discussion Objectives Training Steps and Check List Reading Assignments Interpretive Exercises Competency Evaluation Supervised Casework Mock Trial Court *Written Examination*	**65**
Appendix I	**Reading Assignments**	**(pages 1 - 24)**
Appendix II	**Practical Exercises**	**(pages 1 - 128)**

Chapter 1 Introduction

Scope of Training Program

1. This training program manual is intended as a trainer's and trainee's guide through the training process. It contains pertinent assignments and exercises to be distributed to the trainee as required. Literature and sources of information other than those listed should also be considered. As time and monetary resources permit, trainees should be provided with appropriate additional training experiences such as off-site academic courses and exposure to industrial manufacturing and analysis of materials.

This manual is designed in a modular format to be used as the basis for individual lesson plans, student orientation, training program monitoring and evaluation. It may also be used to record the trainee's progress, and prepare the trainee for assigned projects and examinations.

2. It is the intent of the training program to provide a foundation of theoretical knowledge and basic practical skills for a trainee to become a fully qualified analyst capable of making appropriate analytical decisions, competent examinations, and proper interpretations of the analytical results. The modules cover the topics that should be included in a forensic fiber examiner training program. In some instances, modules or topics may include methods and techniques, or alternative methods and techniques which are not currently employed in the laboratory, or which may be considered antiquated. For those instances, while the trainee may not gain practical skills, the trainee is expected to gain the theoretical knowledge about the alternative methods, their limitations, and/or their appropriate application.

3. Considering the overall possibilities, fibrous trace evidence may originate from many sources (e.g. textiles, cordage, building materials). Several fiber classification schemes exist that are utilized by forensic scientists, based largely on fiber production methods and end uses. It is imperative that the trainee and trainer understand the scope and organization of this training program before commencing. The orientation and the emphasis of this training program is on fibers as used in textiles and textile products. This training program utilizes the general classification scheme of natural versus manufactured fibers, and is further organized around the concepts of how fibers and textiles are identified and compared.

Educational Requirements

The minimum academic level for a fiber trainee is a BA/BS degree in a natural or applied science.

Trainees are expected to have a good theoretical background in pertinent subjects and have successfully completed the following undergraduate or graduate courses:

A. one year (or equivalent) of General Chemistry with laboratory,
B. one year (or equivalent) of Organic Chemistry with laboratory, and
C. General Biology with laboratory.

If the following is not available at the undergraduate or graduate level, then completion through structured course work is required:

A. analytical/instrumental analysis,
B. basic microscopy and polarized light microscopy, and
C. fiber microscopy.

Training Program Objectives

Through completion of this training, the trainee is expected to build on his/her formal educational background and gain theoretical knowledge and practical skills in the following areas:

1. equipment and instrumentation use, routine maintenance, and functionality assessment;
2. fiber and textile history, terminology, and usage including common end uses of different fiber, yarn, fabric and cordage types;
3. fiber and textile chemistry and manufacturing processes including chemical compositions, chemical and mechanical treatments, and manufacturing mechanical, dyeing and finishing processes;
4. search, recovery, preservation, and examination techniques including proper sample handling; packaging and documention for fibrous materials associated with a variety of substrates;
5. classification of natural and manufactured fibers used in textile materials;
6. identification and/or comparison of natural and manufactured fibers by optical, chemical and physical property examinations;
7. examination and comparison of textiles for physical matches, physical construction, and fiber composition;
8. fiber and textile physical wear, damage, and manufacturing artifacts assessment;
9. accessing relevant literature and standards databases;
10. fiber and textile examination, identification and/or comparison results interpretations including factors affecting the analytical interpretation and the significance of the evidence with respect to fiber transfer and persistence;
11. proper laboratory report completion and testimonial evidence presentation; and
12. detection and assessment of other types of physical evidence which may be encountered during fiber and textile examinations.

Training Steps and Schedule

1. With the aid of this manual, the trainee should gain theoretical knowledge and practical skills through the following methods:
   - A. reading
   - B. personal instruction
     1. lectures and discussions
     2. practical demonstration of basic skills
   - C. practical skills
     1. basic skills
     2. practical exercises
     3. assisting in and performing supervised casework
   - D. observing others
     1. in casework
     2. in court
   - E. examinations
     1. oral and/or written quizzes
     2. practical examinations
     3. written examinations
     4. mock trial

2. The expected training period will be a minimum of 12 months, full time, for the inexperienced trainee with no prior forensic experience, and will include casework observation, supervised casework examinations and mock trial. Flexibility in the duration of the expected training period may take into account the trainee's academic background, experience, and aptitude. A written schedule of expected completion dates for training goals shall be determined and provided to the trainee.

Responsibilities

It is the responsibility of each agency/laboratory to define "satisfactory completion" whenever used in this training program. Each trainee shall be assigned a trainer. The assigned trainer may not necessarily be the supervisor of the Section/Unit, but must be a technically competent fiber examiner, internal or external to the agency.

The trainer shall be responsible for:

1. introducing the trainee to the relevant technical literature, procedures, training material, and reference or standards collections; to include the agency's standard operating procedures;
2. assisting the trainee in interpretation of the literature by discussion of technical methods and foundational theory;
3. providing training in the performance of some basic practical skills and methods, and coordinating training in other methods with other experienced analysts;
4. providing instruction in case management, including but not limited to,
    a. chain of custody documentation
    b. evidence processing, preservation and storage
    c. decision making criteria, data interpretation and conclusionary statements
    d. documentation of analyses
    e. report writing
    f. laboratory safety practices
5. providing an example of ethical and proper professional conduct and communications;
6. providing instruction on appropriate quality assurance and quality control procedures;
7. providing instruction on proper court presentation and etiquette; and
8. providing a written schedule of expected completion dates for training goals.

The trainer and supervisor shall be responsible for monitoring the trainee's progress, and the thoroughness and correctness of the trainee's education. All qualified members of the laboratory are expected to make themselves available to the trainee for discussion of training topics.

The trainee shall be responsible for:

1. meeting the training objectives within the specified training schedule;
2. self-study of the relevant technical literature;
3. practicing basic skills, analytical methods and techniques on non-case samples;
4. completing the practical exercises;
5. observing casework;
6. satisfactory completion of sponsored course work or its equivalent; and
7. performing casework under supervision.

Competency Evaluations

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

It is imperative that competency testing, of any type indicated below, be prepared and evaluated by the trainer and/or other technically qualified staff members to ensure the relevancy of questions, as well as the correctness, thoroughness and directness of answers.

The trainer shall discuss the training progress with the trainee and supervisor on a regular basis. The supervisor and trainer shall evaluate training and competency of a trainee by way of:

1. oral and/or written quizzes,
2. written examinations,
3. practical examination of unknowns,
4. supervised casework, and
5. mock trial

Documentation of all of the trainee's tests shall be in written form and retained in the laboratory's files.

Chapter 2 User's Guide

Training Steps and Record Keeping Task Descriptions

The Table of Contents preceding the Introduction outlines the segments of the training program manual by Chapter and Section. Amendments to this manual may be issued periodically or may be developed and issued by a laboratory in order to meet their specific needs. Each laboratory's procedures or methods manuals may be incorporated into this training program. It is the responsibility of each laboratory to maintain a current training program manual, record of amendments, and record of additional requirements beyond those recommended by this program.

Each Chapter is divided into sections including:

1. General Discussion - a general module subject description including a statement of the intended scope of topic coverage
2. Objectives - defined learning results
3. Training Steps and Teaching Point Check List
    a. reading assignments
    b. basic skills table
    c. practical exercises
4. Competency Testing - guide for the topical area in the form of suggested application of quizzes, written examinations, and/or practical examinations

During the training period, the trainer shall:

1. ensure that reading assignments material, supplies and equipment for practical exercises are available;
2. provide personal instruction, discussion and practical demonstrations as necessary;
3. monitor the trainee's check sheets to ensure adequate progress is being made and documented, and schedule expected completion dates;
4. ensure that the trainee adequately reviews previous chapter and section material to reinforce topics, introduce new perspectives to topics, and adequately integrate topics that were studied separately;
5. be available to answer trainee questions;
6. critically evaluate when a trainee can satisfactorily perform each of the basic skills;
7. review and critically discuss completed practical exercises, then initial the appropriate space on the list when satisfactorily completed;
8. prepare, administer and grade oral/written quizzes, written examinations and practical examinations, discuss the examination results with the trainee and rectify any deficiencies;
9. keep management informed of the trainee's progress; and
10. communicate the agency's policy on the requirements for satisfactory completion of the training modules.

During the training period, the trainee shall:

1. complete all required reading assignments and take notes as appropriate;
2. carefully observe all demonstrated practical basic skills;
3. practice the basic skills until they are competent in the skill;
4. perform the practical exercises and make appropriate notes on such;
5. satisfactorily complete oral and written examinations, discussing and correcting any deficiencies; and
6. provide a critical evaluation of the training program.

Reading assignments are selected to give the trainee a broad range of understanding of current and past thoughts on various topics. The reading assignments intended to give the trainee a sound theoretical background are designated as required, and are considered mandatory. Supplemental readings should be sought out by the trainee and may be specified by the trainer or laboratory policy. Continued exposure to the forensic literature should encourage further professional development, reveal alternate opinions in certain subject areas, and present some possible research topics. Appendix I provides the reading assignments and a format to record completion of required and supplemental readings (see Appendix I Reading Assignments-User's Guide).

Basic skills are clearly itemized in a practical skills list. The trainer should provide a practical demonstration of each skill to the trainee, and initial and date column **"D"** for demonstrated on the basic skills list. The trainee should practice these basic skills until s/he is proficient in performing these techniques, and initial and date the basic skills list column **"P"** for practiced. When the trainee has satisfactorily demonstrated proficiency in performing the basic skill to the trainer, the trainer should initial and date column **"C"** for having demonstrated competency in performance.

Practical exercises are designed to expose the trainee to the basic skills learned within each chapter, and to develop observational and interpretational skills related to the data collected by use of the basic skills. All references provided with each practical exercise are considered mandatory readings and should be initialed and dated within the exercise as the trainee completes each. All practical exercises should be completed by the trainee with full and complete note taking, and report writing if appropriate. The exercises should be reviewed by the trainer with particular attention to development of the trainee's critical thinking skills, as well as continuous review of learned basic skills. A record of satisfactory practical exercise completions shall be kept. The "Record of Practical Exercise Completion" table located in Chapter 2 may be used for this purpose.

These practical exercises are not comprehensive for the totality of practical exposure a fiber examiner trainee should receive. Rather, these exercises should serve as a stimulus for the trainee to extend their practical experience through their own curiosity and intellect in self-tutorials of their own design, or by expansion or variations on the existing exercises. Toward this goal of gaining additional practical experience, the trainee should be given reasonable and adequate time to continue their experimentation beyond these required practical exercises.

Quizzes are recommended with each Chapter of the training program manual as a mechanism to keep the trainee's attention focused on the relevant topical areas, as well as providing continuous feedback to the trainee with respect to his/her comprehension of the material, success in meeting the objectives, and indications of deficiencies. Quizzes should include questions which will demonstrate the trainee's mastery of practical basic skills as well as theoretical knowledge. A record of satisfactory quiz completions shall be kept. The "Record of Quiz Completion" table located in Chapter 2 may be used for this purpose.

Practical examinations should take the form of exercises in which the trainee is expected to perform all aspects of the work, administrative and technical, as if it were actual case material and should be evaluated as such (including such things as maintaining chain of custody, marking exhibits, note taking and report writing, etc.). The test should evaluate critical thinking skills for decision making and conclusionary statements. A record of satisfactory practical examination completions shall be kept. The "Record of Examination Completion" table located in Chapter 2 may be used for this purpose.

Written examinations are used to test the trainee's knowledge. It is recommended that the final written examination and practical examination for the training program be prepared with substantial input from a technical peer of the trainer in order to ensure objectivity in testing. A record of satisfactory final written examination completion should be maintained. The "Record of Examination Completion" table located in Chapter 2 may be used for this purpose.

Court testimony observation is a valuable learning tool for the trainee. The trainee should attempt to observe a variety of examiners testify on a range of offenses and types of examinations. The number and frequency of such observations will be dictated by circumstances and the trainer's judgement. The testimony should be discussed with the trainee after each observation. A record of the observed court testimony shall be kept. Recommended points of observation and discussion, and a "Record of Observed Court Testimony" form located in Chapter 2 may be used for this purpose. The trainer should consider using review of trial transcripts to supplement court testimony observations when an adequate number of trial observations are logistically difficult in your laboratory's circumstances (e.g. testimony is too infrequent or excessive distances).

Supervised casework and assisting in casework is expected throughout the training period. Initially, the trainee will observe other members of the laboratory who are working relevant cases. As training progresses, the trainee may be asked to assist others in their casework. It is expected that the levels of responsibility and assistance will be increased until the trainee is able to perform supervised casework, and complete cases independently by the end of the training period. A record of supervised casework shall be kept. The "Record of Supervised Casework" form located in Chapter 2 may be used for this purpose.

If laboratory policy does not permit trainees to assist in casework prior to performing supervised casework, then the trainer should expose the trainee to this facet of training by inserting simulated casework practical exercises designed to develop reasoning skills, analytical performance and responsibility.

Mock trials should be held using separate individuals for the various roles, and using simulated casework material analyzed and interpreted by the trainee. Satisfactory completion of the mock trial should be by concurrence of all participants, including the trainee. Some suggested evaluation points are provided on the "Record of Mock Trial Evaluation" form located in Chapter 2. A written record of satisfactory completion of the mock trial shall be kept and the Mock Trial Evaluation Form may be used for this purpose.

Final competency evaluation for satisfactory completion of the training program shall be in written form and maintained in the laboratory files. The Certification of Competency form located in Chapter 2 may be used for this purpose.

RECORD OF PRACTICAL EXERCISE COMPLETION

Name of Trainee: _____ Date training commenced: _____

Name of Trainer: _____

Name of Supervisor: _____

Chapt	Satisfactory Practical Exercise Completion	Trainee Initials and Date	Trainer Initials and Date
5	5-1 Fiber Transfer and Persistence		
	5-2 Collecting Fibers on Tape		
6	6-1 Familiarization with the Stereomicroscope		
	6-2 Familiarization with the Compound Light Microscope		
	6-3 Familiarization with the Polarized Light Microscope		
7	7-1 Fiber Manipulations		
	7-2 Observing Effects of Mounting Media		
	7-3 Observing Fiber Shape, Surface and Internal Structure		
	7-4 Observing Color and Pleochroism		
	7-5 Distinguishing Natural and Manufactured Fiber Classes		
8	8-1 Microscopy of Non-woody Vegetable Fibers		
	8-2 Determining Natural Fiber Twist		
9	9-1 Examining the Cuticle of Animal Hairs		
	9-2 Introduction to Examining Fibers of Animal Origin		
10	10-1 Identification of Asbestos Fibers		
11	11-1 Determining the Sign of Elongation		
	11-2 Measuring Birefringence		
	11-3 Measuring Refractive Indices by the Immersion Method		
12	12-1 Cross-sectioning and Interpretation of Cross Sections		
	12-2 Determining the Modification Ratio		
13	13-1 Solubility Testing of Acetate and Triacetate Fibers		
14	14-1 Thermal Microscopy - Use of a Hot Stage		

(Continued on next page)

Chapt	Satisfactory Practical Exercise Completion	Trainee Initials and Date	Trainer Initials and Date
15	15-1 Sample Preparation for FTIR-Microscopy		
	15-2 The Transmission/Reflection Technique		
	15-3 Interpretation of Fiber FTIR Spectra		
16	16-1 Comparison Microscopy with Brightfield Illumination		
17	17-1 Fluorescence Microscope: Set-up and Operation		
	17-2 Effect of Mounting Media in Fluorescence Microscopy		
	17-3 Observing Fluorescence on Fibers		
18	18-1 Microspectrophotometer Set-up and Operation		
	18-2 Acquiring Spectra from Single Fibers		
	18-3 Acquiring Known Spectral Sets and Comparing Curves		
	18-4 Examining Metameric Fibers		
19	19-1 Classification of Fiber Dyes		
	19-2 Thin Layer Chromatography of Fiber Dyes		
21	21-1 Examining Fabric Damage		
	21-2 Environ., Chemical and Mechanical Effects on Fabrics		
22	22-1 Composition and Construction of Cordage		
	22-2 Environ., Chemical and Mechanical Effects on Cordage		
23	Interpretive Exercise		

RECORD OF QUIZ COMPLETION

Name of Trainee: _____ Date training commenced: _____

Name of Trainer: _____

Name of Supervisor: _____

Satisfactory Quiz Completion by Chapter	Trainee Initials and Date	Trainer Initials and Date
3		
4		
5		
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12		
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15		
16		
17		
18		
19		
20		
21		
22		

RECORD OF EXAMINATION COMPLETION

Name of Trainee: _____ Date training commenced: _____

Name of Trainer: _____

Name of Supervisor: _____

Satisfactory Examination Completion	Trainee Initials and Date	Trainer Initials and Date
Practical Examination A Evidence Recovery (at end of Chapter 6)		
Practical Examination B Fiber Identification (at end of Chapter 14)		
Practical Examination C Fiber Identification and Comparison (at end of Chapter 16)		
Practical Examination D Fiber Identification and Comparison (at end of Chapter 19)		
Practical Examination E Textile and Cordage Examinations (at end of Chapter 22)		
Final Written Examination (at end of Chapter 23)		

RECORD OF OBSERVED COURT TESTIMONY

Name of Trainee: _____ Date training commenced: _____

Name of Trainer: _____

Name of Supervisor: _____

The trainee should discuss the salient points of providing court testimony such as:

1. What did you learn about legal proceedings?
2. What questions were asked by the prosecutor?
3. What questions were asked by the defense attorney?
4. Were there redirect questions and, if so, what were they?
5. What were the salient points of the testimony?

I have observed and evaluated______________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

I have observed and evaluated______________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

I have observed and evaluated______________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

I have observed and evaluated____________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

I have observed and evaluated____________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

I have observed and evaluated____________________________________in the management and analysis of
(Name of trainee)
casework from case____.

S/he competently performed all duties of a fiber examiner throughout this casework.
____(signed and dated by evaluator).

or

S/he displayed some inadequacies in the management and analysis of this casework. I recommend that there be remedial training in the area(s) of: ____
____
____ (signed and dated by evaluator).

RECORD OF MOCK TRIAL EVALUATION

Name of Trainee: _____ Date Held: _____

Other Participants: _____

Suggested evaluation points include:

1. Was the witness able to clearly state his/her qualifications?

2. Was the witness prepared for testimony?

3. Was the testimony clear and explicit?

    A. -at the lay juror level?

    B. -at the expert technical level?

    C. Were the limitations/significance of the evidence properly explained?

4. How was the witness's demeanor and appearance?

5. Any suggested points of improvement?

Date and participant signatures upon satisfactory completion: _____

_____

_____

_____

TRAINING PROGRAM CRITIQUE

Name of Trainee: _____ Date training commenced: _____

Name of Trainer: _____ Date training completed: _____

Name of Supervisor: _____

Part I: Circle the letter of the response which most closely fits your impression.

A. Personal Instruction

1. The amount of personal instruction received from your supervisor/trainer and others in the section was:
   (a) about right (b) too little (c) too much

2. The quality of personal instruction received from your supervisor/trainer and others in the section was:
   (a) very good (b) adequate (c) poor

B. Reading Assignments

1. The number of papers included in the reading assignments was:
   (a) about right (b) too few (c) too many

2. In general, I thought that the reading assignments were:
   (a) excellent (b) adequate (c) irrelevant

C. Practical Exercises

1. The number of practical exercises was:
   (a) about right (b) too few (c) too many

2. In general, I thought that the practical exercises were:
   (a) excellent (b) adequate (c) irrelevant

D. External Resources

1. The number of external resource people and materials used was:
   (a) about right (b) too few (c) too many

2. The external resource materials were:
   (a) excellent (b) adequate (c) irrelevant

3. Specify external resource people or materials that were particularly noteworthy, good or bad:

E. Audio/visual Materials

1. The audio/visual materials used were:
   (a) excellent (b) adequate (c) irrelevant

2. Specify audio/visual materials that were particularly noteworthy, good or bad:

F. In-house Discussions

1. The frequency of in-house discussions and round-tables was:
   (a) about right (b) too few (c) too many

2. The in-house discussions and round-tables were:
   (a) excellent (b) adequate (c) irrelevant

G. Practical Examinations

1. The number of practical examinations was:
   (a) about right (b) too few (c) too many

2. The level of complexity of the practical examinations was:
   (a) about right (b) too easy (c) too difficult

3. In general, I thought that the practical examinations were:
   (a) valuable (b) irrelevant

H. Quizzes/Written Examinations

1. The number of quizzes/written examinations was:
   (a) about right (b) too few (c) too many

2. The level of complexity of the quizzes/written examinations was:
   (a) about right (b) too easy (c) too difficult

3. In general, I thought that the quizzes/written examinations were:
   (a) relevant (b) irrelevant

I. Mock Trial(s)

1. The number of mock trials was:
   (a) about right (b) too few (c) too many

2. The level of complexity of the mock trials was:
   (a) about right (b) too easy (c) too difficult

3. In general, I thought that the mock trials were:
   (a) valuable (b) irrelevant

J. General

1. The length of training was:
   (a) about right (b) too short (c) too long

2. The coverage of the material was:
   (a) good (b) adequate (c) deficient in areas

3. In general, I thought that this training program was:
   (a) good (b) adequate (c) needs improvement

Part II: Use the back of this sheet or additional pages if necessary.

A. Reading Assignments

1. The following reading assignments, if any, were particularly good:

2. The following reading assignments, if any, should be deleted:

3. The following reading assignments or papers, if any, should be added:

B. General

1. The coverage of the following topics or areas, if any, should be expanded:

2. The coverage of the following topics or areas, if any, should be reduced:

3. Additional comments by trainee:

Part III Comments by the trainer and supervisor:

1. Indicate any areas in Part I or II where you strongly agree or disagree with the trainee.

2. Make any additional, general comments on the training program.

Signature of Trainee: _____

Signature of Trainer: _____

Signature of Supervisor: _____

Date of Training Program : _____

CERTIFICATION OF COMPETENCY

I have reviewed the training records, skills inventory, practical exercises, examinations, supervised casework, and court presentation skills, and have discussed the final assessment of competence for fiber and textile examinations with the trainee _____ and trainer _____.
(Name) *(Name)*

_____ is approved to independently conduct fiber and textile examinations.
(Name)

_____

Supervisor Date

Chapter 3 Introduction to Fibers and Textiles

General Discussion

This training module will introduce the trainee to the basic concepts and theoretical knowledge of fiber and textile product manufacture and use, commercial and forensic classifications, and an overview of forensic examinations for identification and comparisons. It is intended to be historical as well as contemporary, and to provide the theoretical foundation upon which practical analytical skills will be built in the subsequent modules.

Objectives

Through completion of this module the trainee will develop the theoretical knowledge to be conversant in:

1. fiber and textile history, usage and manufacturing,
2. fiber and textile technology and terminology,
3. chemistry and manufacturing processes of fibers and dyes,
4. fiber classification schemes, and
5. identification vs. comparison of fibers and textiles.

Training Steps and Check List

1. Reading Assignments: Complete Chapter 3 reading assignments listed in Appendix I.

2. Additional training may be obtained from textile museum or industrial manufacturing plant tours, videotapes of textile processing, visits to fabric and carpet stores, etc. A record of additional training resources used (e.g. tours, museums) should be noted and maintained.

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge as specified in the module objectives to demonstrate comprehension of the reading assignments. The quizzes should include any questions which will demonstrate theoretical or practical knowledge acquired through additional training means such as industrial and museum tours.

Chapter 4 Fiber Transfer and Persistence

General Discussion

This training module will introduce the trainee to the basic concepts and theoretical knowledge of fiber transfer and persistence from two perspectives. First, it is transfer and persistence that imparts a value to textile fibers as associative trace evidence. Second, it is transfer and persistence that can result in fibrous evidence contamination and loss. It is intended that the trainee demonstrate a sound theoretical foundation of the contamination and loss concepts before exposure to actual evidentiary materials and practical basic skills.

Objectives

Through completion of this module the trainee will develop the theoretical knowledge to be conversant in:

1. Locard's Exchange Principle;
2. the potential significance of fibers as associative trace evidence in forensic cases;
3. textile fiber sheddability;
4. fiber transfer mechanisms and factors affecting transfer;
5. fiber persistence mechanisms and factors influencing persistence, and
6. techniques utilized to prevent or reduce fibrous evidence contamination and loss from the time of field examinations through laboratory analyses including:
    a. limiting contacts between items and individuals,
    b. wearing appropriate protective apparel,
    c. proper packaging, handling and labeling,
    d. cleaning equipment and work surfaces,
    e. maintaining controlled environments,
    f. separation of evidence from different sources by location and/or time.

Training Steps and Check List

1. Reading Assignments: Complete Chapter 4 reading assignments listed in Appendix I.

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge as specified in the module objectives to demonstrate comprehension of the reading assignments.

Chapter 5 Casework Familiarization/Search and Recovery

General Discussion

This training module serves as a guide for introducing the basic procedures and techniques for proper documentation, detection, collection and preservation of fibrous and other trace evidence from crime scenes, individuals and items submitted to the laboratory for examination. The trainee should be exposed to a wide variety of casework throughout the training period. This exposure will include the trainee's assistance in exhibit searching, observation of subsequent analyses, discussion of data generated, results obtained, and conclusions drawn. This module should be completed in conjunction with Chapter 6 Microscopy Review for proper operation of stereomicroscopes.

The trainee should become proficient with the format and procedures pertaining to casework administrative and technical documentation, and report writing as outlined in the laboratory's procedures manual.

Objectives

Upon satisfactory completion of this training module, the trainee will have acquired introductory theoretical and practical knowledge of:

1. the significance and use of trace physical evidence from the scientific perspective, and an overview of the types of information one may obtain from analyzing different types of trace evidence;
2. general evidence detection, collection and preservation techniques;
3. guidelines for evaluating, prioritizing and coordinating processing of items for multiple types of evidence from the administrative and scientific perspectives;
4. the administrative, legal and scientific requirements for case documentation and reporting;
5. evidence security requirements and practices;
6. health and safety requirements and practices; and
7. quality assurance requirements and practices.

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and practical skills to:

1. search, recover, and preserve fibrous evidence from a variety of substrates;
2. prevent contamination and loss of evidence during search and recovery;
3. document searches and recoveries; and
4. provide testimony on the learned practical skills.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 5 reading assignments listed in Appendix I.

2. Observe casework in progress to include examination of case documentation and final reports. Discuss work in progress with the examiner regarding decision making criteria in case processing and reporting. The trainee and examiner should use the following topical outline as a reference for discussion:

1. significance and use
    a. integrity of evidence
    b. other forms of trace evidence

2. documentation
    a. case file documentation per laboratory policy to include notes containing: date, initials, item description, unique identifier, sketches, measurements and images
    b. chain of custody
    c. labeling items and packages
    d. record maintenance, storage and security
3. contamination and loss practices
    a. limiting contact between items and individuals
    b. appropriate protective apparel
    c. limiting evidence handling and exposure to contaminants
    d. collecting, packaging and sealing in appropriate packaging
    e. controlled environments
    f. clean equipment and work surfaces
    g. separation of evidence from different sources by location and/or time
    h. documenting any situation which could have contaminated or compromised the evidence
    i. consideration of associated evidentiary items from other disciplines
4. 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
5. questioned versus known samples, and collection of representative known samples
6. 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
7. health and safety in laboratory and non-laboratory settings
    a. mechanical hazards
    b. chemical hazards
    c. biological hazards
8. crime scene/field evidence
    a. site and item identification
    b. observations and documentation
    c. searches/collections
    d. interpretations
    e. evidence disposition
9. laboratory examination report writing
    a. case identification
    b. items received and methods of analysis used
    c. examination results and conclusions
10. court testimony
11. quality assurance/quality control of examinations, reports and testimony

Trainee record of observed and discussed casework in progress (adequate number specified by trainer or laboratory policy):

Case Identifier	Date and Examiner Observed
1.	
2.	
3.	
4.	
5.	
6.	

3. Basic Skills

Basic Skills in Exhibit Searching	**D**	**P**	**C**
1. contamination and loss prevention techniques when actually manipulating evidentiary items			
2. visual searching, using alternate light sources			
3. stereomicroscopical searching			
4. fibrous evidence recovery by: a. picking b. tape lifting c. scraping d. vacuuming e. combing f. cutting			
5. documenting and packaging			

4. Practical Exercises: Complete these exercises located in Appendix II.

5-1 Fiber Transfer and Persistence
5-2 Collecting Fibers on Tape

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include questions on the significance and use of trace evidence, and questions that assess the trainee's ability to properly reason through and perform general trace evidence detection, collection, preservation, and documentation.

Chapter 6 Microscopy Review

General Discussion

This module is intended as a guide to familiarize the trainee with the operating theory and care of microscopes used in the laboratory. Emphasis is placed on proper illumination, calibration and preliminary observations made with stereomicroscopes and polarized light microscopes (PLM), which are the instruments typically used first in any fibrous evidence examinations.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. understand microscope optics;
2. properly operate and maintain each microscope and its accessories including adjustments, cleaning and diagnosing problems;
3. establish proper microscope illumination including adjustment for Köhler illumination;
4. calibrate the ocular micrometer, and acquire proper measurements utilizing ocular and stage micrometers; and
5. select appropriate microscopes and accessories for the observational task required.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 6 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in Microscopy:	D	P	C
1. operating, adjusting and cleaning a microscope			
2. centering stages and objectives			
3. setting up Köhler illumination			
4. measuring with ocular and stage micrometers			
5. observing relative refractive index by Becke line			
6. distinguishing isotropic and anisotropic substances			
7. determining extinction positions			
8. observing interference colors			
9. taking photomicrographs			

3. Practical Exercises: Complete these exercises located in Appendix II.

6-1 Familiarization with the Stereomicroscope
6-2 Familiarization with the Compound Light Microscope
Part 1 Köhler Illumination
Part 2 Basic Micrometry and Calibrating an Ocular Micrometer
6-3 Familiarization with the Polarized Light Microscope
Part 1 Refractive Index by Becke Line Observations
Part 2 Observation of Extinction Positions and Interference Colors in Crossed Polars

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as measuring fiber dimensions of length and diameter.

Practical Examination A: Trace Evidence Detection, Collection and Preservation

The trainee should be given simulated case materials to screen for trace evidence. This competency test is to be general in nature and should not cover fiber examination analytical techniques. The trainee should have the skills to perform generalized trace evidence searches and collections upon satisfactory completion of the practical examination.

Chapter 7 Fiber Examinations/Preliminary Observations

General Discussion

This module is intended as a guide to familiarize the trainee with fibrous evidence handling and mounting techniques. The trainee will be taught the proper visual and microscopical preliminary observations and examinations to distinguish between natural and manufactured fibers as broad categories.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. select proper mounting media and tools for making these preliminary observations;
2. properly handle fibrous evidence during recovery, mounting, examination, and de-mounting while maintaining control and integrity of the items;
3. discern fiber shape, surface features, internal structure, and color, and apply accepted terminology to the features observed;
4. classify fibers as manufactured, animal or vegetable based on preliminary observation of the macroscopic and microscopic fiber features; and
5. measure fiber diameter and observe color and pleochroism.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 7 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in preliminary fiber examinations:	D	P	C
1. removing fibers from tape			
2. mounting fibers for microscopical examination			
3. qualitative bright field and PLM examinations			
4. assessing color and pleochroism			
5. assessing shape, surface and internal features by optical cross-sectioning			
6. de-mounting and securing fibers			

3. Practical Exercises: Complete these exercises located in Appendix II.

7-1 Fiber Manipulations: Removing Fibers from Tape and Mounting
7-2 Observing Effects of Mounting Media
7-3 Observing Fiber Shape, Surface and Internal Structure
7-4 Observing Color and Pleochroism
7-5 Distinguishing Natural and Manufactured Fiber Classes

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as distinguishing natural from manufactured fibers and vegetable and animal fibers, making qualitative PLM observations, etc.

Chapter 8 Identification of Vegetable Fibers

General Discussion

This module is intended as a guide to familiarize the trainee with techniques utilized in the identification of the plant fibers which are commonly used in textile products and cordage (cotton, flax, ramie, jute, hemp, etc.). The scope of this module is not intended to include other botanical identifications. This module should be reviewed/reworked in conjunction with several other chapters of the training program manual in order to fully integrate all topics.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. use bright field and polarized light microscopy to identify plant tissue and cellular structural features that allow for the classification of the fiber(s) as vegetable, and as bast (stem) fibers, leaf fibers, or seed (fruit) fibers;
2. determine the plant cell wall spiral thickening's direction of twist and apply this information to a botanical identification;
3. make a botanical identification of the fiber source as specifically as possible using reference sources and comparisons; and
4. have an understanding of the processing, dyeing techniques, and end use products of the various vegetable fibers.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 8 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in vegetable fiber examinations:	D	P	C
1. identifying and measuring cell structures: a. entire cell length and width, cell wall, lumen b. spiral thickenings, pits, dislocations c. cytoplasmic remnants, crystals, resins			
2. identifying basic plant tissues including epidermis, xylem, phloem, seed hairs, leaf hairs			
3. cross-sectioning plant cells and examining relative lumenal dimension			
4. macerating plant cells			

(continued next page)

Basic Skills in vegetable fiber examinations:	D	P	C
5. determining sign of elongation and direction of twist with first order red plate/PLM			
6. determining direction of twist with the drying twist test			
7. microchemical testing for degree of lignification			
8. ashing			

3. Practical Exercises: Complete these exercises located in Appendix II.

8-1 Microscopy of Non-woody Vegetable Fibers
8-2 Determining Natural Fiber Twist
Part 1 The Herzog Effect
Part 2 The Drying Twist Test

(Note: these exercises should be completed in conjunction with or subsequent to learning the material presented in Chapter 11 and Chapter 12.)

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as vegetable fiber identification.

Chapter 9 Identification of Animal Textile Fibers

General Discussion

This module is intended as a guide to familiarize the trainee with techniques used in the identification of animal textile fibers. These may include silk, leather and animal hairs. This module is not intended to include animal hair species identifications. The trainee will find it useful to review animal hair features from various domestic and local wild animals as an educational exercise in comparison and exclusion of these animals as sources of textile fibers.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. identify and distinguish “wild type” and “cultivated type” silk by physical and optical properties;
2. identify the major morphological and structural features of animal hairs including, but not limited to, root, cortex, medulla, scales, and shield as appropriate for fur hairs or guard hairs;
3. distinguish human from non-human animal hairs, and identify and/or be conversant in the 3 major human hair “look-a-likes” of cattle, horse and bear (some of which may be used in textile products such as felts);
4. identify the major animal hairs and hides which are commonly used in textile products by their distinguishing morphological features, including but not limited to, wool, fibers from the goat family (mohair, cashmere), fibers from the camel family (camel, alpaca, vicuna), rabbit (angora), and fur animals (such as mink, ermine and chinchilla);
5. have an understanding of processing, grading, finishing, and dyeing techniques, and end use products of the various animal hairs; and
6. have an understanding of appropriate animal taxonomy and morphological terminology.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 9 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in animal textile fiber examinations:	D	P	C
1. determining optical and physical properties (e.g. wild and cultivated silk and leather)			
2. identification and descriptive classification of major hair morphological features			
3. scale casting			

(continued next page)

Basic Skills in animal textile fiber examinations:	D	P	C
4. scale counting techniques			
5. cross-sectioning hairs			
6. medullary clearing techniques			
7. measuring shield size and sub-shield strictures			

3. Practical Exercises: Complete these exercises located in Appendix II.

9-1 Examining the Cuticle of Animal Hairs
Part 1 Scale Margin Distance by Scale Counts
Part 2 Scale Margin Appearance and Pattern by Scale Casting
9-2 Introduction to Examining Natural Fibers of Animal Origin
Part 1 Examining Animal Hairs
Part 2 Examining Silk and Leather
(Note: this exercise should be completed in conjunction with or subsequent to learning the material presented in Chapter 11 and Chapter 12.)

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as animal textile fiber identification.

Chapter 10 Identification of Inorganic Fibers

General Discussion

This module is intended to familiarize the trainee with inorganic fibers. Some classification schemes utilize the term “mineral” fiber which can include fiber types encountered in textile products such as asbestos, glass wool, some anti-static fibers, and metallic fibers. As used in this training program manual, these fibers are considered manufactured and should be studied within the manufactured fiber chapters.

The use of asbestos in textile products has declined significantly due to health risks associated with these fibers. Nevertheless, the trainee should be familiar with these inorganic fibers for thoroughness in theoretical knowledge and for instances in which they may be encountered as fibrous evidence from sources such as building and insulation products, in old textile products or the few textile products in which chrysotile may occur. This module should be completed in conjunction with Chapter 11 in which the trainee is exposed to the determination of fiber optical properties.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. be conversant in processing practices and end uses of the asbestos minerals including chrysotile, amosite, crocidolite, fibrous tremolite/actinolite, and fibrous anthophyllite from both historical and current perspectives;
2. be conversant in the crystalline nature, chemistry and differences between layer silicates and chain silicates;
3. determine the optical properties of asbestos fibers by polarized light microscopy, and use of the dispersion staining technique;
4. identify and classify asbestos, particularly chrysotile, based on optical properties; and
5. be conversant in the applicability of infrared spectroscopy, X-ray diffraction and elemental composition examinations with respect to inorganic fiber identification.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 10 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in asbestos optical property examinations:	D	P	C
1. determining refractive indices, sign of elongation, birefringence by PLM (see also Chapter 11)			
2. dispersion staining			
3. asbestos type identification by comparison to reference materials			

3. Practical Exercise: Complete this exercise located in Appendix II.

    10-1 Identification of Asbestos Fibers

    (Note: this exercise should be completed in conjunction with or subsequent to learning the material presented in Chapter 11.)

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as determination of optical properties and fiber identification.

Chapter 11 Manufactured Fiber Examinations - Optical Properties

General Discussion

This module is intended to teach the trainee proper techniques and the appropriate observations for determining the optical properties of manufactured fibers. Manufactured fibers include fibers that are man-made by chemical synthesis (e.g. thermoplastics, glass, steel), regenerated natural polymers (e.g. rayons), or derived from chemically modified natural polymers (e.g. cellulose acetates). Textile fibers such as fiberglass, anti-static fibers, ceramic fibers, metal fibers or metal-coated decorative threads should be included within the scope of the training. The appropriate determination and interpretation of optical properties with reference to natural fibers is addressed in the applicable natural fiber chapters of this training program manual.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. determine refractive indices, sign of elongation and birefringence of fibers;
2. reveal internal structures in fibers by use of appropriate mounting media;
3. obtain optical property values of reference materials from literature and/or standard collection; and
4. classify manufactured fibers into generic classes based on optical properties.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 11 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in optical property examinations:	D	P	C
1. determining refractive indices (parallel and perpendicular) by immersion method			
2. determining sign of elongation with compensators			
3. determining birefringence with compensator and quartz wedge			

3. Practical Exercises: Complete these exercises located in Appendix II.

11-1 Determining the Sign of Elongation
Part 1 Using the First Order Red Compensator
Part 2 Using the Quartz Wedge
11-2 Measuring Fiber Birefringence
11-3 Measuring Fiber Refractive Indices by the Immersion Method

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as determination of optical properties and fiber identification.

Chapter 12 Manufactured Fiber Examination - Cross Sections

General Discussion

This module is intended to familiarize the trainee with various fiber cross-sectioning techniques and the type of information obtained from these techniques. Along with physical shape, cross sections reveal the distribution of internal structures and dye penetration. The trainee will develop proficiency in optical and physical cross-sectioning techniques.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. obtain satisfactory physical cross sections from single fibers, multiple fibers and fiber tufts;
2. determine the modification ratio of multi-lobed fibers;
3. describe observed fiber features, such as: shape, delustrant, pigment particle distribution, presence and size of spherulites or voids, dye penetration depth, and bi-component fibers;
4. compare and contrast the types and quality of information obtained from optical cross-sectioning versus physical cross-sectioning; and
5. observe the relationship of fiber cross-sectional shape to generic class and end usage.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 12 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in cross-sectioning:	D	P	C
1. techniques for cross-sectioning multiple fibers and fiber tufts			
2. techniques for cross-sectioning single fibers			
3. measuring fiber dimensions in cross section			
4. measuring fiber diameter and determining shape from longitudinal sections			
5. calculating modification ratio from cross sections			
6. observing, in cross and longitudinal sections: a. delustrant, voids, spherulites, b. pigment particles, dye penetration, c. shapes and surface treatment			

3. Practical Exercises: Complete these exercises located in Appendix II.

12-1 Cross-Sectioning Fibers and Interpretation of Cross Sections
12-2 Determining the Modification Ratio of Multi-lobed Fibers

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as determination of optical properties and modification ratios from fiber cross sections.

Chapter 13 Manufactured Fiber Examination - Solubility

General Discussion

This module is intended to familiarize the trainee with solubility testing and the judicious use of this destructive technique. The trainee should be aware of the applicability of solubility testing in which it can provide information for fiber identification or distinctions that cannot easily be provided by other techniques.

Objectives

Upon satisfactory completion of this training module the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. understand the practice and applications of solubility testing;
2. use solubility testing to determine fiber generic class distinctions;
3. identify those situations in which solubility testing is appropriate and select appropriate tests; and
4. recognize solvent reactions indicative of bi-component/bi-constituent fiber compositions.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 13 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in solubility testing:	D	P	C
1. micro-sampling an appropriately sized fiber segment			
2. observing and describing solubility test reactions (total/partial solubility, insolubility, swelling, shrinking, gelling, color change, and bi-component fiber differential reactions).			
3. solvent washing and clearing on slide and cover slip			
4. side-by-side solubility testing for comparisons			

3. Practical Exercise: Complete this exercise located in Appendix II.

    13-1 Solubility Testing of Acetate and Triacetate Fibers

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions such as preliminary fiber identification and distinction based on performance of solubility testing.

Chapter 14 Manufactured Fiber Examination - Thermal Microscopy

General Discussion

This module is intended to familiarize the trainee with the use of a polarized light microscope equipped with a hot stage to observe the effect of heat on thermoplastic fibers and to determine fiber melting point. The trainee should be aware of the applicability and the judicious use of this destructive technique.

Objectives

Upon satisfactory completion of this training module the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. set up, operate and calibrate a hot stage;
2. use the hot stage for melting point determinations and observe reactivity (e.g. softening, charring, melting, etc.);
3. identify those situations in which thermal microscopy is appropriate;
4. obtain melting point values from reference materials and the literature; and
5. understand alternative methods of melting point determination.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 14 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in melting point range determination:	D	P	C
1. proper hot stage and microscope set-up, operation, and calibration			
2. micro-sampling an appropriately sized fiber segment			
3. observing and describing thermal reactions: a. droplet formation, contraction, softening, charring, melting, and b. differential reactions in bi-component or bi-constituent fibers			
4. evaluating and comparing data			

3. Practical Exercise: Complete this exercise located in Appendix II.

14-1 Use of a Hot Stage
- Part 1 Determining Melting Range of a Manufactured Fiber
- Part 2 Identifying and Discriminating Nylon 6 and 6,6 by Melting Points
- Part 3 Comparing Fibers by Melting Points

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions on the determination of melting point ranges and fiber classifications using literature references and comparison to the generated data.

Practical Examination B:

The trainee shall complete a fiber identification practical examination. The test fibers should include natural and manufactured fibers. The trainee should be instructed to identify the fiber as completely as possible based on the appropriate application of all methods and techniques learned to date and your laboratory's identification protocol. Some fibers may be identified as to generic type, sub-generic type, or manufacturer.

Chapter 15 Manufactured Fiber Examination - Infrared Spectroscopy

General Discussion

This module is intended to familiarize the trainee with the use of infrared spectroscopy (IR) for fiber identification and comparison by interpretation of absorption spectra. The IR is typically used in the identification of manufactured fibers. Cellulosic fibers are indistinguishable from one another using IR. While this technique does not provide sufficient information for a complete identification of cellulosic fibers, the trainee will find it useful to use IR on natural fibers to gain experience from the spectral information obtained by this technique.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. properly operate and maintain an IR and its accessories;
2. have an understanding of the optical and vibrational physics of infrared absorption;
3. have an understanding of the operational capabilities and limitations of the spectrometer and its accessories;
4. understand the differences between conventional dispersive IR and Fourier Transform IR;
5. identify those situations in which infrared analysis is appropriate;
6. prepare samples by a variety of techniques; and
7. obtain spectra from samples, run spectral library searches, and interpret the spectra.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 15 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in infrared spectroscopy:	D	P	C
1. setting up and operating the bench and microscope, checking performance and calibration			
2. adjusting apertures, objectives and condensers for optimum performance			
3. sample preparation techniques			
4. sample alignment and acquiring spectra			
5. interpreting spectra and searching reference libraries			

3. Practical Exercises: Complete these exercises located in Appendix II.

15-1 Sample Preparation for FTIR-Microscopy
Part 1 The Effect of Fiber Thickness
Part 2 Reducing the Interference Fringes in Spectra
15-2 The Transmission/Reflection Technique
15-3 Interpretation of Fiber FTIR Spectra

(Note: These exercises require substantial preparation by the trainer, see exercise preparation section.)

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include basic skills questions such as obtaining acceptable spectra from samples and comparisons to reference spectra.

Chapter 16 Fiber Examination - Comparison Microscopy

General Discussion

This module is intended to familiarize the trainee with the use of the comparison microscope and techniques for comparisons. This method is essential for the comparisons of physical and optical properties of fibers.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. prepare known fiber samples;
2. properly operate and maintain the comparison microscope and its accessories;
3. have an understanding of the comparison microscope optics (e.g. magnification, balancing illumination);
4. perform comparison of fiber features (e.g. morphology, color, delustering agents, diameter); and
5. interpret the significance of the compared fiber features.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 16 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in comparison microscopy:	D	P	C
1. setting up, operating and adjusting the microscopes			
2. color balancing the light sources for similar visual response to color, clarity, brightness			
3. performing side-by-side fiber comparisons			
4. taking photomicrographs			

3. Practical Exercise: Complete this exercise located in Appendix II.

16-1 Using the Comparison Microscope with Brightfield Illumination
Part 1 Making Slides to Assist in Balancing the Illumination
Part 2 Color and Morphological Feature Comparison

(Note: This exercise requires substantial preparation by the trainer, see Part 2 preparation section.)

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include basic skills questions on fiber comparisons.

Practical Examination C:

The trainee shall complete a fiber identification and comparison practical examination. The trainee should be instructed to identify and compare the fibers based on the appropriate application of all methods and techniques learned to date and your laboratory's identification protocol. The test fibers should include natural and manufactured fibers. The quantity and quality of test fibers provided for the practical examination should be varied, but within the realm of realistic case submissions. The trainee shall be evaluated not only for achieving a correct answer as to fiber identification and comparison of known and questioned fibers, but also for critical thinking in the selection of tests performed and the sequence in which they are performed.

Chapter 17 Fiber Examination - Fluorescence Microscopy

General Discussion

This module is intended to familiarize the trainee with the use of the fluorescence microscope and fluorescence microscopy techniques. Fluorescence microscopy can be applicable to natural as well as manufactured fibers.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. prepare samples and select appropriate mounting media;
2. properly operate and maintain the fluorescence microscope and its accessories;
3. have an understanding of the optical properties of fluorescence and the fluorescence microscope;
4. perform visual examinations and comparisons to assess the presence/absence of fluorescence and its dependence on various excitation conditions;
5. distinguish between fluorescence originating from dyes and that originating from optical brighteners;
6. recognize fluorescence from adherent material;
7. interpret the significance of the fluorescence observed during comparison; and
8. understand the factors which may or may not affect fluorescence.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 17 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in fluorescence microscopy:	D	P	C
1. setting up and operating the fluorescence microscope			
2. optimizing the high intensity light source			
3. using excitation and barrier filters			
4. observing fluorescence, noting both color and intensity			
5. taking photomicrographs			

3. Practical Exercises: Complete these exercises located in Appendix II.

17-1 The Fluorescence Microscope: Set-up and Operation
17-2 The Effects of Mounting Media in Fluorescence Microscopy
17-3 Observing Fluorescence on Fibers
Part 1 Optical Brighteners
Part 2 Dyed Fibers
Part 3 Contaminants

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include basic skills questions on practical examination and comparison of fibers, using fluorescent properties.

Chapter 18 Fiber Examination - UV/VIS Microspectrophotometry

General Discussion

This module is intended to familiarize the trainee with the use of ultraviolet/visible light microspectrophotometry as a qualitative, quantitative and objective method of color analysis. Color analysis can be applicable to natural as well as manufactured fibers. The calculation of complementary chromaticity coordinates is not required for color comparisons. However, the trainee should obtain theoretical knowledge of colorimetry for thoroughness in understanding color analysis.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. properly operate and maintain the microspectrophotometer and its accessories;
2. understand the optical properties of the microspectrophotometer;
3. identify those situations in which microspectrophotometry is appropriate;
4. prepare samples and select appropriate mounting media;
5. evaluate the number of fibers required within a control sample to yield representative spectra;
6. interpret and compare spectra; and
7. have an understanding of colorimetry (e.g. color intensity, hue, calculation of chromaticity coordinates).

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 18 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in UV/VIS microspectrophotometry:	D	P	C
1. setting up and operating the instrument, checking performance and calibration			
2. adjusting illumination and detector apertures			
3. setting up Köhler illumination			
4. sample preparation and selection of mounting media			
5. acquiring spectra and maintaining measuring parameters throughout analysis			
6. interpreting and comparing spectra			

3. Practical Exercises: Complete these exercises located in Appendix II.

18-1 Microspectrophotometer Set-up and Operation
18-2 Acquiring Spectra From Single Fibers
18-3 Acquiring Known Spectral Sets and Comparing Spectral Curves
18-4 Examining Metameric Fibers

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include basic skills questions on obtaining acceptable spectra and spectral curve comparisons.

Chapter 19 Fiber Examination - Dye Analysis/Thin Layer Chromatography

General Discussion

This module is intended to familiarize the trainee with the proper application of thin layer chromatography (TLC) for fiber dye analysis and comparison. The use of TLC is applicable to natural as well as manufactured fibers.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. understand the physical and chemical principles of TLC;
2. identify those situations in which TLC analysis is appropriate;
3. use standard dye mixtures for testing eluent, extraction chemical and system performance;
4. classify dyes based on fiber type and extraction reactions in various solvents;
5. select optimum eluent systems;
6. extract dyes from a variety of sample sizes;
7. record and compare chromatograms for colors, fluorescence, position and intensity of bands; and
8. interpret the significance of the observed chromatogram.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 19 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in thin layer chromatography:	D	P	C
1. extracting dye from single fibers and bulk samples			
2. effective sample application to plates			
3. developing plates using appropriate eluents			
4. evaluating plates with UV and VIS light			
5. comparing results			

3. Practical Exercises: Complete these exercises located in Appendix II.

19-1 Classification of Fiber Dyes
19-2 Thin Layer Chromatography of Fiber Dyes
Part 1 Testing of Eluents and Extractant
Part 2 TLC of Basic Dyed Acrylic Fiber Bulk Samples
Part 3 TLC of Basic Dyed Acrylic Fibers of Differing Lengths

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include basic skills questions on sample application, plate development and interpretation of bands.

Practical Examination D:

The trainee shall complete a fiber identification and comparison practical examination. The trainee should be instructed to use all applicable and appropriate methods and techniques learned to date. The test should include natural and manufactured fibers. The quantity and quality of test fibers provided for the practical examination should be varied, but within the realm of realistic case submissions. The trainee shall be evaluated not only for achieving a correct answer as to fiber identification and comparison, but also for critical thinking in the selection of tests and the sequence in which they were performed.

Chapter 20 Fiber Examination - Other Techniques

General Discussion

This module is intended to familiarize the trainee with a variety of other instrumental techniques that may be applied to fiber and textile examinations. This module is written as a development of the trainee's theoretical knowledge of these techniques. If your laboratory uses any of these methodologies, then the trainer shall amend this module to include specifically stated learning objectives, reading assignments, basic skills demonstrations, and practical exercises.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed theoretical knowledge of the applicability and use of alternative methodologies in fiber examinations including, but not limited to, the use of:

1. scanning electron microscopy/energy dispersive spectroscopy;
2. pyrolysis-gas chromatography, pyrolysis-mass spectrometry, pyrolysis-FTIR;
3. capillary electrophoresis and high-performance liquid chromatography for dye analysis; and
4. Raman spectroscopy and emission spectroscopy.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 20 reading assignments listed in Appendix I.

Competency Evaluation

The trainee shall be evaluated by quizzes on the theoretical knowledge as specified in the module objectives. If this module has been amended (as described above) to include specifically stated objectives, reading assignments and basic skills, then the quizzes should include basic practical skills questions relevant to the amendment.

Chapter 21 Textile Examinations

General Discussion

This module is designed to familiarize the trainee with the examination and comparison of yarns, threads, fabrics and buttons. This will include the identification and possible cause of physical damage to textile materials, physical matching, and fabric impressions.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. understand textile terminology, examination and comparison techniques for physical construction and composition of yarns, threads, fabrics and buttons;
2. identify and assess characteristics of physical damage to textile materials;
3. describe characteristics used to assess taphonomy of physical damage;
4. examine and compare fabric impressions; and
5. examine textile materials for physical matches.

Training Steps and Check List

1. Reading Assignments: Complete Chapter 21 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in textile examinations:	D	P	C
1. examining and comparing textile yarns and threads			
2. examining and comparing fabrics			
3. comparing buttons			
4. determining type of physical damage			
5. assessing taphonomy of physical damage			
6. examining and comparing fabric impressions			
7. examining textiles for physical matches			

3. Practical Exercises: Complete these exercises located in Appendix II.

21-1 Examining Fabric Damage
21-2 Environmental, Chemical and Mechanical Effects on Fabrics

Competency Evaluation

The trainee shall be evaluated by quizzes on the theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions on identification of fabric: physical construction, pattern, identification of warp and weft, fiber content, and fabric impressions, etc.

Chapter 22 Cordage Examinations

General Discussion

This module is designed to familiarize the trainee with the examination and comparison of cordage, to include construction and fiber content. This module is not intended to teach the trainee identification of knots. The cordage terminology used may vary within the literature.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. understand cordage terminology, examination and comparison techniques for physical construction and composition;
2. identify and assess characteristics of physical damage to cordage materials;
3. determine characteristics used to assess taphonomy of physical damage;
4. examine and compare cordage impressions; and
5. examine cordage for physical matches.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 22 reading assignments listed in Appendix I.

2. Basic Skills

Basic Skills in cordage examinations:	D	P	C
1. physical description of cordage			
2. examining and comparing cordage			
3. examining and comparing cordage impressions			
4. determining types of physical damage			
5. assessing taphonomy of physical damage			
6. examining cordage for physical matches			

3. Practical Exercises: Complete these exercises located in Appendix II.

22-1 Composition and Physical Construction of Natural and Manufactured Fiber Cordage
22-2 Environmental, Chemical and Mechanical Effects on Natural and Manufactured Fiber Cordage

Competency Evaluation

The trainee shall be evaluated by quizzes on theoretical knowledge and basic practical skills as specified in the module objectives. The quizzes should include practical skills questions on the determination of cordage construction and fiber content.

Practical Examination E:

The trainee shall complete a textile and cordage practical examination which should include construction and composition, physical matching, physical impressions, and assessment of damage and wear. The trainee should be instructed to use all applicable and appropriate methods and techniques in compliance with laboratory protocol. The textile and cordage samples should include those composed of natural fibers as well as those composed of manufactured fibers. The quantity and quality of test materials provided for the practical examination should be varied, but within the realm of realistic case submissions. The trainee shall be evaluated not only for achieving a correct answer, but also for critical thinking in the selection of tests performed and the sequence in which they are performed.

Chapter 23 Interpretation and Presentation

General Discussion

This module is designed to serve as a mechanism for the trainee to integrate the factors that affect evidence interpretations and the significance of fiber evidence. Although these topics should have been routinely addressed in each chapter throughout the training, the intent is to perform some academic exercises to draw all topics together at the end of the training period. The trainee is directed to review and interpret case documentation. The trainee is then to write a report of his/her findings. The trainer should review and discuss these results with the trainee.

Objectives

Upon satisfactory completion of this training module, the trainee will have developed and demonstrated theoretical knowledge and/or practical skills to:

1. draw appropriate conclusions from analytical data;
2. understand the factors affecting the data and its interpretation;
3. understand the significance of fiber evidence;
4. understand the application of statistics;
5. write complete and unbiased analytical reports with appropriate conclusions and opinions; and
6. provide appropriate testimony.

Training Steps and Check Lists

1. Reading Assignments: Complete Chapter 23 reading assignments listed in Appendix I.

2. Interpretive Exercises:

The trainee should be given a predetermined number of “cases” for analysis, composed of either analytical data, case evidence or simulated evidence depending on what is available in the laboratory. Any material given should be handled as evidence under laboratory protocol. The number of interpretive exercises or re-examinations should be determined by the trainer and laboratory policy.

Based on the provided or generated information, the trainee should prepare an analytical report for each case. The examination notes and report prepared by the trainee should be evaluated for completeness and discussed with the trainee. The trainee’s report should be compared to the report prepared by the experienced examiner and discussed with the trainee as part of the evaluation when appropriate.

A written record of the satisfactory completion of this Interpretive Exercise should be maintained and may be recorded in the Practical Exercise completion table provided in Chapter 2. Individual exercise assignments may be noted below as a record of casework interpretive exercises or casework re-examinations.

Case or Exercise Identifier	Date Assigned	Satisfactory Completion (date and initials of trainer and trainee)
1.		
2.		
3.		
4.		
5.		
6.		

Competency Evaluation

The final progression to certification of competency as an independent fiber examiner shall be reviewed as specified in Chapter 2 of this training program manual. In accordance with those specifications, the trainee shall complete:

1. Supervised Casework

The trainee should be prepared to perform all aspects of supervised casework after satisfactory completion of all readings, basic skills acquisitions, practical exercises and examinations specified in this training program to this point. Upon concurrence of the trainer and laboratory management, the trainee should begin all aspects of supervised casework. The number of cases to be completed by the trainee should be determined by your laboratory's protocol. A complete record of these cases and casework evaluations should be maintained and may be recorded as provided for in Chapter 2.

2. Mock Trial

At least one mock trial should be conducted as part of this training program. Mock trial(s) shall be conducted in accordance with your laboratory's protocol and may be recorded as provided for in Chapter 2.

3. Written Examination

The final written examination for this training program shall be administered and may be recorded as provided for in Chapter 2.

4. Certification of Competency

The trainee shall be evaluated by review of all training records generated through this training program, and shall receive a written certificate of competency before performance of independent casework. A suggested format for final competency certification is provided in Chapter 2.

APPENDIX I
READING ASSIGNMENTS

TOPIC		PAGE
READING ASSIGNMENT USER'S GUIDE		2
READING ASSIGNMENTS		
Chapter 3	Introduction to Fibers and Textiles	3
Chapter 4	Fiber Transfer and Persistence	4
Chapter 5	Casework Familiarization/Search and Recovery	5
Chapter 6	Microscopy Review	6
Chapter 7	Fiber Examinations/Preliminary Observations	7
Chapter 8	Identification of Vegetable Fibers	8
Chapter 9	Identification of Animal Textile Fibers	9
Chapter 10	Identification of Inorganic Fibers	10
Chapter 11	Manufactured Fiber Examination - Optical Properties	11
Chapter 12	Manufactured Fiber Examination - Cross Sections	12
Chapter 13	Manufactured Fiber Examination - Solubility	13
Chapter 14	Manufactured Fiber Examination-Thermal Microscopy	14
Chapter 15	Manufactured Fiber Examination - Infrared Spectroscopy	15
Chapter 16	Fiber Examination - Comparison Microscopy	16
Chapter 17	Fiber Examination - Fluorescence Microscopy	17
Chapter 18	Fiber Examination - UV/VIS Microspectrophotometry	18
Chapter 19	Fiber Examination - Dye Analysis/TLC	19
Chapter 20	Fiber Examination - Other Techniques	20
Chapter 21	Textile Examinations	22
Chapter 22	Cordage Examinations	23
Chapter 23	Interpretation and Presentation	24

READING ASSIGNMENTS USER'S GUIDE

Reading assignments are selected to give the trainee a broad range of understanding of current and past thoughts on various topics. The reading assignments intended to give the trainee a sound theoretical background are designated as required, and are considered mandatory. Supplemental readings should be sought out by the trainee and may be specified by the trainer or laboratory policy. Continued exposure to the forensic literature should encourage further professional development, reveal alternate opinions in certain subject areas, and present some possible research topics. Some reading assignments may appear redundant. However, re-reading some assignments is thought to be beneficial because the trainee will have some newly learned skills, practical experience and/or perspectives which will make the literature more meaningful.

The reading assignments are designated by Chapter. The trainee should place their initials and date in the space provided to the left of each *Required Reading* reference upon completion of the reading assignment as part of the training record. Space is provided for addition of a written record of *Supplemental Readings* completed by the trainee. The references and bibliographies in the required readings are a good source of material for the supplemental readings.

A number of general reference texts, including dictionary and encyclopedic sources, should be made available for the trainee's use throughout the training period. The trainee should refer to the *General Reference* books for all material relevant to each Chapter topic. These readings should also be recorded as Supplemental Readings in the space provided upon completion. The following texts are recommended:

American Fabrics and Fashions Magazine, editor. Encyclopedia of Textiles, 3rd edition. Englewood Cliffs, NJ: Prentice-Hall, 1980.

Brady GS, Clauser HR, Vaccari JA. Materials Handbook, 15th edition. New York: McGraw-Hill Professional Publishing, 2002.

Dictionary of Fiber and Textile Technology. Charlotte, NC: Hoechst-Celanese Corp.,1990.

Grayson M, editor. Encyclopedia of Textiles, Fibers, and Nonwoven Fabrics. New York: John Wiley & Sons, Inc., 1984.

Hall D. Practical Fiber Identification Auburn, AL: Auburn University, 1982.

Hatch KL. Textile Science. Minneapolis, MN: West Information Publishing Group, 1993.

Heyn ANJ. Fiber Microscopy: A Textbook and Laboratory Manual. New York: Interscience Publishers, 1954.

Kuehni RG. Color: An Introduction to Practice and Principles. New York: Wiley Interscience, Inc., 1997.

Luniak B. Identification of Textile Fibres, Qualitative and Quantitative Analysis of Fibre Blends, 2nd edition. London: Pitman, 1953.

Mauersberger H, editor. Matthews' Textile Fibers, 6th edition. New York: John Wiley & Sons, Inc., 1975.

Raheel M, editor. Modern Textile Characterization Methods, New York: Marcel Dekker Publisher, 1996.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975.

Chapter 3 Introduction to Fibers and Textiles Reading Assignment

Completion Date & Initials	Reference

Required Readings

David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1-31.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 209-214.

Grieve MC, Wiggins KG. Fibers under fire: suggestions for improving their use to provide forensic evidence. J Forensic Sci 2001;46(4):835-843.

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

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 1 Introduction to fiber examinations. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Supplemental Readings

Chapter 4 Fiber Transfer and Persistence Reading Assignment

Completion Date & Initials | **Reference**

Required Readings

Akulova V, Vasiliauskiene D, Talaliene D. Further insights into the persistence of transferred fibres on outdoor clothes. Sci Justice 2002; 42(3):165-171.

Annis PA, Bresee RR, Cooper TR. Influence of textile structure on single fiber transfer from woven fabrics. Textile Research J 1992; 65(2):293-301.

Coxon A, Grieve M, Dunlop J. A method of assessing the fibre shedding potential of fabrics. J Forensic Sci Soc 1992; 32:151-158.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 214-221, 255-259.

Grieve MC, Dunlop J, Haddock PS. Transfer experiments with acrylic fibres. Forensic Sci Int 1989; 40:267-277.

Palmer R. The retention and recovery of transferred fibers following the washing of recipient clothing. J Forensic Sci 1997; 43(3):502-504.

Pounds C A, Smalldon KW. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part I. J Forensic Sci Soc 1975;15:17-27.

Pounds C A, Smalldon KW. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part II. J Forensic Sci Soc 1975;15:29-7.

Pounds C A, Smalldon KW. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part III. J Forensic Sci Soc 1975;15:197-207.

Robertson J, Roux C. Transfer, persistence and recovery of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 89-100.

Robertson J, Lloyd AK. Redistribution of textile fibres following transfer during simulated contacts. J Forensic Sci Soc 1984; 24:3-7.

Salter MT, Cook R, Jackson AR. Differential shedding from blended fabrics. Forensic Sci Int 1987; 33:155-164.

Scientific Working Group for Materials Analysis (SWGMAT). Trace Evidence Recovery Guidelines, Part 4 Contamination and loss. Forensic Sci Commun Oct 1999; 1(3) available on the Internet at www.fbi.gov .

Supplemental Readings

Chapter 5 Casework Familiarization/Search and Recovery Reading Assignment

Completion Date & Initials **Reference**

Required Readings

All applicable parts of your laboratory's policy and standard operating procedures manuals covering the topics of:

- health and safety practices
- quality assurance
- general evidence handling, analytical, and reporting procedures

Biermann TW. Fiber finder systems. In: In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;135-152.

Farley MA. Legal standards for the admissibility of novel scientific evidence. In: Saferstein R, editor. Forensic Science Handbook, Vol. III. Englewood Cliffs, NJ: Prentice-Hall Inc., 1993; 1-23.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 218-272.

Dignan SJ, Murphy KJ. Fibre evidence from fingernail clippings. Can Soc For Sci J 2002; 35(1):17-21.

Grieve MC Influential factors, quality assurance, report writing and case examples. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 343-361.

Moore JE, Jackson G, Firth M. Movement of fibres between working areas as a result of routine examination of garments. J For Sci Soc 1986; 26:433-440.

Palenik S. Microscopy and microchemistry of physical evidence. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 161-208.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;116-134.

Roux C, Huttuen J, Rampling K, Robertson J. Factors affecting the potential for fibre contamination in purpose-designed forensic search rooms. Sci Justice 2001; 41:135-144.

Springer F. Collection of fibre evidence from crime scenes. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;101-115.

Scientific Working Group for Materials Analysis (SWGMAT). Trace Evidence Recovery Guidelines. Forensic Sci Commun Oct 1999; 1(3) available on the Internet at www.fbi.gov .

Scientific Working Group for Materials Analysis (SWGMAT). Trace Evidence Quality Assurance Guidelines. Forensic Sci Commun Jan 2000; 2(1) available on the Internet at www.fbi.gov .

Supplemental Readings

Chapter 6 Microscopy Review Reading Assignment

Completion Date & Initials | **Reference**

Required Readings

DeForest PR. Foundations of forensic microscopy. In: Saferstein R, editor. Forensic Science Handbook, Vol. I, 2nd edition. Englewood Cliffs, NJ: Regents/Prentice-Hall Inc., 2002; 215-319.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Research Institute, 2002.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres; 2nd edition. London: Taylor & Francis, 1999; 153-177.

The instruction manuals provided by the manufacturer(s) of your laboratory's stereomicroscope, transmitted light and polarized light microscopes.

Your laboratory's standard operating procedures and quality assurance procedures governing microscope operation and maintenance.

Supplemental Readings

Chapter 7 Fiber Examinations/Preliminary Observations Reading Assignment

Completion Date & Initials **Reference**

Required Readings

David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1-31.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 222-230, 238-241.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;153-177.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;116-134.

Scientific Working Group for Materials Analysis (SWGMAT), Forensic fiber examination guidelines, Chapter 2 Microscopy of textile fibers. Forensic Sci Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Your laboratory's fiber standard operating procedures governing preliminary examinations, interpretation and reporting.

Supplemental Readings

Chapter 8 Identification of Vegetable Fibers Reading Assignment

Completion Date & Initials **Reference**

Required Readings

Catling D, Grayson J. Identification of Vegetable Fibres. London: Chapman and Hall, 1982.

David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1, 8-15.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;156-159.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;124-128.

Wiggins K. Ropes and cordage. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 59.

Your laboratory's fiber standard operating procedures governing vegetable fiber examinations, interpretation and reporting.

Supplemental Readings

General References Specific to Natural Fibers

Cook JG. Handbook of Textile Fibres-Natural Fibres, 5th edition, Durham UK: Merrow, 1984.

Friesen PL Natural Fibers Information Guide, 2nd edition, Dennisport, MA: Crane, 1994.

Chapter 9 Identification of Animal Textile Fibers Reading Assignment

Completion Date & Initials **Reference**

Required Readings

David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1-31.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 209-214, 239-241.

Your laboratory's fiber standard operating procedures governing animal fiber examinations, interpretation and reporting.

Supplemental Readings

General References Specific to Fibers of Animal Origin

Appleyard HM. Guide to the Identification of Animal Fibres. Leeds, UK: WIRA, the research and services centre for textiles and clothing, 1978.

Debrot S. Atlas Des Poils De Mammiferes D'Europe. Pereux, Suisse: Imprimerie de l'Ouest S.A., 1982.

Moore TD, Spence LE, Dubnolle CE. Identification of the Dorsal Guard Hairs of Some Mammals of Wyoming. Cheyenne, WY: Wyoming Game and Fish Department, Bulletin No. 14, 974.

Chapter 10 Identification of Inorganic Fibers Reading Assignment

Completion Date & Initials	Reference

Required Readings

Deer WA, Howie RA, Zussman J. An Introduction to the Rock-Forming Minerals. New York: Addison-Wesley Publishing, 1992. (Note: read sections on asbestiform minerals; specific pages vary in different editions.)

McCrone WC, Asbestos Identification, 2nd edition. Chicago: McCrone Research Institute, 1987.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Research Institute, 2002; 169-196.

Your laboratory's fiber standard operating procedures governing inorganic fiber examinations, interpretation and reporting.

Supplemental Readings

Chapter 11 Manufactured Fiber Examination - Optical Properties Reading Assignment

Completion Date & Initials **Reference**

Required Readings

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 223-230.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Research Institute, 2002; 142-149.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;153-177.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;116-134.

Your laboratory's fiber standard operating procedures governing fiber microscopical examinations, interpretation and reporting.

Supplemental Readings

Chapter 12 Manufactured Fiber Examination - Cross Sections Reading Assignment

Completion Date & Initials	Reference

Required Readings

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Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;161-163.

Your laboratory's fiber standard operating procedures governing fiber crosssection examinations, interpretation and reporting.

Supplemental Readings

Chapter 13 Manufactured Fiber Examination - Solubility Reading Assignment

Completion Date & Initials **Reference**

Required Readings

David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1-31.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 236-237.

Your laboratory's fiber standard operating procedures governing solubility examinations, interpretation and reporting.

Supplemental Readings

Chapter 14 Manufactured Fiber Examination - Thermal Microscopy Reading Assignment

Completion Date & Initials	Reference

Required Readings

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 233, 236.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;170-172.

The instruction manuals provided by the manufacturer(s) of your laboratory's microscope(s) and hot stage apparatus.

Your laboratory's fiber standard operating procedures governing thermal microscopy examinations, interpretation and reporting.

Supplemental Readings

Chapter 15 Manufactured Fiber Examination - Infrared Spectroscopy Reading Assignment

Completion Date & Initials **Reference**

Required Readings

Bartick EG, Tungol MW. Infrared microscopy and its forensic applications. In: Saferstein R, editor. Forensic Science Handbook, Vol. III. Englewood Cliffs, NJ: Prentice-Hall Inc., 1993: 196-252.

Cho L, Reffner JA, Gatewood BM, Wetzel DL. Single fiber analysis by internal reflection microspectroscopy. J Forensic Sci 2001; 46(6):1309-1314.

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Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 6 Infrared analysis of textile fibers. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

The instruction manuals provided by the manufacturer(s) of your laboratory's IR instrumentation.

Your laboratory's fiber standard operating procedures governing infrared spectroscopy examinations, interpretation and reporting.

Supplemental Readings

Chapter 16 Comparison Microscopy Reading Assignment

Completion Date & Initials	Reference

Required Readings

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Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 241-245.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;121-124.

The instruction manuals provided by the manufacturer of your laboratory's comparison microscope.

Your laboratory's fiber standard operating procedures governing comparison microscopy examinations, interpretation and reporting.

Supplemental Readings

Chapter 17 Fluorescence Microscopy Reading Assignment

Completion Date & Initials	Reference

Required Readings

Abramowitz M. Fluorescence Microscopy, The Essentials", Vol. 4, Basics and Beyond Series. Melville, NY: Olympus America Inc., 1993; 1-22.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Research Institute, 2002; 64-68.

The instruction manuals provided by the manufacturer of your laboratory's fluorescence microscope.

Your laboratory's fiber standard operating procedures governing fluorescence microscopy examinations, interpretation and reporting.

Supplemental Readings

Chapter 18 Fiber Examination - UV/VIS Microspectrophotometry Reading Assignment

Completion Date & Initials **Reference**

Required Readings

Adolf FP, Dunlop J. Microspectrophotometry/colour measurement. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;1252-289.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 245-248.

Grieve MC, Biermann TW, Davignon M. The evidential value of black cotton fibres. Sci Justic 2001; 41(4):245-260.

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 3 Visible spectroscopy of textile fibers. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

The instruction manuals provided by the manufacturer of your laboratory's instrumentation.

Your laboratory's fiber standard operating procedures governing microspectrophotometry examinations, interpretation and reporting.

Supplemental Readings

General References Specific to Color

McLaren K. The Colour Science of Dyes and Pigments. Bristol, UK: Adam Hilger, 1983.

Venkataraman K. The Analytical Chemistry of Synthetic Dyes. New York: John Wiley & Sons, 1977.

Chapter 19 Fiber Examination - Dye Analysis/TLC Reading Assignment

Completion Date & Initials	Reference

Required Readings

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 248-254.

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 4 Thin layer chromatography of non-reactive dyes in textile fibers. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Wiggins KG. Thin layer chromatographic analysis for fibre dyes. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 291-310.

Your laboratory's fiber standard operating procedures governing fiber dye analysis and TLC examinations, interpretation and reporting.

Supplemental Readings

General References Specific to TLC or Dyes

Hamilton R, Hamilton S. Thin Layer Chromatography. Chichester, UK: John Wiley & Sons, Inc., 1987.

Sherma J, Fried B, editors. Handbook of Thin Layer Chromatography. New York: Marcel Dekker Publishers, 1990.

Society of Dyers and Colourists. The Colour Index, 4th edition, Vols. 1-6, Research Triangle Park, NC: AATCC, 1985.

Stahl E. Thin Layer Chromatography. New York: Springer Verlag, 1969.

Chapter 20 Fiber Examination - Other Techniques Reading Assignment

Completion Date & Initials **Reference**

Required Readings

The instruction manuals provided by the manufacturer(s) of your laboratory's instrumentation which may be applicable under this chapter topic.

Your laboratory's standard operating procedures governing any examinations, interpretation and reporting that may be applicable under this chapter topic.

CE

Robertson J. Capillary electrophoresis. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 328-336.

HPLC

Davila-Jimenex, Elizalde-Gonzalez, Gutierrez-Gonzalez, Pelaez-Cid. Electrochemical treatment of textile dyes and their analysis by high-performance liquid chromatography with diode array detection. J Chromatography 2000; 889 (1-2): 253-259.

Kretschmer K, Kelbig W. Differentiation of commercial polyester fibers using two dimensional high-performance liquid chromatography (HPLC) and multivariate pattern recognition techniques. J Forensic Sci 1992; 37(3):727-737.

Griffin R, Speers J. High-performance liquid chromatography. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 311-327.

Pyrolysis

Challinor JM. Fibre identification by pyrolysis techniques. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 223-238.

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 5 Pyrolysis-gas chromatography of textile fibers. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

SEM

Roux C. Scanning electron microscopy and elemental analysis. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 239-250.

Spectroscopy:

Cho L, Reffner JA, Gatewood BM, Wetzel DL. A new method for fiber comparison using polarized infrared microspectroscopy. J Forensic Sci 1999; 44(2):275-282.

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Keen I, White G, Fredericks P. Characterization of fibers by Raman microprobe spectroscopy. J Forensic Sci 1998; 43(1):82-89.

Kokot S, Tuan NA, Rintoul L. Discrimination of reactive dyes on cotton fabric by Raman spectroscopy and chemometrics. Applied Spectroscopy 1997; 51(3):387-395.

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White P. Surface enhanced resonance Raman scattering spectroscopy. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 337-342.

Supplemental Readings

General Reference Specific to Other Techniques Topic

Mastura R, editor, Modern Textile Characterization Methods. New York: Marcel Dekker Publisher, 1996;

Chapter 3 Surface Characterization of Textiles Using SEM
Chapter 4 Investigation of Textiles by Analytical Pyrolysis
Chapter 5 Liquid Chromatographic Techniques in Textile Analysis

Chapter 21 Textile Examinations Reading Assignment

Completion Date & Initials	Reference

Required Readings

Adolf FP. The structure of textiles: an introduction to the basics. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 33-54.

Amick JF, Beheim CW. Screen-printing ink transfer in a sexual assault case. J Forensci Sci 2002: 47(3):619-624.

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 7 Fabrics and Cordage. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Taupin JM, Adolf FP, Robertson J. Examination of damage to textiles. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 65-87.

Your laboratory's fiber standard operating procedures governing textile, fabric impressions and physical match examinations, interpretation and reporting.

Supplemental Readings

Chapter 22 Cordage Examinations Reading Assignment

Completion Date & Initials	Reference

Required Readings

Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 7 Fabrics and Cordage. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Wiggins KG. Ropes and cordage. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 55-64.

Your laboratory's fiber standard operating procedures governing cordage and physical match examinations, interpretation and reporting.

Supplemental Readings

Chapter 23 Interpretation and Presentation Reading Assignment

Completion Date & Initials | **Reference**

Required Readings

Champod C, Taroni F. The Bayesian approach. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 379-398.

Cook R, Evett IW, Jackson G, Jones P, Lambert JA. A model for case assessment and interpretation. Sci Justice 1998; 38(3):151-156.

Evett IW, Jackson G, Lambert JA, McCrossan S. The impact of the principles of evidence interpretation on the structure and content of statements. Sci Justice 2000: 40:233-239.

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Grieve MC. A survey on the evidential value of fibres and on the interpretation of the findings in fibre transfer cases. Part 2-interpretation and reporting. Sci Justice 2000; 40(3):201-209.

Grieve MC. Influential factors, quality assurance, report writing and case examples. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 343-361.

Houck M. Statistics and trace evidence: the tyranny of numbers. Forensic Sci Commun Oct 1999; 1(3) available on the Internet at www.fbi.gov .

Sapir G. Legal aspects of forensic science. In: Saferstein R, editor. Forensic Science Handbook, Vol. I, 2nd edition. Englewood Cliffs, NJ: Regents/Prentice-Hall Inc., 2002; 1-39.

Starrs J. Mountebanks among forensic scientists. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 1-37.

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Web-Salter M, Wiggins KG. Aids to interpretation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 364-378.

Your laboratory's fiber standard operating procedures governing examination, interpretation and reporting.

Supplemental Readings

APPENDIX II
PRACTICAL EXERCISES

TOPIC		PAGE
PRACTICAL EXERCISE USER'S GUIDE		3
PRACTICAL SAFETY ISSUES		4
PRACTICAL EXERCISES		
Chapter 5	**Casework Familiarization/Search and Recovery**	
5-1	Fiber Transfer and Persistence	5
5-2	Collecting Fibers on Tape	7
Chapter 6	**Microscopy Review**	
6-1	Familiarization with the Stereomicroscope	10
6-2	Familiarization with the Compound Light Microscope	13
6-3	Familiarization with the Polarized Light Microscope	17
Chapter 7	**Fiber Examinations/Preliminary Observations**	
7-1	Fiber Manipulations: Removing Fibers from Tape and Mounting	22
7-2	Observing Effects of Mounting Media	25
7-3	Observing Fiber Shape, Surface and Internal Structure	28
7-4	Observing Color and Pleochroism	31
7-5	Distinguishing Natural and Manufactured Fiber Classes	33
Chapter 8	**Identification of Vegetable Fibers**	
8-1	Microscopy of Non-woody Vegetable Fibers	36
8-2	Determining Natural Fiber Twist	39
Chapter 9	**Identification of Animal Textile Fibers**	
9-1	Examining the Cuticle of Animal Hairs	42
9-2	Introduction to Examining Natural Fibers of Animal Origin	45
Chapter 10	**Identification of Inorganic Fibers**	
10-1	Identification of Asbestos Fibers	49
Chapter 11	**Manufactured Fiber Examination - Optical Properties**	
11-1	Determining the Sign of Elongation	52
11-2	Measuring Fiber Birefringence	56
11-3	Measuring Fiber Refractive Indices by the Immersion Method	59
Chapter 12	**Manufactured Fiber Examination - Cross Sections**	
12-1	Cross-sectioning Fibers and Interpretation of Cross Sections	62
12-2	Determining the Modification Ratio of Multi-lobed Fibers	65
Chapter 13	**Manufactured Fiber Examination - Solubility**	
13-1	Solubility Testing of Acetate and Triacetate Fibers	68

	TOPIC	PAGE
Chapter 14	**Manufactured Fiber Examination - Thermal Microscopy**	
	14-1 Use of a Hot Stage	71
Chapter 15	**Manufactured Fiber Examination - Infrared Spectroscopy**	
	15-1 Sample Preparation for FTIR-Microscopy	74
	15-2 The Transmission/Reflection Technique	78
	15-3 Interpretation of Fiber FTIR Spectra	81
Chapter 16	**Fiber Examination - Comparison Microscopy**	
	16-1 Using the Comparison Microscope with Brightfield Illumination	87
Chapter 17	**Fiber Examination - Fluorescence Microscopy**	
	17-1 The Fluorescence Microscope: Set-up and Operation	91
	17-2 The Effects of Mounting Media in Fluorescence Microscopy	94
	17-3 Observing Fluorescence on Fibers	96
Chapter 18	**Fiber Examination - UV/VIS Microspectrophotometry**	
	18-1 Microspectrophotometer Set-up and Operation	99
	18-2 Acquiring Spectra from Single Fibers	102
	18-3 Acquiring Known Spectral Sets and Comparing Spectral Curves	105
	18-4 Examining Metameric Fibers	109
Chapter 19	**Fiber Examination - Dye Analysis/TLC**	
	19-1 Classification of Fiber Dyes	111
	19-2 Thin Layer Chromatography of Fiber Dyes	114
Chapter 21	**Textile Examinations**	
	21-1 Examining Fabric Damage	118
	21-2 Environmental, Chemical and Mechanical Effects on Fabrics	121
Chapter 22	**Cordage Examinations**	
	22-1 Composition and Construction of Cordage	124
	22-2 Environmental, Chemical and Mechanical Effects on Cordage	127

PRACTICAL EXERCISE USER'S GUIDE

These practical exercises are designed to expose the trainee to the basic skills learned within each chapter, and to develop observational and interpretational skills related to the data collected by use of the basic skills. These practical exercises are not comprehensive for the totality of practical exposure a fiber examiner trainee should receive. Rather, these exercises should serve as a stimulus for the trainee to extend their practical experience through their own curiosity and intellect in self-tutorials of their own design, or by expansion or variations on the existing exercises. Toward this goal of gaining additional practical experience, the trainee should be given reasonable and adequate time to continue their experimentation beyond these required practical exercises.

All practical exercises should be completed by the trainee with full and complete note-taking, and report writing, if appropriate. The exercises should be reviewed by the trainer with particular attention to development of the trainee's critical thinking skills, as well as continuous review of learned basic skills.

The practical exercises are all organized with the following format and intent:

- **Subject** the title and topic of the exercise
- **Time** the anticipated minimum time required to perform the hands-on work; additional time shall be allotted for reading, preparation and note-taking
- **Objective** brief statement of the purpose of the exercise
- **Theory** brief statement(s) of the theoretical knowledge basis for the exercise, meant to direct the trainee's attention and to highlight the relevant points in the literature and exercise
- **References** mandatory readings that should be initialed and dated when completed
- **Preparation** statement of activities required prior to beginning the exercise
- **Materials** statement of supplies and equipment needed
- **Safety** good laboratory safety practices are always expected, a brief statement here is meant to alert a trainee to some safety issues that they may not have routinely encountered before (see comments below)
- **Directions** instructions for performing the exercise
- **Observations** statement(s) to direct the trainee's attention to appropriate types of observations, note-taking and data organization; statements may be reiterative with instructions given in the Directions section in some exercises
- **Discussion** a brief discussion of the pertinent observations, information, interpretations and conclusions that the trainee should have made if the exercise was performed correctly; including a statement of the exercise's relevance to other topics in the training program and suggestions for additional self-directed explorations in some exercises

A record of satisfactory practical exercise completions shall be kept. The "Record of Practical Exercise Completion" table located in Chapter 2 may be used for this purpose.

PRACTICAL SAFETY ISSUES

Fiber examiners (and trainees) come into contact with virtually all of the potentially dangerous physical (temperature, radiations and voltages), chemical and biological hazards present in the laboratory. The following brief reminders are made to draw particular attention to a few issues that a trainee should be aware of as a supplement to any existing laboratory policies and procedures, and/or safety manuals.

1. Appropriate health and safety practices should always be considered mandatory. Review your laboratory's policies and procedures on these topics.

2. Know where your laboratory's safety equipment (e.g. eye washes, safety showers, first aid kits, spill kits, fire extinguishers, etc.) is located, how to properly use the equipment and when/under what circumstances to use the equipment.

3. Always wear any personal protective equipment that is appropriate for the task(s) at hand. Utilize any appropriate engineering and/or work place practices to minimize risk.

4. All instrumentation should be inspected at least annually to ensure that all optical, mechanical and electrical components are in clean and safe operating condition. Heed all manufacturer's warnings about the safe handling, use and maintenance procedures of any instrumentation.

5. Material Safety Data Sheets (MSDS) or other relevant safety information should be reviewed for all chemicals an individual intends to use.

6. Virtually all mounting media could be considered hazardous. Different mountants contain volatile materials, corrosive compounds, teratogenic or carcinogenic materials, acute poisons, halogenated hydrocarbons or polychlorinated biphenyls (PCB's). Individuals should consider that microscopy will bring them, and others working near them, into close tactile and respiratory contact with these chemicals.

7. Be cognizant of the possibility that the evidentiary item(s) you are working with may have biological and chemical hazards associated with them, or may have chemical residues from previous examinations.

8. Special eye protection should be worn whenever using some alternate light sources such as intense UV light and lasers.

9. Explosion proof face shielding and hand protection should be worn while handling high pressure lamp assemblies such as xenon or mercury vapor lamps or hot lamp housings. The protection should be sufficient to protect against both thermal and fragmentation wounds. Do not attempt to handle or replace these or other light sources until you have been properly trained to do so.

10. Always use "sharps" with extra caution, following any recommended engineering and work practices to minimize the hazard they pose. Dispose of "sharps" with extra caution in a proper "sharps" disposal container.

11. Warning signs should be posted whenever chemicals or radiation may pose a hazard to individuals entering the work area.

12. Immediately report any health and safety problem of which you become aware. Take other action to remedy the problem or minimize risk in those situations in which it is appropriate and possible.

Chapter 5 Practical Exercise 5-1

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.

References:
Kidd C, Robertson J. The transfer of textile fibres during simulated contacts. J For Sci Soc 1982; 3:301-308.

Roux C, Langdon S, Waight D, Robertson J. The transfer and persistence of automotive carpet fibres on shoe soles. Sci Justice 1999; 39(4):239-251.

Scott H. The persistence of fibers transferred during contact of automobile carpets and clothing fabrics. Can Soc For Sci J 1985; 18(4):185-199.

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

Safety:
Use standard laboratory safety procedures according to the rules prescribed by your laboratory.

Directions:

1. 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.

2. 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.

3. Dress in your spare clothes after removing the evidence clothing.

4. 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.

5. Hold all evidence for use in Practical Exercises 5-2 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. If it is desirable to practice other collection methods, then more "evidence" should be generated at this time. If a variety of methods are used, then comparing the efficiency and discrimination of those methods will be useful.

Chapter 5 Practical Exercise 5-2

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.

References:
Fong W. Fiber evidence laboratory methods and observations from casework. J Forensic Sci 1984; 29(1):55-63.

Grieve MC. The role of fibers in forensic science examinations. J Forensic Sci 1983; 28:877-887.

Grieve MC, Garger BS. An improved method for rapid and accurate scanning of fibers on tape. J Forensic Sci 1981; 26(3):560-563.

McKenna FJ, Sherwin JC. A simple and effective method for collecting contact evidence. J For Sci Soc 1985; 30:485-493,

Robertson J, Roux C. Transfer, persistence and recovery of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 95-98.

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 5-1
- 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:

1. 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.

2. 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.

3. 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.

4. 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.

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

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

7. 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?

8. Save all of your tape-lift samples for use in Practical Exercise 7-1.

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 5-1 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.

Chapter 6 Practical Exercise 6-1

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 stereo-microscope 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.

References:
Scientific Working Group for Materials Analysis (SWGMAT). Forensic Fiber Examination Guidelines, Chapter 2 Microscopy of textile fibers. Forensic Sci Commun Apr 1999; 1(1) available on the Internet at www.fbi.gov .

Taupin J M, Adolf FP, Robertson J. Examination of damage to textiles. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 66, 82.

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**.**

1. 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.
2. 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 use to increase and decrease overall magnification. Select an objective magnification. Your selection will depend upon the detail necessary for the examination.
3. 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.
4. 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.
5. Separating out a single fiber, note the color and compare this to your color interpretation for the complete piece of fabric.
6. 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.
7. 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 physical matches. 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.

Chapter 6 Practical Exercise 6-2

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.

References:
Goldberg O. Köhler illumination. The Microscope 1980;28:15-2.

McCrone W C. Checklist for true Köhler illumination. American Laboratory 1980;12(1):96-98.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Reasearch Institute, 2002; 30-34 (illumination), 99-107 (micrometry).

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

1. 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 microcope 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?

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

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

4. Remove the fiber from the optical path.

5. 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.

6. 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.

7. 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.

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

9. 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.

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

11. 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.

1. 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.

2. 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).

3. 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:

$$\text{Observe \# osd} = \text{\# ssd} \times 100\ \mu m$$
or
$$1\ \text{osd} = \#\text{ssd} \times 100\ \mu m / \text{\# osd}$$

   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.

4. 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.

5. 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)
4 X				
10 X				
20 X				
40 X				
100 X				

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. These concepts will be explored further in later Practical Exercises.

Chapter 6 Practical Exercise 6-3

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. (This concept will be discussed further in Chapter 11.) 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" (the numerical difference between the refractive indices which is covered in greater detail in Chapter 11).

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.

References:

Delly JG. Photography Through the Microscope, 9th edition. Rochester, NY: Eastman Kodak Company, 1988.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Reasearch Institute, 2002; 69-94, 167-168 (photomicrography), 126-127 (Becke test), 143 (extinction), 145 (interference colors).

Preparation:

It is necessary for polarizied 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

Safety:
Use standard laboratory safety procedures according to the rules prescribed by your laboratory.

Directions:

1. 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

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

3. 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

1. Determine the orientation of the polarizer on your microscope.
2. Using the mounted nylon fiber, place the fiber parallel to the orientation of the polarizer (analyzer should be out).
3. 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.
4. 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 "=".
5. Rotate the fiber perpendicular to the orientation of the polarizer and record the refractive index (as <, >, or =) again in the n-perpendicular column.
6. Repeat this procedure with the remaining fibers and record your observations.

Part 2 - Observation of Extinction Positions and Interference Colors in Crossed Polars

1. Using the mounted nylon fiber, align the fiber with the orientation of the polarizer.
2. Place the analyzer in position (the field of view, including the fiber, should appear black).
3. 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.
4. 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.
5. 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.

Upon completion of this practical exercise, you will have had initial exposure to the following:

- polarized light
- components of a polarized light microscope
- anisotropic and isotropic substances
- the effect of polarized light on anisotropic and isotropic substances
- refractive indices and the Becke line method of observing refractive indices
- extinction positions
- interference colors
- the practical use of and problems with your image capture system in polarized light

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?

Chapter 7 Practical Exercise 7-1

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.

References:
Choudhry MY. A novel technique for the collection and recovery of foreign fibers in forensic science case work. J Forensic Sci 1988; 33(1):249-253.

Grieve MC, Garger EF. An improved method for rapid and accurate scanning of fibers on tape. J Forensic Sci 1981; 26:560-563.

Preparation:
Thoroughly clean the work area inside a fume hood or an exhaust cabinet large enough to accommodate a stereomicroscope. Set-up the stereomicroscope and allow spacing to scan the fiber tapings. Pour a fresh supply of mounting medium in a working container.

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 5-2

Safety:
Know the hazards associated with the mounting media and chemicals used, and handle them according to the rules prescribed in your laboratory. The entire procedure must be conducted under

vented conditions to prevent exposure to xylene fumes. Always clean your forceps with disinfectant and change your scalpel blade prior to beginning any procedure. After completion, any unwanted microscope slides and cover slips should be put in the used glass container. Scalpel blades should be discarded in a sharps disposal container.

Directions:

1. 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.

2. 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.

3. 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.

4. 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.

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

6. 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.

7. 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.

8. 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.

9. Prepare microscope slides of each of the known fiber samples taken from the source items in Practical Exercise 5-2. 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.

10. 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.

If the known yarns were a blend of fiber types, then what sampling techniques and strategies could be used to ensure all component fiber types are revealed during an examination?

Chapter 7 Practical Exercise 7-2

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.

References:
Cook R, Norton D. An evaluation of mounting media for use in forensic textile fibre examinations. J For Sci Soc 1982; 22(1):57-63.

Grieve MC, Deck S. A new mounting medium for the forensic microscopy of textile fibers. Sci Justice 1995; 35(2):109-112.

Loveland RP, Centifano YM. Mounting media for microscopy. The Microscope 1986; 34:181-242.

Roe GM, Cook R, North C. An evaluation of mountants for use in forensic hair examinations. J For Sci Soc 1991; 31:59-65.

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)

(list continues)

- 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

Safety:
Know the hazards associated with the mounting media and chemicals used, and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

1. 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.]

2. 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?

3. 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. These concepts will be explored more in Practical Exercise 11-3.

Chapter 7 Practical Exercise 7-3

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.

References:
Longhetti A, Roche G. Microscopic identification of man-made fibers from the criminalistics point of view. J Forensic Sci 1958; 3(2):303-329.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;160-161, 163.

Petraco N, DeForest P. A guide to the analysis of forensic dust specimens. In: Saferstein R, editor. Forensic Science Handbook, Vol. III. Englewood Cliffs, NJ: Prentice-Hall Inc., 1993.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975; 51-127.

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

Safety:
Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

1. 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 $\pm$ .05.

2. 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.

3. 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.

Evaluate your observational skills with your trainer by comparing your observations of shape and structure to those known or previously documented for the fiber samples you were given.

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 12-1.

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 12-1. This will be useful from two perspectives. First, you can re-examine the longitudinal mounts and review your notes from this exercise. Hopefully you will demonstrate to yourself that your observational skills have improved over time and practice. Second, you can physically cross section the unmounted samples and compare your longitudinal view observations to the cross-sectional view as additional practice and experience.

Chapter 7 Practical Exercise 7-4

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.

References:
Aspland JR. What are dyes? What is dyeing?. AATCC Dyeing Primer. Research Triangle Park,NC: American Association of Textile Chemists and Colorists, 1981.

Connelly RL. Colorant formation for the textile Industry. In: Color Technology in the Textile Industry, 1997; 91-96.

Frei-Sulzer M. Coloured fibres in criminal investigation with special reference to natural fibers. In: Curry AS, editor. Methods of Forensic Science, Vol. IV. New York: Interscience Publishers Inc. 1965;152-164.

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

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

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

2. 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

3. 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.

Chapter 7 Practical Exercise 7-5

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.

References:
Carroll G R. Forensic fibre microscopy. In: Roberston J, editor. Forensic Examination of Fibres, 1st edition. London: Ellis Horwood Ltd.,1992; 99-105.

Gaudette B. The forensic aspects of textile fiber examination. In: Saferstein R, editor. Forensic Science Handbook, Vol. II. Englewood Cliffs, NJ: Prentice-Hall Inc., 1988; 211-213, 238-241.

Joseph ML. Introductory Textile Science, 3rd edition. New York: Holt, Rinehart and Winston, 1977; 41-50, 88-111,184-187.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975; 136.

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

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

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

2. 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.

3. 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.

4. 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.

Chapter 8 Practical Exercise 8-1

Subject: **Microscopy of Non-woody Vegetable Fibers**

Time: 8 hours

Objective: To learn microscopic features of bast, leaf and seed fibers used in differentiation or identification of the source

Theory:
Differentiation and identification of non-woody vegetable fibers is largely based on the recognition of distinguishing microscopic features of the fiber cells. Observations of the crystalline inclusions and the morphological appearance of the cells in longitudinal and cross-sectional mounts can provide sufficient discriminating and identifying features of these fiber types, whether the fiber cells occur individually or grouped in bundles.

References:
Catling D, Grayson J. Identification of Vegetable Fibres. London: Chapman and Hall, 1982.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth, NJ: Grosvenor Press 1975;14-20, 74-91, 136-137, 167-170, 223-225.

Preparation:
Obtain authenticated fiber samples of cotton, coir, flax, hemp, ramie, jute, sisal, henequen, and manila hemp. Note that you will need a sufficient amount of fiber samples to use in this exercise as longitudinal mounts and for cross-sectioning, and still have some unmounted fiber sample for use in Practical Exercise 8-2.

Materials:
- stereomicroscope (for initial observation and mounting slides)
- polarized light microscope with various objectives (e.g. 4X,10X, 20X, 40X and 100X) and calibrated ocular micrometer
- microscope slides and cover slips
- suitable mounting medium
- dissecting needles and fine tip forceps
- fiber cross-sectioning supplies
- authenticated fiber samples

Safety:
Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container. Use caution when using razor blades and place used blades in a sharps disposal container.

Directions:

1. Prepare longitudinal mounts of the known fiber samples by mounting several (~ two centimeters long) fiber strands of each on microscope slides using Permount or another appropriate mounting medium (i.e. XAM). Some of the "fibers" will actually be bundles of multiple cells and may require teasing of the sample to obtain a reasonable microscopic mount for proper observations.

2. Examine each fiber sample in longitundinal mount. Note:
   - the presence or absence of transverse dislocations
   - the longitudinal variation in the cell wall thickness and lumen dimension
   - the fiber cell wall and lumen diameters
   - the relative size of the lumen compared to the fiber diameter
   - whether the cells readily separate out of the bundles, or not
   - shape of the cell tips
   - the presence of any other striations, markings, pits or spiral cell wall thickenings
   - the presence, shape and position of crystalline inclusions [Note that some crystalline inclusions are more readily visible with crossed polars.]

3. Record your observations for each fiber type.

4. Compare and contrast your observations for each fiber type. Can you or can you not distinguish among all of the fiber types by longitudinal observations? Re-examine each fiber sample in longitudinal mount from the perspective of comparing and contrasting among the samples. Save your longitudinal mounts for use in Practical Exercise 8-2.

5. Prepare acceptable cross sections of each fiber type and mount them for microscopical examination.

6. Examine each of the fiber types in cross section with at least 200X magnification. Note:
   - how each of the features viewed in longitudinal mount appear in cross section
   - the thickness of the cell and the cell wall relative to the lumen size and shape
   - cross-sectional shape of the single fiber cells (=ultimates)
   - presence of any radial cracks in the cell wall
   - how the ultimates are arranged within the bundles
   - variation of ultimates within the fiber bundles of the same plant species

7. Record and diagram your observations and differentiate the samples based on the features revealed in the cross sections.

Observations:

Prepare a table of observed morphological features for each fiber type including appropriate diagrams for both the longitudinal and cross-sectional views. Re-examine the fiber samples comparing and contrasting morphological features. Specifically identify any morphological features that:

- differentiate the bast, leaf or seed fiber types as to their plant part origin
- would allow discrimination or segregation among fibers with similarly appearing features
- are sufficiently distinctive for identification of a particular fiber type

Discussion:
Vegetable fibers encountered in forensic casework can originate from a variety of end use products including cordage, burlap bags, carpet backing, and clothing articles. Some of the most commonly encountered vegetable fibers originate from textile articles composed of cotton, ramie, flax and hemps.

Microscopical observation of plant cell cross sections and longitudinal mounts should be used in conjunction with microchemical methods to identify vegetable fibers. Bast fibers (ramie, flax, jute, hemp, kenaf, roselle, sunn hemp, urena) originate from the plant stem vascular tissue (phloem and xylem) and have microscopically distinctive structures characteristic of the tissue and of the plant when seen in longitudinal view. However, discrimination among these fibers is perhaps better accomplished with the best-view observation of the plant cell wall and lumen in prepared cross sections. Particularly important are the shape of the fiber bundles, shape of the cells, size and shape of the cell lumen, size of lumen relative to the overall cell size, and thickness of the cell wall.

As technical fibers, flax, ramie and jute are examples of fiber types that may appear similar in longitudinal mount, but can be differentiated by observation of the cross section. Ramie fiber ultimates are thick walled with slit-like lumina, and have flattened oval cross-sectional shapes. Flax fiber ultimates are very thick walled with very small rounded lumina, and have polygonal cross-sectional shapes. Jute fiber ultimates appear similar to flax fibers except that jute fibers have larger rounded lumina.

Distinctive crystals are often visible in longitudinal mounts making destructive ashing of samples unnecessary. Sisal, henequen and manila hemp may appear very similar morphologically, but some discrimination is possible based upon crystalline inclusions. Sisal and henequen have similar fusiform shaped crystals usually occurring singly, while manila has silica-based crystals of rectangular shape with a peripheral central depression usually occurring in multiples along a row.

The trainee is encouraged to examine more of the vegetable fiber types than were specifically covered in this exercise, and to examine dyed or pigmented fiber samples. Familiarity and experience of the examiner with the observation of the microscopical features of vegetable fibers are the primary analytical tools used for fiber discrimination and identification. There can be significant variation within some of the fiber types due to the inherent variability of a natural source fiber, and differences in the quality controls in fiber preparation or manufacturing processes. In addition, possible artifacts can be caused by manufacturing processes (e.g. commercial bleaching, mercerization) or poor sample preparation (e.g. incomplete mountant infiltration, fiber bundle compression distortions during cross-sectioning).

Chapter 8 Practical Exercise 8-2

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.

References:
Carroll GR. Forensic fibre microscopy. In: Robertson J, editor. Forensic Examination of Fibres, 1st edition. London: Ellis Horwood Ltd., 1992; 101-102.

DeForest PR. Foundations of forensic microscopy. In: Saferstein R, editor. Forensic Science Handbook, Vol. I. Englewood Cliffs, NJ: Prentice-Hall Inc., 1982; 487-488.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth, NJ: Grosvenor Press 1975;168-169, 225.

Valaskovic GA. Polarized light in multiple birefringent domains: a study of the Herzog effect. The Microscope 1991; 39:269-286.

Preparation:
You should use remaining unmounted fiber samples and the longitudinal mounts of fibers prepared in Practical Exercise 8-1. If the mounted samples are no longer available or could be improved, then prepare new 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

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container. Use caution when holding material over the hot plate.

Directions:

Part 1 - The Herzog Effect

1. Place the mounted fiber sample on the microscope stage between crossed polars.
2. Orient the longitudinal axis of the fiber ultimate parallel to the vibration direction of the analyzer.
3. Insert the compensator plate into the microscope between the sample and the analyzer.
4. Observe and record the resultant color of the fiber ultimate. It should be either orange or blue.
5. Repeat steps 1-4 for each of the mounted fiber samples.

Part 2 - The Drying Twist Test

1. 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.
2. 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.
3. As the fiber dries note whether it rotates clockwise, counterclockwise or has some other response.
4. 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 tests consistent with these reported data? Note that cotton exhibits both S and Z twist characteristics.

Chapter 9 Practical Exercise 9-1

Subject: **Examining the Cuticle of Animal Hairs**

Time: 8 hours

Objective: To learn scale casting and counting techniques used in the examination of animal hair cuticles

Theory:
Natural fibers of animal origin that are typically used in textile products include the animal hairs referred to as the under hairs (as opposed to guard hairs), the specialty furs, silk and leather. Detailed microscopical examination of the morphological features is the basic analytical tool used for identification of these natural fiber types.

For the examination of fibers that are of animal hair origin, the cuticular scales are examined for scale margin appearance, scale margin distance and scale pattern. Scale margin appearance and scale pattern are the physical appearance or characterization of the scale shapes. Scale margin distance is the physical separation of the scales, determined by counting the number of scales present on a hair for a given length.

Examining cuticular scales can be accomplished by mounting the hairs in a medium with a refractive index that allows visualization of the hair exterior (but hinders the examination of the hair interior structures), or by scale casting. Two commonly employed casting techniques use either clear fingernail polish or Polaroid film coater as the replicating medium.

References:
Bruner H, Coman B. The Identification of Mammalian Hair. Melbourne:Inkata Press, 1974; 1-18.

Ogle RR, Mitosink BA, Mitosinka GT. A rapid technique for preparing hair cuticular scale casts. J Forensic Sci 1973;18:82.

Petraco N. The replication of hair cuticle scale patterns in meltmounts. The Microscope 1986; 34: 341-345.

Petraco N. A microscopical method to aid in the identification of animal hair. The Microscope 1987; 35:83-92.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 128-133.

Preparation:
Calibrate the ocular micrometer for the microscope and objectives that you will be using if this has not already been done.

Materials:

- stereomicroscope
- transmitted light microscope with 10X, 20X and 40X objectives and rotatable stage
- a focusing ocular with calibrated micrometer scale
- microscope slides and cover slips
- fine forceps
- glycerin
- clear fingernail polish or Polaroid film coaters
- authenticated fiber samples of
    - < various grades of sheep wool including fine, medium, coarse and kemp
    - < camel, llama and/or alpaca
    - < cashmere goat
    - < angora goat (=mohair)
    - < rabbit
- reference books depicting scale patterns of animal hair

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

Part 1 - Scale Margin Distance by Scale Counts

1. Mount the hair in glycerin (n = 1.46).
2. Align the fiber parallel to the ocular micrometer and count the number of scales on the hair spanning the distance of the ocular scale. Calculate the number of scales per centimeter.
3. Rotate the stage so that the hair is perpendicular to the ocular micrometer and measure the hair fiber diameter.
4. Repeat this measurement/count at 3 different locations along the hair fiber.
5. Observe the distal margins of the scales and note if they are protruding (prominent) or show any type of damage.
6. Demount the hair, clean it and blot it dry. Properly secure the hair in identified packaging for use in Part 2 or immediately move on to the Part 2 examination for this hair.
7. Follow the above steps for all the hair samples you have to examine.

Part 2 - Scale Margin Appearance and Pattern by Scale Casting

1. Coat a microscope slide with either scale cast medium (fingernail polish or Polaroid coater).
2. While still wet, firmly place hair into medium leaving one end free in order to facilitate removal.
3. Mark the slide to indicate location of root and/or tip.
4. Allow casting medium to dry and remove the hair by pulling upward from free end.
5. Properly secure the hair in identified packaging. Save the hair for use in Practical Exercise 9-2.
6. Observe hair scale cast under microscope and sketch your observations. Characterize the scale distal margin appearance and the scale pattern using available literature references to apply the appropriate terminology. Be sure to examine the full length of the scale cast as the scale type can change along the length of the hair. Note whether the scale distal margins are prominent.

Observations:
Record your scale counts from each fiber type. Did the count vary significantly along the length of one hair, between hairs from different grades of wool, among hairs from different animals? Note the wide variation in diameters among different grades of wool and the large medullary diameter in kemp fibers. Is there a relationship between scale count and diameter?

Sketch your observation of scale margin pattern and scale pattern observed in the casts you made. Identify the scale pattern types. Are protruding scale margins more readily visible in the whole mount or the cast?

Discussion:
There can be considerable variation in the diameter of wool and "wool-like" fibers because this characteristic can be affected by the climate and diet or genetic differences among the source animals. The length and diameter data reported in the literature vary. Some reported diameter differences from wools of various breeds of sheep are listed below. The size and number of scales varies in comparing fine to coarser wools. For example, fine wools may have 790 scales per cm, coarser wools may have as few as 275 scales per cm. It is not possible to accurately identify the breed from processed wool. However, the diameter can be a characteristic of wool fibers which may help the examiner in identifying possible end uses. For example, Merino wool is used for fine wool and worsted yarns and medium wools are used in the manufacture of woolens, knitting yarns, hosiery, blankets, etc.

Type	Breed	Ave. Length (cm)	Diameter (μm)
Fine	Merino	3.7 - 10	10 - 20
Medium	Southdown, Cheviot	5.0 - 10	20 - 40
Med. Crossbred	Corriedale, Polwarth	7.5 - 15	20 - 40
Coarse - Long	Lincoln	12.5 - 35	25 - 50
Kemp			70 - 200

Wool is commonly defined as the fibrous covering from sheep, and is noted for prominent distal scale margins. Animal fibers from goats (cashmere, mohair), camels, alpaca, etc., can also be referred to as wool or wool-like fibers. Note the diameter differences you observed among all these samples. How do your values compare to literature reference values?

Scale patterns, along with other hair morphological features, among the non-human animal hairs are used for species identification. In this exercise you should have observed scale pattern types known as irregular mosaic on sheep wool, irregular waved mosaic on angora goat hair, regular waved mosaic on cashmere goat hair, and pectinate on rabbit hair.

Chapter 9 Practical Exercise 9-2

Subject: **Introduction to Examining Natural Fibers of Animal Origin**

Time: 12 hours (Part 1 ~8 hrs, Part 2 ~4 hrs)

Objective: To learn the basic morphological and optical features used to discriminate and identify the natural fibers of animal origin

Theory:
Natural fibers of animal origin that are typically used in textile products include the animal hairs referred to as the under hairs (as opposed to guard hairs), the specialty furs, silk and leather. Detailed microscopical examination of the morphological features is the basic analytical tool used for identification of the fiber types which originate from animal hairs. Determination of the optical and physical properties is the basic analytical tool used for discrimination and identification of silk and leather.

All mammalian hair has three basic morphological components: the cuticle, the cortex and the medulla. Hair is primarily composed of the protein keratin and is considered a staple fiber. Once a fiber is recognized as a hair, the next task is to determine if the hair is of human or non-human origin. The distinction between human and non-human hairs as well as species of origin among the non-human hairs is made based on the detailed examination and characterization of all of the morphological components.

Silk and leather originate as protein secretions from animals and, therefore, are not hairs. Silk is composed of the protein fibroin and leather is composed primarily of the protein collagen. These two fiber types of animal origin are typically identified by their optical and physical properties observed with polarized light microscopy and cross-sectional shape.

References:
Bruner H, Coman B. The Identification of Mammalian Hair. Melbourne:Inkata Press, 1974; 1-18.

Hicks J. Microscopy of Hairs: A Practical Guide and Manual. Washington DC: FBI, 1977; 1-5, 28-40.

Palenik S. Light microscopy of medullary micro-structure in hair identification. The Microscope 1983; 31:129-136.

Robertson J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 128-133.

Preparation:
Calibrate the ocular micrometer for the microscope and objectives that you will be using if this has not already been done. This exercise assumes that the trainee can identify the basic structural features of hair and has an understanding of the characteristics used to distinguish human from non-human hair. It should be noted that for some hairs, infiltration of the mounting media may be slow. If you are working with hairs in which this appears to be the case, then it may be necessary to allow the preparations to sit for an extended period of time, continuing with the microscopical examination on another day.

Materials:

- stereomicroscope
- polarized light microscope with 10X, 20X and 40X objectives and rotatable stage
- a focusing ocular with calibrated micrometer scale
- microscope slides and cover slips
- adhesive tape
- fine forceps
- hot plate
- glycerin
- glycerin-alcohol mix (65:35)
- scale casting supplies
- fiber cross-sectioning supplies
- mounting media with n~1.52-1.54
- authenticated fiber samples of:
    - < various grades of sheep wool including fine, medium, coarse and kemp
    - < camel, llama and/or alpaca
    - < cashmere goat
    - < angora goat (=mohair)
    - < rabbit
    - < any specialty fur hairs that your agency or environment is likely to encounter
    - < human head hair and pubic hair
    - < gummed and de-gummed silk (wild and cultivated types)
    - < suede leather fibers
- reference books depicting animal hair structures and morphology of cuticle, cortex and medulla

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. Use appropriate safety precautions when working with sharps and the hot plate. After completion, any unwanted microscope slides and cover slips should be put in the used glass container and used razor blades should be discarded in sharps disposal.

Directions:

Part 1 - Examining Animal Hairs

1. Examine each hair with a stereomicroscope. Identify and characterize the root, shaft, shield, tip, overall profile and curl, and color banding. Measure the hair length for complete hairs.
2. Examine each hair cuticle by the methods learned in Practical Exercise 9-1 for those hairs which you did not previously examine in that exercise. If your preparations in Practical Exercise 9-1 could be improved upon, then make new scale casts as these may be kept in your personal collection for later reference.
3. Mount each hair longitudinally in an appropriate medium with n~1.52-1.54. Observe the root, shaft, shield, tip and coloration along the length of the hair at higher magnification. Characterize the medullary features along the length of the hair, such as size, form or type, and pigmentation. Characterize the cortical features along the length of the hair, including size, texture and pigmentation.
4. For each hair type you have (each species of origin), take another hair and clear the medulla by splitting the hair and mounting in glycerin/alcohol according to the instructions found in Palenik, 1983.
5. Compare and contrast the morphological features visible in the cleared hair with observable features in the uncleared hair.

Part 2 - Examining Silk and Leather

1. Observe and characterize the appearance of each fiber type with a stereomicroscope.
2. Mount each fiber type longitudinally in an appropriate medium with n~1.52-1.54.
3. Examine each fiber type by transmitted light microscopy and characterize the fiber color, shape, surface and internal structure by optical sectioning. Measure the fiber diameter. (Recall the preliminary observational techniques you learned in Practical Exercises 7-3 and 7-4.)
4. Examine each fiber type by polarized light microscopy and characterize their optical properties (refractive index, birefringence and sign of elongation) utilizing skills learned in Practical Exercise 6-3, and found in Practical Exercises 11-1, 11-2 and 11-3.
5. Cross section each fiber type, mount the sections and observe the cross-sectional shape, size, internal structure and surface materials. (Refer to techniques found in Practical Exercise 12-1.)

Observations:

Record and diagram your observations for each animal hair. Using appropriate reference literature apply the correct terminology to each of the characterizations. Knowing the species of origin, do your observations match illustrations from the literature used to typify hair fibers from those animals?

Record your observations of the optical and physical properties of silk and leather. Although these are considered natural fibers, their examination is similar to what you have learned for the characterization of manufactured fiber optical and physical properties. How do your observational data compare to published data for these fiber types?

Discussion:

Familiarity with the longitudinal view of animal hair morphological features, and the optical/physical properties of silk and leather will often suffice for the rapid categorization and generic identification of these natural fiber types for the most commonly encountered textile products. Keratin, fibroin and collagen will produce characteristic infrared spectra but they are not species specific.

Although this training program emphasizes fibers associated with textile products, reality is that most fiber examiners will encounter animal hairs in casework as associative trace evidence which originate from common wild and domestic animals. The identification of species of origin requires substantial additional exposure to animal hair identification methods and taxonomy, and is usually considered a sub-specialty area of expertise. As time permits, the trainee can continue to examine animal hairs, and to explore the information revealed by physical cross sections.

Silk is usually recognized by its characteristic optical properties, rounded deltoid cross section, lack of visible internal structure, and presence of surface cross-over marks. Leather originates from the part of animal skin known as the dermis. Dermis is composed of bundles of collagen fibers which are very low birefringent fibers. The dimensions and orientations of these fibers varies with the animal species of origin, the somatic site of origin, and even environmental stress that the site underwent during and after the fiber deposition. Collagen fibers are not cells. Collagen is a repeating protein structure so there may be faint transverse striations visible on the fibers due to the molecular organization. In some animals there may be strands of skeletal muscle extending into the dermis which remain in the leather product. These muscle fibers may appear microscopically similar or indistinguishable to collagen to the untrained observer, but they are in fact different. These muscle fibers also have a transverse banding pattern originating from the molecular alignments for muscle contraction.

Any natural fibers of animal origin may be artificially dyed or pigmented, or may receive other processing for use in textile products. These treatments, as with any other natural or manufactured fiber type, are also subject to characterization, identifications and/or comparisons.

Chapter 10 Practical Exercise 10-1

Subject: **Identification of Asbestos Fibers**

Time: 4 hours

Objective: To learn to identify different types of asbestos fibers by using polarized light microscopy with dispersion staining and other optical techniques, and to learn to distinguish asbestos from other fibrous materials used in insulation, construction, textiles and papers

Theory:
Most natural and manufactured fibers can be conclusively identified and distinguished from one another based upon their morphological and optical properties as examined using a polarized light microscope. Among the optical properties that can be used to identify and distinguish fibers are refractive indices, birefringence, sign of elongation, extinction, pleochroism and dispersion staining colors. The various types of asbestos (which include a number of naturally occurring fibrous mineral silicates) are readily identified in bulk samples by polarized light microscopy, particularly when used in conjunction with dispersion staining.

References:
Deer WA, Howie RA, Zussman J. An Introduction to the Rock-Forming Minerals. New York: Addison-Wesley Publishing, 1992. [Note: read sections on asbestiform minerals; specific pages vary in different editions.]

McCrone WC. Asbestos Identification, 2nd edition. Chicago: McCrone Research Institute, 1987.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Research Institute, 2002; 169-196.

Preparation:
Before performing dispersion staining examinations on the asbestos reference materials, it may be useful for the trainee to practice judging dispersion staining colors using known isotropic materials for which dispersion curves are available.

Materials:
- stereomicroscope
- polarized light microscope, dispersion staining objective, dispersion staining color chart
- forceps, glass slides, cover slips,
- high dispersion refractive index oils (including 1.550, 1.605 and 1.680)
- authenticated standards of asbestos, other natural and manufactured fibers and building/insulation materials. The following standards should be available:
  - < Asbestos Materials: Crocidolite, chrysotile, amosite, fibrous tremolite, fibrous actinolite, fibrous anthophyllite
  - < Natural and manufactured fibers: Cotton, leather, paper, polyethylene (pulped), Kevlar (pulped), glass wool, mineral wool, refractory ceramic fibers, Ryton (polyphenylene sulfide), other manufactured textile fibers as needed
  - < Other Fibrous Mineral Materials: Brucite, wollastonite, talc

Safety:
Asbestos samples and materials known to contain asbestos fibers should be handled and prepared in a hood with sufficient airflow to prevent the introduction of asbestos fibers into the laboratory environment. Trainees should be familiar with the proper procedures before attempting to mount these fibrous materials.

Directions:

1. Prepare microscopical mounts of known asbestos materials, as well as other standards of synthetic fibers and fibrous mineral materials. Use oils of one or more refractive indices, such as 1.53 or 1.55.

2. Examine the morphological characteristics of each type of fiber and note the features that tend to distinguish asbestos fibers from most other types:
   a. the presence of fiber bundles of widely varying diameters; and
   b. fiber ends which tend to fray down to individual fibrils smaller than 1 µm in diameter.

   Also note differences in optical properties such as refractive indices relative to the mountant, birefringence and sign of elongation.

3. Prepare mounts of each of the six asbestos materials in high dispersion refractive index oils with indices of 1.550, 1.605 and 1.680. Observe each sample using the dispersion staining objective (central stop) with fibers oriented both parallel and perpendicular to the polarizer.

   Using the dispersion staining color chart as a reference, record the colors observed in the parallel and perpendicular positions for each asbestos fiber type in each mountant. Note that for some of the sample preparations, the matching wavelength for the fibers and oil will be well outside the visible region, giving either black or very pale dispersion staining colors.

   Perform the same observations for any of the fibrous mineral materials or other references that were difficult to distinguish from asbestos by normal PLM examination.

4. Compare the dispersion staining colors that you recorded for the samples to those listed in Table XIV, p 127, in *Asbestos Identification*. If your recorded colors differ from those in the Table, then re-examine your samples and the dispersion staining color chart. It may take some practice to properly judge the colors.

5. When you are satisfied that you can correctly judge the parallel and perpendicular dispersion staining colors for each asbestos sample, work through the Analytical Scheme for Asbestos supplied on page 157 of *Asbestos Identification* with each sample, until you are able to distinguish each of the six types. Alternatively, the condensed flowchart on page 167 for abestos identification or another appropriate analytical scheme may be used.

6. Have your trainer prepare a series of unknown samples from the asbestos and other reference sets. Using the asbestos identification flowcharts and your experience from examining the reference samples, identify each unknown.

Observations:
The manufactured fibers that are most likely to be confused with asbestos based on morphology are pulped polyethylene and pulped Kevlar. Leather may also be confused with asbestos. The trainee should be familiar with the features that distinguish these fibers from asbestos. The differences in

optical properties such as refractive indices relative to the mountant, birefringence and sign of elongation should generally serve to fully distinguish asbestos fibers from all other fiber types.

Discussion:
Having completed this exercise, the trainee should be capable of determining whether a fibrous sample contains asbestos or other types of natural or manufactured fibers. The trainee should also be capable, if necessary, of determining what particular type(s) of asbestos fibers are present. The identification of specific asbestos types may be important in comparing evidence samples of insulation material, old textile products or the few textile products in which chrysotile may currently occur.

Chapter 11 Practical Exercise 11-1

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.

References:

Heyn AJN. Fiber Microscopy. New York:Interscience Publishers, 1954:289-351.

McCrone WC, McCrone LB, Delly JB. Polarized Light Microscopy. Chicago: McCrone Reasearch Institute, 2002; 142-149.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres; 2nd edition. London: Taylor & Francis, 1999; 163-170.

Stoeffler S. A flowchart for the identification of common synthetic fibers by polarized light microscopy. J Forensic Sci1996; 41:297-299.

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 11-2.

1. 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.

2. 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

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

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

1. 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.]

2. 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.

3. 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.

4. Note the interference color of the acetate fiber.

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

6. 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.

7. 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

1. 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.

2. 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.

3. 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.

4. 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?

5. 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.

6. Repeat steps 3-5 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 actetate 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.

Chapter 11 Practical Exercise 11-2

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:

$r = 1000\ t \times B$ 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.

References:
Carroll G. The use of optical compensators in forensic fiber examination. Proceedings of the 10th Meeting of the International Association of Forensic Sciences, Oxford, England, 1983.

Gorski A, McCrone W. Birefringence of fibers. The Microscope 1998; 46:3-16.

Johri MC, Jatar DP. Identification of some synthetic fibers by their birefringence. J Forensic Sci 1979; 24:692-697.

McCrone W. Refractive indices and birefringence of fibers. The Microscope 1991; 39:57-58.

Sieminski MA. A note on the measurement of Birefringence. The Microscope 1975; 23:35-36.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975; 138-141.

Preparation:
You should still have the microscope slides that you prepared from authenticated fiber samples in Practical Exercise 11-1. 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 11-1.

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

Safety:
Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory. After completion, any unwanted microscope slides and cover slips should be put in the used glass container.

Directions:

1. 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.

2. 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.

3. 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.

4. 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.

5. Determine and record the sign of elongation.

6. 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.

7. 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.

8. 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.

Chapter 11 Practical Exercise 11-3

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.

References:
Carroll GR, Demers J. Technical note: a new method for determining the refractive indices and birefringence of textile fibres. Can Soc For Sci J 1993; 26:15-117.

Grieve,MC. The use of melting point and refractive index determination to compare colourless polyester fibres. For Sci Int 1983: 22:31-48.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975; 217-219.

Heyn AJN. Fiber Microscopy. New York Interscience Publishers 1954; 298-302.

Heuse O, Adolf FP. Non-destructive identification of textile fibres by interference microscopy. J For Sci Soc 1982; 22:103-122.

Preparation:
None.

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

Safety:
Know the hazards associated with the Cargille refractive index liquids and handle them according to the rules prescribed in your laboratory. The Cargille liquids must be kept in a safety storage cabinet. Only one bottle of the liquids should be opened at a time and handled under a portable fume hood. Slides contaminated with the liquids should be put in a closed chemical waste container after use.

Directions:

1. 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.

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

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

4. 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.

5. 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.

6. 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.

7. 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?

8. 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).

9. Record the refractive indices for the two positions.

10. Repeat the exercise steps 2-9 using the partially oriented polyester yarn (POY).

11. 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.

Chapter 12 Practical Exercise 12-1

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.

References:
Barna CE, Stoeffler SF. A new method for cross-sectioning single fibers. J Forensic Sci 1987; 32:761-767.

Craven BJ. Cross-sectional measurement of cellulose acetate fibers using scanning electron microscopy and image analysis. The Microscope 1993; 41:115-117.

Fong W, Inami S. Simple, rapid, and unique hand techniques for cross-sectioning fibers and hairs. J Forensic Sci 1988; 33:305-309.

Mauersberger H, editor. Matthews' Textile Fibers, 6th edition. New York: John Wiley and Sons, Inc., 1975; 987-989.

Palenik S, Fitzsimmons C. Fiber cross-sections: Part I. The Microscope 1990; 38:187-195.

Palenik S, Fitzsimmons C. Fiber cross-sections: Part II A simple method for sectioning single fibers. The Microscope 1990; 38:313-320.

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 steps 1 through 5 below. After you have obtained satisfactory cross sections of single, multiple and tufts of fibers, practice using case-sized samples of fibers (~ 4 mm).

1. Mount fibers to examine them longitudinally.

2. 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.

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

Polyethylene Method

a. Heat hot plate to approximately 150 ^{O}C, just enough to melt polyethylene (PE).
b. Cut two small pieces of PE, slightly larger than your fiber sample.
c. Place one piece of PE at one end of a microscope slide.
d. 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.
e. Place a second microscope slide or a small piece of glass over the PE sandwich.
f. Place the slide with the PE sandwich on the hot plate. Let the PE sheets melt together. 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. Wait until the PE turns clear, and wait a few additional seconds.
g. Remove the slide with the PE sandwich from the plate. Allow the PE sandwich to cool. Remove the top microscope slide.
h. Trim away the excess PE around the fiber that will be sectioned.
i. 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.
j. Mount the cross sections in xylene or Permount and examine under the polarizing scope.

Jolliff Method

a. Slide your finger across the Jolliff plate. The smooth side is considered the front. Push the threader through the back of the slide so it comes out the front.
b. Cut an approximately 5 inches long piece of filler yarn. The color of the filler yarn should contrast the color of the fiber.
c. 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.
d. Pull the threader down through the hole leaving a tuft on the upper side of the Jolliff slide.
e. 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.
f. Pull the filler down, drawing the sample into the hole.
g. 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.
h. Lay the Jolliff plate flat on the desk, with the front side up. Trim off the top.
i. 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.

4. 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.

5. 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 though 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.

Chapter 12 Practical Exercise 12-2

Subject: **Determining the Modification Ratio of Multi-lobed Fibers**

Time: 4 hours

Objective: To learn how to measure a multi-lobed fiber's cross section and calculate its modification ratio

Theory:
The cross-sectioning and subsequent microscopical examination of manufactured fibers can reveal a great deal of information about the fibers in question. The cross section of a fiber can provide important information not always observed from a longitudinal microscopic examination. Cross sections can provide information about the size, shape, delustrant, dye penetration, bicomponent nature, structural deformities and end use of the fiber. The modification ratio is the geometrical parameter used in the characterization of non-circular fiber cross sections. The modification ratio can be used to suggest a possible manufacturer of a particular multi-lobed fiber.

The modification ratio is the ratio of the outside diameter of the fiber to the diameter of the core. It may also be called "aspect ratio". The different cross-sectional shapes of multi-lobed fibers are created by the design of the spinneret. These spinnerets are shaped in a particular way to give the fiber its desired shape for a particular purpose, i.e., trilobal fibers hide soil better than round fibers. The spinnerets are under patent protections; thus locating a particular manufacturer is possible.

References:
Grieve MC, Kotowski TM. An improved method of preparing fiber cross sections. J Forensic Sci 1986; 26:29-34.

Palenik S, Fitzsimmons C. Fiber cross-sections: Part I. The Microscope 1990; 38:187-195.

Preparation:
Obtain a minimum of 10 different known carpet fibers from a variety of carpeting and fiber types, and different manufacturers. Prepare cross sections of these fibers and properly mount them for microscopical examination.

Materials:
- stereomicroscope and cross-sectioning materials
- compound microscope with transmitted light and accessories capable of taking photographs or electronic images of your fiber cross sections
- scanning electron microscopy (SEM) if it is capable of capturing cross-sectional images
- photocopier or tracing paper
- a professional decimal circle template or a metric circle template
- fibers from a variety of carpets

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

Illustration:

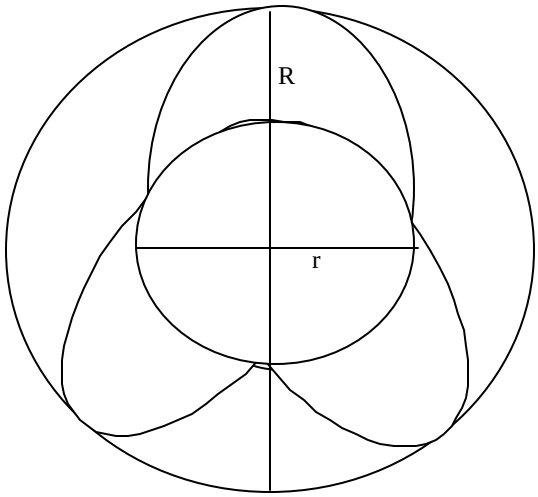


Figure 1. Modification Ratio, M.R. = R/r

Directions:
Once you have prepared the cross sections from an adequate number of known carpet fibers you may begin to examine them under the microscope:

1. Place the properly mounted cross sections on the microscope stage or appropriate SEM sample holder. Focus on the cross section and adjust the magnification until the image is large enough for you to make measurements from the photomicrographs (typically between 200X and 600X).

2. Take a photomicrograph or digital image of the cross section. (Remember to refocus the cross-sectional image for the purpose of image capture.)

3. Make a photocopy or paper tracing of the cross-sectional shape from the photomicrograph or digital image.

4. Using the circle template, find the circle size that fits best into the fiber core and draw the circle (see Figure 1). Again, using the circle template, find the circle size that best fits around the outside circumference of the fiber and draw that circle (see Figure 1).

5. With a ruler, draw and determine the length of **R** and **r**. Using the formula **M.R.= R/r**, calculate the modification ratio of that particular fiber.

6. Repeat this procedure for the rest of your known samples. When you have completed calculating all of the modification ratios for your samples, compile a table of your results.

Observations:
Calculate the modification ratio from the geometric determination of the diameter of the best-fit circles for the fiber inner core and outer circumference. Carefully document or diagram how you made these measurements.

In some instances, you may have difficulty in selecting the “best fit” circle. What happens to the calculated modification ratio if you use these alternate choices in finding the best fit? How do your modification ratio values compare to ones calculated from the same images by an experienced examiner?

Discussion:

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.

Chapter 13 Practical Exercise 13-1

Subject: **Solubility Testing of Acetate and Triacetate Fibers**

Time: 1 hour

Objective: To learn a microchemical technique for distinguishing acetate and triacetate fibers by solubility testing

Theory:
Certain polymers can be distinguished from one another by their solubility behavior when mounted in specific organic liquids. Acetate (diacetate) and triacetate fibers exhibit similar optical properties and infrared spectra, making it potentially difficult to distinguish between these two generic fiber classes using these techniques alone. The chemical differences between these two fibers are due to the percent of acetylated hydroxyl groups on the cellulose backbone of these two polymers. In normal cellulose acetate (diacetate) approximately 60% of the hydroxyl groups are acetylated while in the triacetate more than 90% of these sites are acetylated. The percentage of hydroxyl groups that have been acetylated affects the solubility behavior of these polymers. Although solubility testing is destructive, when carried out according to the microchemical procedure described here, the loss of material is negligible in relation to the certainty of information gained.

References:
David SK, Pailthorpe MT. Classification of textile fibres: production, structure, and properties. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 26-30.

Dupont Technical Information Bulletin X-156. Identification of Fibers in Textile Materials. Textile Fibers Dept., Technical Service Section, E.I. DuPont de Nemours & Company, Inc., 1961.

Hartshorne AW, Wild FM, Bab NL. The discrimination of cellulose di- and tri-acetate fibers by solvent tests and melting point determination. J For Sci Soc 1991; 31(4):457-461.

The Textile Institute. Identification of Textile Materials, 7th edition. Portsmouth NJ: Grosvenor Press, 1975; 28-29, 181-187.

Longhetti A, Roche G. Microscopic identification of man-made fibers from the criminalistics point of view. J Forensic Sci 1958; 3(2):303-329.

Stratmann M. Identification of textile fibers. In: Mitchell J, editor Applied Polymer Analysis and Characterization. New York: McMillan Publishing Co., 1987; 387-411.

Tucker PA. Fibers, identification. In: Mark HF, editor. The Encyclopedia of Polymer Science and Engineering, 2nd edition, Vol. 8. New York: John Wiley and Sons, Inc., 1987.

Preparation:
Prepare 5 ml of a 75% aqueous acetone solution (v/v).

Materials:

- stereomicroscope, compound microscope with transmitted light
- glass slides, cover slips
- 75% aqueous acetone
- chloroform
- razor or scalpel blades, forceps
- double-drawn pipettes or capillary tubes
- authenticated and commercial samples of di- and triacetate fibers

Safety:
Know the hazards associated with the solvents listed above and handle them according to the rules prescribed in your laboratory. Use caution when using razor or scalpel blades and place used blades in a sharps disposal container.

Directions:
Perform steps 1-5 using authenticated di- and triacetate fibers. After you obtain accurate results with these known samples, practice using commercial acetate fibers provided by your trainer.

1. Place a microscope slide under a stereomicroscope (~40X to 80X magnification). Focus on the slide where you intend to position the fibers to be tested. Using the forceps, place a single fiber on the slide and cover with a cover slip.

2. Transfer the slide to the transmitted light microscope stage, being careful not to lose the fiber and cover slip. Focus on the fiber and check for Köhler illumination. With a capillary tube or pipette, place a drop of the acetone reagent next to the cover slip and allow it to flow beneath it by capillary action.

3. Observe the fiber as the reagent flows over the fiber and record your observations.

4. Repeat steps 1-3 on new preparations using chloroform as the reagent.

5. Practice and improve your technique by cutting small pieces from single fibers as follows:
   - Under the stereomicroscope, practice cutting off different size segments of a single fiber on a microscope slide with a sharp blade, and performing steps 1-4 on the segments.
   - Document any differences in the behavior due to the size of the segment tested.
   - To prevent a fiber from blowing away while cutting, it may help to wet it down with a temporary mountant. Once the fiber fragment is in place, allow the temporary mountant to evaporate, apply a cover slip fragment and perform the test.

Observations:
Diacetate fibers dissolve in 75% acetone while the triacetate fibers do not. Triacetate fibers may swell to as much as twice their diameter, but do not lose their crenulated appearance.

Triacetate fibers are immediately soluble in chloroform. Diacetate fibers will retain some crenulation for at least 15 seconds in chloroform, eventually swelling 5 to10 times their normal diameter.

Discussion:
Di- and triacetate fibers are not easily differentiated by infrared microspectroscopy. In infrared spectral data bases for fibers, diacetate fibers show slightly more absorbance at 3489 cm^{-1} than triacetate fibers. Measurement of the actual refractive indices or melting behavior can also serve to distinguish these fibers. Acetates typically show low birefringence and a crenulated cross section, whereas the birefringence of triacetates is usually very close to zero. Heavily colored fibers may cause difficulties determining the refractive indices. Solubility testing may be the easiest method for rapidly making this determination.

Diacetate is the only manufactured fiber soluble in 75% acetone. Triacetate is the only manufactured fiber soluble in chloroform. Triacetate fibers are no longer manufactured in the United States, but are still imported to the United States.

Prior to running solvent tests on questioned/known casework samples, solvents should be tested on authenticated fiber samples to insure reliability. Use only single fibers or small pieces of individual fibers. Fiber cross sections may also be used for this purpose. If several fibers are used, then the analyst should be aware that overloading can affect the solvent composition and give anomalous results. The analyst should disregard results obtained from fibers in contact with air bubbles at the edges of the cover slip.

Chapter 14 Practical Exercise 14-1

Subject: **Use of a Hot Stage**

Time: 6 hours

Objective: To learn how to use the hot stage for determining the melting point range of manufactured fibers, and identifying and differentiating fibers by means of their melting points

Theory:
The determination of melting point ranges may be used to distinguish among certain fibers in the same generic class, or may be used as part of an identification confirmation. Although a melting point test is destructive, fiber samples previously prepared for non-destructive methods (e.g. microscopical examination of cross sections or fluorescence) can be used.

Manufactured fibers are a mixture of a base polymer, additives and sometimes finishes and contaminants, rather than being composed of a single pure compound. These fibers are also a mixture of crystalline and amorphous regions. The higher the degree of crystallization present, the higher the melting point. Many fibers melt over a temperature range rather than at one specific temperature point because of these factors. Polymer ratios, molecular weights, rate of temperature rise and type of hot stage can also affect melting point.

References:
Grieve MC. The use of melting point and refractive index determination to compare colourless polyester fibres. For Sci Int 1983; 22:31-48.

Hartshorne AW, Wild FM, Bab NL. The discrimination of cellulose di- and tri-acetate fibers by solvent tests and melting point determination. J For Sci Soc 1991; 31(4):457-461.

Hartshorne AW, Laing DK. The identification of polyolefin fibres by infrared spectroscopy and melting point determination. For Sci Int 1984; 26:45-52.

McCrone WC, Delly JG, editors. Hot and cold stage methods. The Particle Atlas, 2nd editon, Vol. 1 Principles and Techniques. Ann Arbor, MI: Ann Arbor Science Publishers,1973; 114-116.

Preparation:
Assemble materials and review your instrument's operator manual. Prepare clean slides and micro-cover slips for mounting materials. Fiber samples to be tested should be cut under the stereomicroscope. A very small length (~ 2 mm) is all that is needed because the melting point is observed through a microscope.

Materials:
- stereomicroscope and light source
- compound microscope adapted with long working distance objective
- hot stage and apparatus
- razor or scalpel blades,
- glass cutter or diamond-tip scribe

(list continues)

- glass slides, glass cover slips, forceps, tissues
- authenticated fiber samples
- kit of calibration materials (see comments in Directions, Part 1)
- acetone for slide cleaning
- high-temperature stable silicone oil
- reference literature containing melting point data

Safety:
Be aware that the hot stage will reach high temperatures and the glass slide will be extremely hot. Use caution when using scalpel or razor blades and cutting slides or cover slips. Place used blades in a sharps disposal container, and used glass slides and cover slips in a glass waste container. Follow standard laboratory safety procedures for working with chemical compounds.

Directions:

Part 1- Determining the Melting Range of a Manufactured Fiber

1. Dry mount a fiber of known melting point on a glass slide.
2. Insert slide in the hot stage and center fiber.
3. The temperature of the hot stage unit can be raised quickly to approximately 15 ^{O}C lower than the anticipated melting point, and then the rate of temperature rise lowered to about 2 ^{O}C/minute. If the test is a general melting point determination with no generic class identified and no melting point anticipated, then a preliminary rapid increase of temperature can be used to find an approximate melting point to use as an anticipated melting point.
4. The melting range is determined by observation of the fiber through the microscope from the temperature at which melting begins to the temperature at which no more changes occur. Also note when approximately 50% of the fiber has melted, and note other thermal reactions such as contraction of the fiber, charring, bubbling, and softening.
5. Repeat the test. Report the melting point as the average of the start and end points to the nearest degree Celsius. How reproducible were your values?
6. If available, repeat this test observing the fiber through slightly uncrossed polars and note the changes in interference colors as the fiber melts. Does this type of viewing affect your ability to observe melting behavior?
7. Repeat this test using fiber sections mounted in a minimal drop of high-temperature stable silicon oil instead of dry mounts. Does this mountant make any difference in your ability to observe melting or in your test results?

Part 2 - Identifying and Discriminating Nylon 6 and 6,6 by Melting Points

1. Mount a nylon 6 fiber and a nylon 6,6 fiber on one slide using silicone oil, cutting one “long” and one “short” so that you can visually discriminate them.
2. Insert slide in the hot stage and center fibers.
3. Raise temperature quickly to approximately 195-200 ^{O}C.
4. Lower the temperature rate to increase at about 2 ^{O}C/minute.
5. Observe and record melting ranges and behaviors of the two fibers. Repeat the test. Report the melting point as the average of the start and end points to the nearest degree Celsius.
6. Compare results to published melting point data.

Part 3 - Comparing Fibers by Melting Points

1. Mount several short lengths of one fiber type on one slide.
2. Heat, observe and record melting range and behavior as outlined above. Do all of the fiber fragments on one slide react the same way at the same time?
3. Repeat the test. Report the melting point as the average of the start and end points to the nearest degree Celsius.
4. Repeat test with at least ten different types of manufactured fibers such as acetate, modacrylic, other nylons, olefins (polyethylene and polypropylene), polyesters (PET and PCDT), saran, spandex, and triacetate.
5. Compare results to published melting point data.

Observations:
Observing through slightly uncrossed polars may aid in pinpointing the start and end of the melting range because the interference colors will decrease as the fiber melts. The fiber becomes isotropic under crossed polars at the melting end point. High-temperature stable silicone oil added to the sample provides a more even heat distribution from the hot stage to the sample than a dry mount does.

It is always recommended to repeat the test and average the results. One operator's duplicate observations optimally should be within 2 ^{O}C, however, there is an inherent variability associated with this method.

Discussion:
A melting point determination should generally be used when an identification cannot be achieved by non-destructive methods. Although the melting point test has largely been replaced by more modern instrumental methods, it can be a valuable tool in certain cases.

The melting points of the calibration materials will be much more discrete than for most of the manufactured fibers. Calibration should be performed at least annually or more often if critical refractive index work is being done. It can be seen from the published fiber melting point data tables that extreme accuracy with this method is not always necessary. For example, acetate and triacetate, nylon 6 and nylon 6,6, polyethylene and polypropylene can be easily distinguished. Nylon 6 and nylon 6,6 have respective melting points of approximately 213 ^{O}C and 250 ^{O}C, a difference of 37 ^{O}C, which easily distinguishes the two fibers by this method.

Fibers from a common source should exhibit the same or very close melting points and behaviors. They should be tested under exactly the same conditions, preferably side by side on the same slide, and slight differences may be noted. A significant difference observed between two fibers would indicate two different sources. Fibers of the same generic class that appear microscopically similar may exhibit differences in melting characteristics and these should be noted.

Fibers that do not melt under 300 ^{O}C include the following: acrylic, aramid, azlon, PTFE fluorocarbon, glass, acrylonitrile/vinyl chloride modacrylic, novoloid, rayon and natural fibers.

If using polarized light microscopy, then temperatures can be correlated to changes in fiber retardation and appearance. The isotropic point will be the point in which no retardation colors exist.

Chapter 15 Practical Exercise 15-1

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.

References:

Bartick EG. Considerations for fiber sampling with infrared spectroscopy, ASTM STP 949. In: Roush P, editor. The Design, Sample Handling and Application of Infrared Microscopes. Philadelphia PA: ASTM 1987; 64-73.

Tungol M, Bartick EG, Montaser A. Forensic examination of synthetic textile fibers by microscopic infrared spectrometry. Humicki HJ, editor. Practical Guide to Infrared Microspectroscopy. New York: Marcel Dekker 1995; 245-285.

White GW. A simple high-pressure anvil and template device for the production of infrared spectra from microfiber samples. J Forensic Sci 1992; 37:620-631.

Preparation:
It is assumed the trainee has already been taught the standard operating techniques for producing infrared spectra from fibers using the microscope-FTIR instrument available in their laboratory. The detector must be properly cooled, and the instrument purged of carbon dioxide and water vapor before use. 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

Safety:
The microscope uses an MCT - detector which must be cooled with liquid nitrogen. Cryogenic gloves and eye protection should be worn when handling liquid nitrogen. Do not attempt to fill the detector with liquid nitrogen for the first time without supervision. Use caution when using scalpel blades and place used blades in a sharps disposal container. Used glass slides should be put in a glass disposal container.

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 15-2. 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.

Chapter 15 Practical Exercise 15-2

Subject: **The Transmission/Reflection Technique**

Time: 3 hours

Objective: To learn an alternative method for producing infrared spectra from fibers that is particularly suitable for acrylic fibers, and to observe that FTIR-microscopy can be used to identify some manufactured fiber generic sub-classes

Theory:
In the reflection-transmission technique (also known as reflection-absorption or double-pass transmission) the fiber is flattened onto a highly reflective metal substrate. The FTIR-microscope is operated in the reflection mode and the beam passes through the fiber, reflects off the metal surface and passes back through the sample a second time. The fiber should be flattened on a die (such as is used for pressing KBr) or a gold/silver mirrored disk or slide. Dedicated dies should be kept for this purpose because the fibers will "imprint" onto the die surface. With double pass transmission the effective path length is nearly doubled so the technique is not suitable for fibers which are difficult to flatten, or those with a large diameter and high absorption (such as thick nylon carpet fibers which illustrate all of these characteristics). Acrylic fibers, which are normally thin and have low absorption, lend themselves well to this technique.

Acrylic based fibers can be sub-divided according to their polymer composition which may be recognized using infrared spectroscopy. Classification is based on the major co-monomer, the presence or absence of various solvent residues, and the presence or absence of additives used to provide dye sites or to perform special functions (e.g. flame retardancy).

References:
Espinoza EO. Fiber analysis by reflective FTIR: a novel sample technique using copper and steel plates. Crime Scene 1998; 23(4) and 24(1):27.

Grieve MC. Another look at the classification of acrylic fibers using FTIR microscopy. Sci Justice 1995; 35:179-190.

Grieve MC, Griffin RME. Is it a modacrylic?. Sci Justice 1999; 39:151-162.

Grieve MC, Griffin RME, Malone R. Characteristic dye absorption peaks found in the FTIR spectra of coloured acrylic fibres. Sci Justice 1998; 38(1):27-37.

Smalldon KW. The identification of acrylic fibres by polymer composition as determined by infrared spectroscopy and physical characteristics. J Forensic Sci 1973; 18:69-81.

Tungol MW, Bartick EG, Montaser A. Forensic analysis of acrylic copolymer fibres by infrared microscopy. Appl Spect 1993; 47:1655-1658.

Preparation:
It is assumed the trainee has already been taught the standard operating techniques for producing infrared spectra from fibers using the microscope-FTIR instrument available in their laboratory. The detector must be properly cooled, and the instrument purged of carbon dioxide and water

vapor before use. 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, undyed acrylic/modacrylic fibers representing the most common examples of these generic classes:

- acrylonitrile/vinyl acetate co-polymer, e.g. Acrilan (Monsanto)
- acrylonitrile/methylmethacrylate co-polymer, e.g. Orlon (Du Pont), Cashmilon (Asahi), or Dralon (Bayer)
- acrylonitrile/vinyl chloride, e.g. Kanekalon wig fiber (Kaneka Co.)

Other examples may be used or substituted provided that the samples are authenticated.

Materials:

- microscope-FTIR instrument properly cooled and purged
- fine scissors, fine forceps and scalpel blades
- KBr die; gold or silver mirrored surface disks or slides
- micro-press
- authenticated fiber samples
- ethanol for die/disk cleaning

Safety:

The microscope uses an MCT- detector which must be cooled with liquid nitrogen. Cryogenic gloves and eye protection should be worn when handling liquid nitrogen. Use caution when using scalpel blades and place used blades in a sharps disposal container.

Directions :

1. Place a length of fiber (3-5 mm) on a mirror disk (previously cleaned with ethanol) and cover with another mirror disk.

2. Place the disks in a KBr press and apply pressure.

3. Take the disk with the flattened fiber on it and place in the die holder which fits on the microscope stage. Adjust the measurement aperture to fit the fiber.

4. Use the microscope in the reflection mode. Focus on the mirror surface adjacent to the fiber and acquire a background spectrum.

5. Move the fiber into the aperture field. The fiber should be in focus without readjustment being required. Acquire and store a spectrum from the fiber.

6. Repeat the above steps for the other two fiber samples. Always clean and re-use the same mirror disc.

Observations:

Compare the spectral quality obtained in this exercise with the spectral quality obtained in Practical Exercise 15-1, Part 2 for rough pressed acrylic fibers recorded as air-mounts or using the microcompression cell. Decide which technique you think has given you the best result.

Look at the spectra you have produced in this exercise and note the salient differences between them. Can you attribute the differences to the varying polymer composition of the respective fibers?

Discussion:

There is no one correct preparation method for producing spectra from single fibers. Analysts should choose the method which they find works best for them and their particular instrument. Spectral quality will vary according to fiber thickness and cross-sectional shape. With some samples it may be necessary to try different preparation methods until arriving at the one which produces the best quality spectra.

FTIR-microscopy can be used to identify fiber generic sub-classes. You should learn to recognize some of the common examples among acrylic based fibers. If you have time to perform further experiments, then your trainer may be able to provide you with additional varieties of acrylic or modacrylic fibers which will illustrate different spectral features.

You may also try obtaining spectra from a deeply dyed acrylic fiber (black, dark blue, dark green) before and after extracting the dye (see Practical Exercise 19-2). Can you attribute any spectral peaks to the dye which was present in the fiber?

Chapter 15 Practical Exercise 15-3

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.

References:

Bellamy L. Collection of tables of group frequencies. In: Infrared Spectra of Complex Molecules, Spectroscopy Library, Bowdoin College, Maine. London: Chapman and Hall, 1975.

Carton A, Carlsson DJ, Wiles DM . Infrared spectroscopy of polyethylene terephthalate fibres - uses and limitations. Textile Research Journal 1981; 52:28-34.

Grieve MC. New man made fibres under the microscope. Lyocell fibres and nylon 6 block co-polymers. Sci Justice 1996; 36(2):71 -80.

Grieve MC, Cabiness LR. The recognition and identification of modacrylic fibres. For Sci Int 1985; 29129-146.

Grieve MC, Dunlop J, Kotowsk TM. Bicomponent acrylic fibres - their characterisation in the forensic science laboratory. J For Sci Soc 1988; 28:25-34.

Grieve MC, Kotowski TM. The identification of polyester fibres. J Forensic Sci 1977; 22:390-401.

Humecki H. Which nylon are these fibres? The Microscope 1994; 42(3):104.

Tungol MW, Bartick EG, Montaser A. The development of a spectral data base for the identification of fibers by infrared microscopy. Appl Spect 1990; 44:1543-549.

Tungol MW, Bartick EG, Montaser A. Spectral data base of fibers by infrared microscopy. Spectrochim Acta 1991; 46B:535E.

Was JD, Knittel D, Schollmeyer E. The use of FTIR microscopy for the identification of thermally changed fibres. J Forensic Sci 1996; 41(6):1005 - 1011.

Young PH. The characterization of high performance fibers using infrared microscopy. Spectroscopy 1988; 3(9):24-30.

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)

Safety: There are no special safety requirements for this exercise.

Directions :

1. 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.

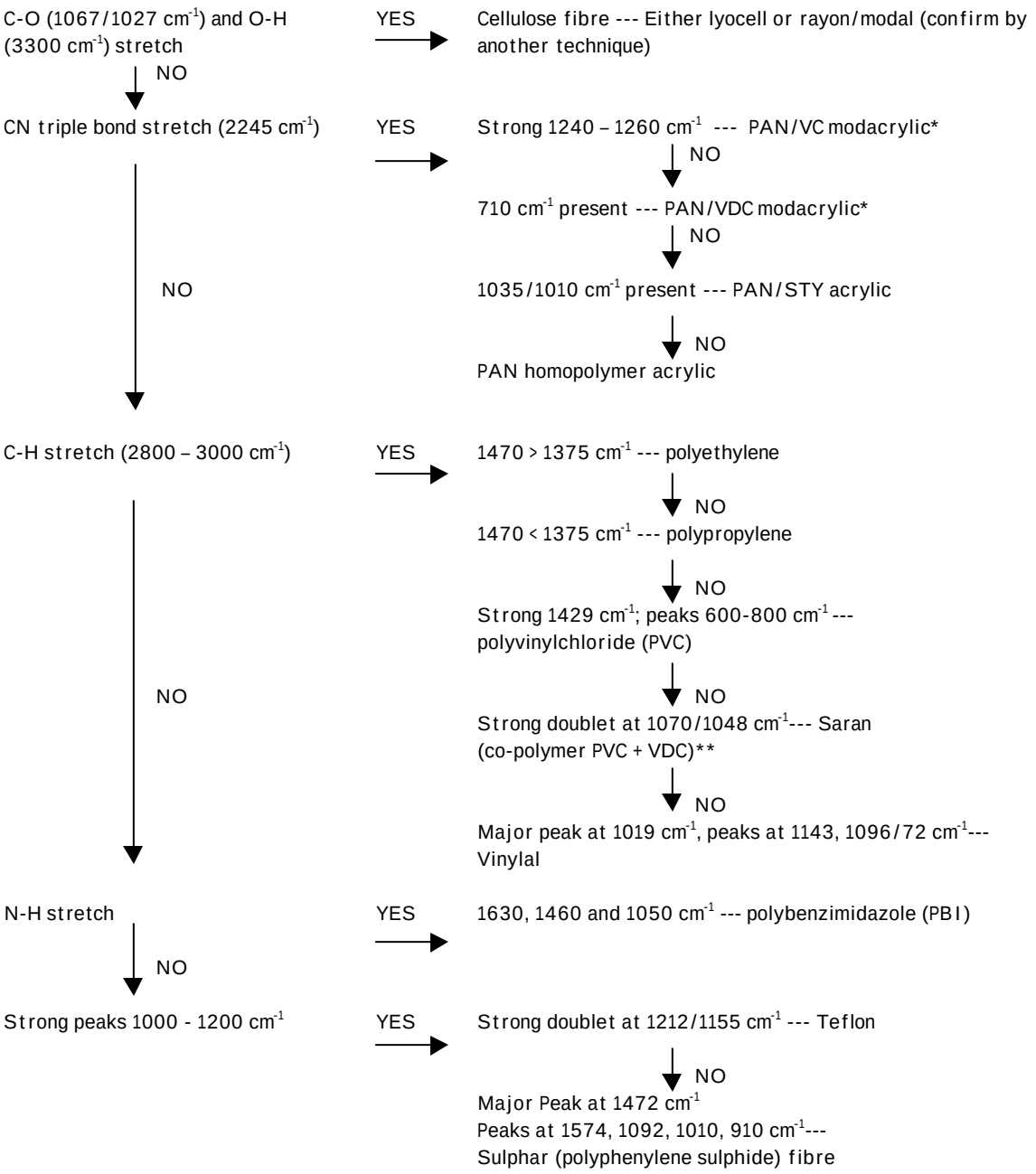
2. Determine which of the peaks and functional groups are crucial to the identification of each of the various fiber generic classes.

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



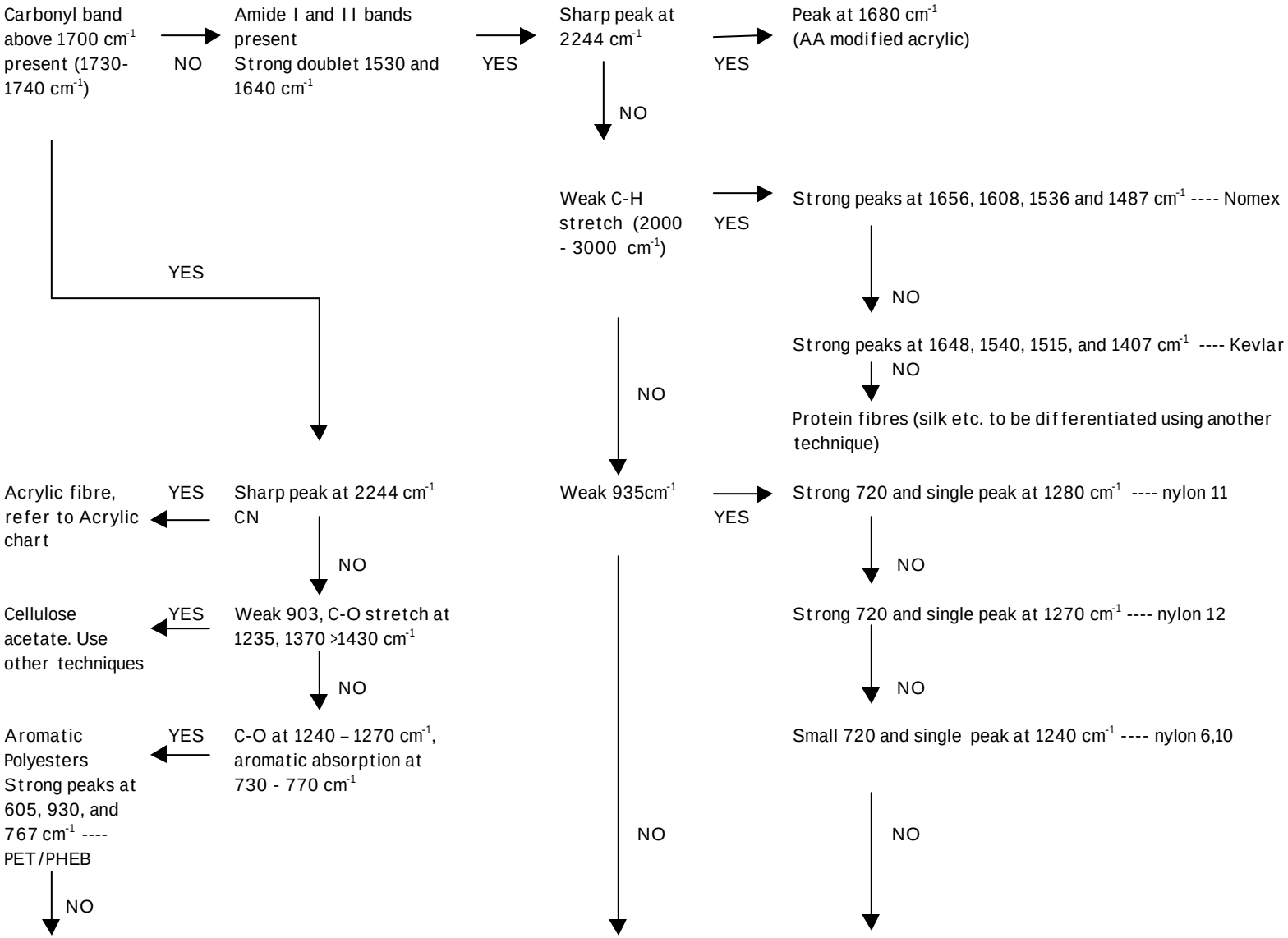
* For further details on modacrylic fibres see :
Grieve MC, Griffin RME. Is it a modacrylic ? Sci Justice. 1999; 39:151-162.

** 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

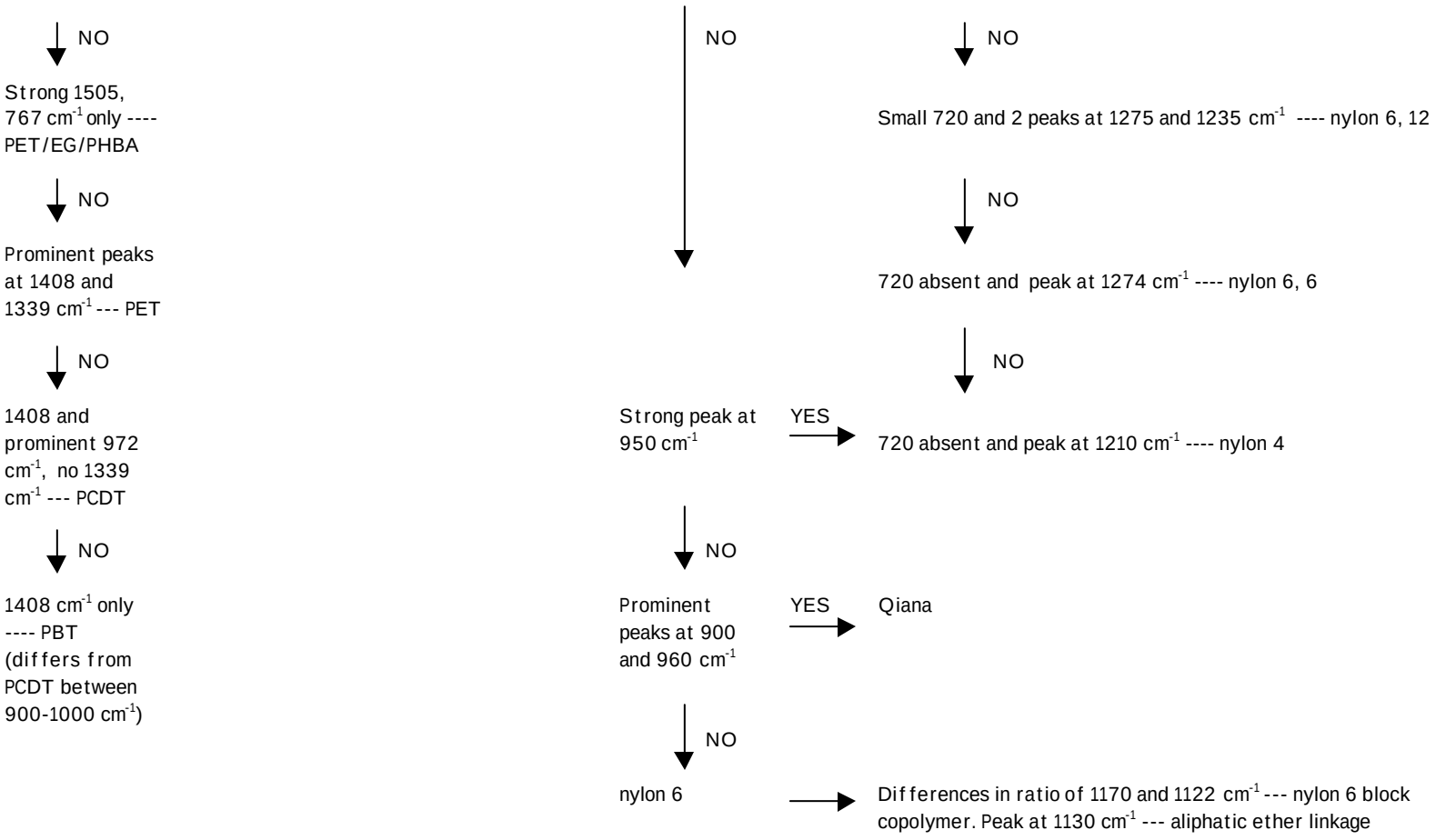
Chart B

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.

Chapter 16 Practical Exercise 16-1

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.

References:
Grieve MC. Fibres and their examination in forensic science. In: Maehly A, Williams RL, editors. Forensic Science Progress, Vol. 4. Berlin: Springer Verlag, 1990.

Robertson, J. Protocols for fibre examination and initial preparation. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 121-123.

Preparation:
Comparison microscopes may vary from laboratory to laboratory. 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:

1. Color matching: The trainer should prepare a labelled 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 exercise in Chapter 18.

2. 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.

3. 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.

4. Morphological feature matching: The trainer should prepare two (2) slides each containing six (6) individually mounted "recovered" fibers, and provide a "known" fabric swatch made of manufactured fibers preferably showing a range of slight variations in morphological features (e.g. in diameter). The mounted fibers should include some fibers that match fibers from the swatch and some which are close, but do not match fibers from the swatch.

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 in Chapter 18.)
- 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 feature 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

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory.

Directions:

Part 1 - Making Slides to Assist in Balancing the Illumination

1. 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.

2. 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.

3. 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.

4. 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.

5. 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.

1. 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.

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

3. 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.

4. 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.

5. 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 (see also Chapter 17 - Examining Fiber Samples Using Fluorescence Microscopy).

Chapter 17 Practical Exercise 17-1

Subject: **The Fluorescence Microscope: Set-up and Operation**

Time: 1 hour

Objective: To learn the techniques required to set up a fluorescence microscope for fiber examination

Theory:
Photoluminescence is described as the absorption and subsequent re-radiation of light from either an organic or inorganic material. Fluorescence is thus a form of photoluminescence in which light emission continues only during the absorption of excitation light originating from an ultraviolet source. The time interval between absorption of excitation light and emission of re-radiated light in fluorescence is of short duration (1×10^{-9} sec). When electrons go from the excitation state to the ground state, there is a loss of vibrational energy (Stokes Law/Stokes Shift).

Primary fluorescence, also called auto-fluorescence, originates from the object itself. Auto-fluorescence may mask materials having a more specific fluorescence in some applications. Various mounting media may exhibit auto-fluorescence (e.g. Permount). The use of these should be avoided. Glass microscope slides and cover slips auto-fluoresce to some degree, but usually work satisfactorily during routine fiber examinations. Quartz slides may be purchased to eliminate any unwanted auto-fluorescence. Secondary, or induced fluorescence, involves non-fluorescent biological materials impregnated with fluorochromes which fluoresce when excited by UV radiation.

In a fluorescence microscope, the specimen is illuminated with transmitted or reflected ultraviolet light from a high intensity light source such as a xenon or mercury arc lamp. Part of this light is absorbed by the specimen and re-emitted as fluorescence. The wavelength of the re-emitted light is longer than that of the incident light. A primary (excitation) filter is placed between the lamp and the specimen. This filter should provide light corresponding to the absorption maximum of the fiber being examined. To observe fluorescence, the light used for excitation is filtered out by a secondary (barrier) filter placed between the specimen and the eye.

The most convenient mode of examining fiber fluorescence is using reflected light. The excitation light is directed down through the objective by means of a semi-transparent mirror (dichromatic mirror). The excitation and barrier filters, together with mirror are mounted in an interchangeable unit in more modern microscopes.

References:
Bradbury S, Evennett PJ. Contrast Techniques in Light Microscopy. Microscopy Handbook 34. Oxford UK: Bios Scientific Publishers, Ltd, 1996; 91-100.

Have your fluorescence microscope owner's manual available during this exercise for any specific set-up requirements.

Preparation: Prepare a standard slide(s) with fibers exhibiting a range of fluorescence from low to high in a mounting medium such as Norland 65 optical adhesive or XAM. These mounting media offer low to almost non-existent auto-fluorescence.

Materials:

- microscope with reflected light fluorescence capability
- authenticated fiber samples exhibiting fluorescence
- standard slide with fibers exhibiting a range of fluorescence from low to high

Safety:

Mercury and xenon arc lamps may explode during operation due to their high internal gas pressure and extreme heat generated during use. Never turn on a lamp outside its housing or observe the lamp directly when it is illuminated. If there is a problem with the lamp, **DO NOT** service it yourself; contact your trainer. Never look at ultraviolet excitation light directly. Ultraviolet light is inherently dangerous and thus precautions **must** be taken in the proper set-up of the fluorescence microscope to prevent inadvertent exposure of the retina to UV light. If supplied, then use the UV protective shade while positioning and viewing microscope slides. The shade will block UV radiation emitted from the mercury/xenon burner.

Directions:

Using the known fiber slides perform the following steps to set-up for reflected light fluorescence microscopy:

1. Place a prepared slide containing a fiber on the microscope stage and focus on the preparation.

2. Set up the microscope for conventional transmitted light microscopy using the Köhler illumination method.

3. Ignite xenon or mercury burner following your microscope instruction manual. To avoid stray light entering from underneath the preparation, remove the condenser and insert a light trap (available on some models) or an opaque material in its place.

4. Switch off transmitted light and dim the room lighting.

5. Depending on your specific model, either select a filter cube containing the dichroic mirror, excitation and barrier filters, or insert a dichroic mirror and a set of excitation and barrier filters into the light.

6. Refocus on the preparation, center and adjust the lamp and the reflected light field diaphragm until it is just outside the field of view.

7. Fully open the reflected light aperture diaphragm and remove all reflected light polarizers and analyzers from the optical path.

Observations:

Observe fluorescence intensity and color using a variety of known fiber standards and record your observations. If a red background is visible, then insert a BG38 (blue filter) or equivalent, which will suppress the red color. If your microscope is so equipped, then change excitation and barrier filters or filter cube to different wavelengths and record fiber fluorescence at various wavelengths.

A uniform fluorescent test object, such as a white piece of paper which is often impregnated with fluorescent brightening agents, can be placed on the stage to test the function of the optical system. It is desirable to make the final microscope image as bright as possible. The brightness of the image can be maximized by ensuring optimal Köhler illumination (no frosted glass filters in the illumination system) and the use of lenses with the highest numerical aperture.

Discussion:

With practice and critical observation you should be able to qualitatively estimate the subtle variations in fluorescence observed between fiber samples. With practice you will be able to prepare cross sections of approximately five micrometers in thickness. The degree, intensity and penetration of fluorescence can be characterized between examined cross sections.

The fluorescence properties between known and questioned samples can be characterized using a comparison light microscope equipped with an appropriate UV light source. Changes in fluorescence at a variety of excitation wavelengths can be evaluated while simultaneously viewing both questioned and known fibers. As in conventional comparison light microscopy, the use of mercury and xenon arc lamps should be assessed for proper Köhler illumination. Mercury burners do not provide an even intensity from ultra-violet to the infrared. It should be noted that both wattage and the size of the arc (the brightness per unit area within the back aperture of the objective) measure the useful brightness of the arc lamp. It is reported that xenon lamps have a more even intensity across the visible spectrum than mercury lamps. Arc lamps displaying amperage, voltage and/or wattage should be individually monitored.

Chapter 17 Practical Exercise 17-2

Subject: **The Effects of Mounting Media in Fluorescence Microscopy**

Time : 2 hours

Objective: To examine and compare a variety of mounting media with respect to auto-fluorescence using a variety of excitation wavelengths

Theory:
The choice of mounting media is critical when preparing samples for fluorescence microscopy. Mounting media exhibiting low auto-fluorescence are recommended, such as XAM or Norland 65 optical adhesive. Occasionally, XAM, which is an aromatic solvent reduced mounting media, can have adverse side effects on fibers and fluorescence brighteners, dissolving them and allowing them to diffuse from the fiber. This normally happens very quickly after mounting. Use another mounting medium if "bleeding" occurs upon sample mounting.

References:
Cook R, Norton D. An evaluation of mounting media for use in forensic textile fibre examination. J Forensic Sci 1982; 22:57-63.

Kubic TA, King JE, DuBey IS. Forensic analysis of colourless textile fibres by fluorescence microscopy. The Microscope 1983; 31:213-222.

Preparation:
glycerin/methanol solution (65:35)

Materials:
- stereomicroscope and fluorescence microscope
- microscope slides, cover slips, forceps
- authenticated fiber samples exhibiting fluorescence
- xylene, methanol, glycerin
- the mounting media Permount, Meltmount $n_{D1.525}$ (hot plate), Norland 65 (UV light source to cure), and XAM

Safety:
Mercury and xenon arc lamps may explode during operation due to their high internal gas pressure and extreme heat generated during use. Never turn on a lamp outside its housing or observe the lamp directly when it is illuminated. If there is a problem with the lamp, **DO NOT** service it yourself; contact your trainer. Never look at ultraviolet excitation light directly. Ultraviolet light is inherently dangerous and thus precautions **must** be taken in the proper set-up of the fluorescence microscope to prevent inadvertent exposure of the retina to UV light. If supplied, then use the UV protective shade while positioning and viewing microscope slides. The shade will block UV radiation emitted from the mercury/xenon burner.

Know the hazards associated with the solvents listed above and handle them according to the rules prescribed in your laboratory. Use standard procedures employed in your laboratory while working with the hot plate.

Directions:

1. Mount several representative fibers exhibiting fluorescence on individual microscope slides in
    - glycerin/methanol
    - xylene
    - methanol
    - Permount
    - Meltmount $n_{D\ 1.525}$
    - Norland 65
    - XAM

2. Using a variety of excitation wavelengths, compare the background auto-fluorescence of each mounting media and record your observations.

Observations:
Observe the prepared slides using all excitation wavelengths (change filter cubes or excitation/barrier filter sets) your microscope has available, and record any changes in auto-fluorescence. An undesirable high to very high auto-fluorescence will be observed using the glycerin/methanol solution, Permount and Meltmount $n_{D\ 1.525}$ as the mounting media. Very low auto-fluorescence will be observed while using methanol, xylene, XAM and Norland 65 as the mounting media.

Discussion:
Mount fibers using a mounting medium with the lowest possible auto-fluorescence. Permount, commonly used in fiber examination, is not recommended as a mountant for fluorescence examination due to its high auto-fluorescence characteristics. Norland 65 has a very low auto-fluorescence, but it is a permanent mounting media. The solvent of choice is methylene chloride if you need to remove a fiber from Norland 65.

Chapter 17 Practical Exercise 17-3

Subject: **Observing Fluorescence on Fibers: Optical Brighteners, Dyes and Contaminants**

Time: 8 hours

Objective: To observe the fluorescence characteristics of optical brighteners, dyes and contaminants of fibers

Theory:

Optical brightening agents form a class of dyes which, when excited with UV light, fluoresce at the short wavelength end of the visible spectrum emitting blue, sometimes violet or greenish-blue light. Brighteners introduced during manufacture are widely used industrially to give a 'bright white' look to textiles. Optical brightening agents present on fibers can also be derived from the consumer use of detergents, bleaches, fabric conditioners and soap. Wear and exposure may lead to further fluorescence modifications.

Manufactured fibers may be dyed in the spinning dope before the filament is formed, a process known as solution dyeing or mass-colored dyeing. If dyes containing fluorescence properties are used, then the cross-sectional view will show homogeneous coloration and fluorescence. Fibers that are dyed after being spun (surface dyeing) will show a range of dye penetration depths which can be observed by fluorescence microscopy.

Fibers may be contaminated with materials such as tape adhesive, oils, fats, pigments, toothpaste, denture cleaners, paints, lacquers, cosmetics, foodstuffs and soil which may fluoresce and interfere with the fiber's fluorescence. These contaminants may be of evidentiary significance in specific cases, and therefore care must be exercised in determining if the contaminants should be removed prior to fluorescence examination.

References:

Hartshorne AW, Laing DK. Microspectrofluorimetry of fluorescent dyes and brighteners on single textile fibres: Part 1-Fluorescence emission spectra. For Sci Int 1991; 51:203-220.

Hartshorne AW, Laing DK. Microspectrofluorimetry of fluorescent dyes and brighteners on single textile fibres: Part 2-Colour measurements. For Sci Int 1991; 51:221-237.

Hartshorne AW, Laing DK. Microspectrofluorimetry of fluorescent dyes and brighteners on single textile fibres: Part 3- Fluorescence decay phenomena. For Sci Int 1991; 51:239-250.

Lloyd LBF. Forensic significance of fluorescence brighteners: their qualitative TLC characteristics in small quantities of fibre and detergents. J For Sci Soc 1997; 17:145-152.

Palenik S. Microscopical examination of fibres. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999;173-175.

Preparation:
You will need to acquire a sufficient quantity of unbleached cotton fabric and a variety of laundry detergents to complete Part 1. You will need to acquire a sufficient quantity of undyed cotton fabric and a variety of "contaminants" to complete Part 3.

Materials:

- stereomicroscope and fluorescence microscope
- microscope slides, cover slips, forceps
- Norland 65 or XAM
- small containers with lids (approximately 30 ml)
- graduated cylinder
- a selection of about eight to ten laundry detergents and fabric softeners such as Tide, All and Bounce (liquid and powder)
- unbleached cotton fabric
- fiber samples containing optical brighteners
- dyed fibers exhibiting fluorescence with a variety of dye penetration depths, and exhibiting solution dyed and surface dyed processes
- fiber cross-sectioning supplies

Safety:
Mercury and xenon arc lamps may explode during operation due to their high internal gas pressure and extreme heat generated during use. Never turn on a lamp outside its housing or observe the lamp directly when it is illuminated. If there is a problem with the lamp, **DO NOT** service it yourself; contact your trainer. Never look at ultraviolet excitation light directly. Ultraviolet light is inherently dangerous and thus precautions **must** be taken in the proper set-up of the fluorescence microscope to prevent inadvertent exposure of the retina to UV light. If supplied, then use the UV protective shade while positioning and viewing microscope slides. The shade will block UV radiation emitted from the mercury/xenon burner.

Know the hazards associated with the solvents listed above and handle them according to the rules prescribed in your laboratory. Use standard procedures prescribed by your laboratory while working with the hot plate. Use caution when using scalpel or razor blades and place used blades in a sharps disposal container.

Directions:

Part 1- Optical Brighteners

1. Characterize the unbleached cotton fabric using reflected light fluorescence microscopy at a variety of excitation wavelengths.
2. Cut small strips of cotton fabric and place each into labeled containers. Add approximately 20 ml of tap water to each container. Add several drops or granules of detergent to each container, close lid, agitate container for approximately 1 minute.
3. Wait one hour and remove each cotton strip.
4. Cut each cotton strip in half. Rinse one of the two halves. Allow all to air dry.
5. Tease one individual thread from each fabric sample and mount in Norland 65 or XAM. Label each slide with the type of detergent used.
6. Examine each thread using a variety of excitation wavelengths. Compare each of the treated threads to the unbleached cotton and to each other, noting intensity and/or color similarities and differences.

Part 2 - Dyed Fibers

1. Cross section dyed fibers (about 5 µm thick is acceptable) and mount in Norland 65 or XAM.
2. Examine the cross sections using a variety of magnifications and excitation wavelengths.
3. Record your observations and note the degree of dye penetration.

Part 3 - Contaminants

1. Characterize the undyed cotton fabric using reflected light fluorescence microscopy at a variety of excitation wavelengths.
2. Apply a small amount of coffee with artificial creamer, watercolor paint, wallboard particles, oil, grease, lubricants, and soil on one side of separate pieces of the cotton fabric. Allow the materials to dry, if possible.
3. Tease a thread from each fabric sample and mount on individual microscope slides in Norland 65 or XAM. Label each slide with the name of the contaminating material used.
4. Examine each contaminated fabric using a variety of excitation wavelengths. Compare each of the contaminated threads to the uncontaminated cotton and to each other, noting intensity and/or color similarities and differences.

Observations:

By comparing the fluorescence properties of a variety of detergents used in the optical brightener experiment, you should be able to observe subtle differences in intensity and possibly color. Many laundry detergents have optical brighteners added to their formulations in amounts ranging from approximately 0.2 to 0.5 percent by weight. Optical brighteners are dyes (usually stilbene derivatives) that always have a blue-white fluorescence with ultraviolet excitation. One commonly used optical brightener is Tinopal whitening agent manufactured by Ciba-Geigy.

Dyes and pigments may fluoresce in a variety of colors with different excitation wavelengths. When dyes or pigments fluoresce, use all available excitation wavelengths with your microscope and note the emission colors observed. Remember that the questioned fiber should exhibit the same fluorescence as the known fiber during comparisons. The characteristics of solution dyed versus surface dyed fibers will be observed in the cross-sectional orientation. Note the variation in dye penetration of the surface dyed fibers.

Granular surface contaminants which may fluoresce, such as soil and wallboard, are easily differentiated from the underlying fabric and can be removed if necessary. Penetrating oils, grease, some foodstuffs such as non-dairy coffee creamer, and bodily fluids will mask the underlying fluorescence properties of fibers and/or create additional anomalous fluorescence characteristics which can be observed on fibers in this experiment.

Discussion:

Fluorescence microscopy can be used to determine if any fluorescing compounds were applied during the manufacturing, finishing or processing stages. Many spinning lubricants are oil based and will fluoresce under UV excitation. It may also be possible to investigate the past chemical history of a fiber. Examples of this may include the changes to fabric due to weathering from exposure to sunlight and oxidation of synthetic polymer fibers such as polyvinyl chloride (PVC).

The trainee should be aware of the effect of fluorescence from a variety of bodily fluids commonly encountered in casework. Fabric and fiber materials contaminated with urine, semen and/or body decompostion fluids may exhibit fluorescence.

Chapter 18 Practical Exercise 18-1

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.

References:
Adolf FP. Remarks on the present state and the methodical limits of microscope photometry. Proceedings of the 10th International Association of Forensic Sciences Meeting, 1984; Oxford.

Eyring M. Spectromicrography and colorimetry: sample and instrumental effects. Analytica Chimica Acta 1994; 288:25-34.

Robson R. A closer look at microspectrophotometric data. Proceedings of the 3rd Meeting of the European Fibres Group, 1995; Linkoping, Sweden:47-50.

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:

1. 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.)

2. 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.

3. 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.

4. 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.

5. 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.

6. 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 dependant, 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.

7. 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.

Chapter 18 Practical Exercise 18-2

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.

References:
Hartshorne AW, Laing DK. The definition of colour for single textile fibres by microspectro-photometry For Sci Int 1987; 34:107-129.

Houck M. Measuring dichroism in fibers by use of the microspectrophotometer. Proceedings of the 5th Meeting of the European Fibres Group, 1997; Berlin:75-82.

Laing DK, et al. Colour measurements on single textile fibres. For Sci Int 1986; 30:65-77.

Robson R. Spectral variation within red cotton dyes. Proceedings of the 5th meeting of the European Fibres Group, 1997; Berlin: 66-74.

Preparation:
Perform instrument set-up, performance check and calibration as described in Practical Exercise 18-1. 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 18-3.

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 18-3.)
- 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 18-3.)

Safety:
Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory.

Directions:

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

2. 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.

3. 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 moderm instruments do not have variable specimen masks and the field diaphragm is used to reduce/eliminate stray light]

4. 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?

5. 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?

6. 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.

7. 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.

8. 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.

9. 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.

10. 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 be 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 inhomogeneities within the area being measured.

Chapter 18 Practical Exercise 18-3

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.

References:
Grieve MC, Biermann TW, Wiggins KG. Fibre comparisons using microspectrophotometry, letter to the editor. Sci Justice 1999: 39(4):273.

Grieve MC, Dunlop J, Haddock P. An assessment of the value of blue, red, and black cottons as target fibres in forensic science investigation. J Forensic Sci 1988; 33(6):1332-1344.

Grieve MC, Dunlop J, Haddock P. An investigation of known blue, red, and black dyes used in the coloration of cotton fibres J Forensic Sci 1990; 35(2):301-315.

Hartshorne AW, Laing DK. Colour matching within a fibre data collection. J Forensic Sci 1988; 33(6):1345-1354.

Laing DK, et al. A fibre data collection for forensic scientists-collection and examination methods. J Forensic Sci 1987; 32:364-369.

Robson R, Wiggins KG. Fibre dye analysis-a comparative study of analytical pathways. Proceedings of the 4th Meeting of the European Fibres Group, 1996; London: 32-37.

Preparation:
Perform instrument set-up and calibration as in Practical Exercise 18-1. 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 18-2 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 18-2) used as the "known"
- electronic and/or hard copy plots of the spectra produced in Practical Exercises 18-2 and the same for any spectra produced in this exercise

Safety:

Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory.

Directions:

Part 1 - Acquiring Known Spectral Sets

1. 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.

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

3. 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.

4. 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 18-2.

5. Randomly pick a group of individual fibers from the known thread or yarn and repeat steps 2 through 4.

Part 2 - Comparing Spectral Curves

1. The "known" fiber spectra obtained from *Part 1* can be printed/plotted on a single graph. "Questioned" fiber spectra from Practical Exercise 18-2 should 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.

2. 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

(evaluation points continue next page)

- peak slope inflection points
- overall peak shapes
- absorbance range(s)
- convoluted peaks
- the relative peak heights within multiple peak spectra

3. 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

4. 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 18-2.

- 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 18-2 (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.

Chapter 18 Practical Exercise 18-4

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.

References:
Wiggins KG, Crabtree SR, Adolf FP, Grieve MC. The importance of analysis of reactive dyes on cotton fibres. Crime Lab Digest 1995; 22:89.

Macrae R, et al. The characterization of dyestuffs on wool fibres with special reference to microspectrophotometry. J Forensic Sci 1979; 24:117-129.

Preparation:
Perform instrument set-up and calibration as performed in Practical Exercise 18-1. This should be done as frequently as is specified by your laboratory's operating procedures.

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

Safety:
Know the hazards associated with the mounting media used and handle them according to the rules prescribed in your laboratory.

Directions:

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

2. 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.

3. 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 18-2 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.

Chapter 19 Practical Exercise 19-1

Subject: **Classification of Fiber Dyes**

Time: 2 hours

Objective: To learn a procedure to classify the dye type on polyacrylonitrile fibers

Theory:
In 1970, Feeman compared classical methods of dye identification with the then more modern methods. Dye identification schemes were based on determining the application class (direct, basic, etc.) and generic structure (anthraquinone, azo, etc.). Successful results depended on the dyes being homogeneous but even these were not identified chemically. Although he worked with large, non-forensic samples, his work has formed the basis of many of the systems now in use for fiber dye analysis in forensic science laboratories.

Generally, dye from single fibers can be sequentially extracted with a range of solvents. This allows not only for the most efficient dye extraction method, but also for the dye to be classified as long as a degree of caution is taken. Dyes used for coloring wool, polyamide, polyacrylonitrile, polyester, cellulosics, polypropylene and acetate fibers have been used in extraction and classification studies.

References:
Beattie IB, Smalldon KW, Dudley RJ. The extraction and classification of dyes on single nylon, polyacrylonitrile and polyester fiber. J Soc Dyers and Colorists 1979; 95:295-301.

Feeman JF. An introduction to modern methods of dye identification-chromatography and spectrophotometry. Canadian Textile J 1970; 87:83-89.

Wiggins KG. Thin layer chromatographic analysis for fibre dyes. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 291-309.

Preparation:
Prepare 10 ml of formic acid/water 1:1.

Materials:
- scissors, forceps
- glass tubes of appropriate diameter, glass pipettes or syringes, fine glass capillaries
- oven, hot plate and hot air stream (e.g. hair dryer)
- TLC plates, 2 x 250 ml glass beakers, 2 petri dishes
- glass scribe
- methyl acetate, methanol, and formic acid/water 1:1
- dyed acrylic fiber

Safety:
Know the hazards associated with the solvents listed above and handle them according to the rules prescribed in your laboratory. If these are not known to you, then consult the laboratory safety officer

before proceeding with this experiment. Follow normal safety rules for handling and cutting glass in your laboratory.

Directions:
Perform the steps below using a known acrylic fiber which has been dyed. The fiber can be identified as acrylic using infrared spectroscopy, if deemed necessary. A single fiber is all that is required to obtain a result as long as sufficient dye is present. The trainee may prefer to start experimentation with a small tuft of fibers, and then repeat with single fibers when the technique has been mastered.

1. Cut a long fiber into two pieces or divide a tuft in two.

2. Place each piece or tuft into a glass capillary tube that is heat sealed at one end, and is about 2.5 cm in length, with an internal diameter of 1.5 mm. A fine wire is used to push the fiber down the tube.

3. Label one tube "water blank" and add about 10 µl of distilled water (sufficient to cover the fiber) using a glass pipette or syringe.

4. Label a second tube "test material" (or similar identifier) and add about 10 µl of formic acid/water 1:1 (sufficient to cover the fiber) using a glass pipette or syringe.

5. Heat seal the tubes to prevent evaporation, and heat at 100 ^{O}C for 20 minutes.

6. The control and test samples are examined and compared visually to check for extraction of the dye. Pigmented or totally delustered acrylic fibers will not extract.

7. The tube containing the test material is cut open using a glass scribe. The extract is drawn up using a finely drawn glass capillary.

8. A TLC plate is placed on a hot plate heated to approximately 70 ^{O}C.

9. The extract is applied 1 cm from the lower edge of the TLC plate to produce a spot of approximately 2 mm diameter. Once the spot is dry, the spotting process is repeated to ensure the dye spot is strongly colored.

10. Dry the plate in an oven heated to approximately 100 ^{O}C for 5 minutes.

11. Place the plate in approximately 10 ml of methyl acetate in a 250 ml glass beaker which is then covered with a glass petri dish.

12. The plate is eluted for about 2 mm above the origin, removed and dried in a hot air stream. The methyl acetate is discarded.

13. If the dye has moved off the origin, then it is performing as a DISPERSE dye. If there is no movement, then the plate should be placed in a clean beaker containing methanol and a fresh attempt at elution made as in step 12.

14. If there is a sharp line at the solvent front, then it is performing as an ACID dye. If there is little or no movement, or if it is smeared, then it is performing as a BASIC dye. The methanol should be discarded.

Discussion:
Having completed this exercise the trainee should be capable of classifying all dyes used to color polyacrylonitrile fibers. Classification procedures for other dyes and fiber types encountered in forensic textile fiber examinations can be found in the supplemental readings listed in Appendix I for Chapter 18.

Dye classification may be useful for comparative purposes, and to assist in identifying a possible manufacturer.

Chapter 19 Practical Exercise 19-2

Subject: **Thin Layer Chromatography of Fiber Dyes**

Time: 6 hours (Part 1 ~2 hrs, Part 2 ~2 hrs, Part 3 ~2 hrs)

Objective: To learn extraction and subsequent TLC procedures for bulk and single fiber samples using acrylic fibers as an example

Theory:
TLC has been used to compare control and recovered fibers in criminal cases for over 25 years. Many of the dyes used to color textile fibers can be extracted using either pyridine/water 4:3 or formic acid/water 1:1.

When dyes have been extracted from textile fibers they can be spotted onto a suitable TLC plate. Solvents (eluents) are then allowed to pass over the spot. Different components travel with the solvents at different rates depending on their chemical and physical properties. Colors that are visually similar can be made up of different component dyes. These can be easily and quickly distinguished using TLC.

References:
Beattie IB, Smalldon KW, Dudley RJ. Thin layer chromatography of dyes extracted from polyester, nylon and polyacrylonitrile fibres. For Sci Int 1981: 117:57-69.

Wiggins KG. Thin layer chromatographic analysis for fibre dyes. In: Robertson J, Grieve MC, editors. Forensic Examination of Fibres, 2nd edition. London: Francis & Taylor, 1999; 291-309.

Preparation:
- Prepare 10ml of formic acid/water 1:1
- Prepare Eluent A = chloroform, methyl ethyl ketone, acetic acid, formic acid 8:6:1:1
- Prepare Eluent B = n-butanol, acetic acid, water 4:1:5
  (Eluent B forms an upper and lower phase, only the upper phase is used as the eluent.)
- Prepare standard dye solutions. Approximately 5 mg of each dye component are made up to a final volume of 25 ml with pyridine/water 4:3 v/v. This can be used until the supply is exhausted.
  - < The standard dye solution for Eluent A is: Solway green G (CI acid green 25), Superacet fast orange G (CI disperse orange 3), Superacet fast violet B (CI disperse violet 8).
  - < The standard dye solution for Eluent B is: Solway green G (CI acid green 25), Solway blue RNS (CI acid blue 47), Naphthalene fast orange 2GS (CI acid orange 10).

Materials:

- scissors, forceps, micropipette tubes, glass pipettes or syringes
- oven, hot plate and hot air stream (e.g. hair dryer)
- TLC plates, 4 x 250 ml glass beakers, 4 petri dishes, fine glass capillaries
- glass scribe or equivalent
- balance
- methyl acetate, methanol, formic acid/water 1:1, chloroform, methyl ethyl ketone, acetic acid, formic acid, n-butanol, water, pyridine/water 4:3, dyes as listed above
- three sources of black 100% acrylic fibers

Safety:

Know the hazards associated with the solvents listed above and handle them according to the rules prescribed in your laboratory. If these are not known to you, then consult the laboratory safety officer before proceeding with this experiment. Follow normal safety rules for handling and cutting glass in your laboratory.

Directions:

Classify the dye on each of the acrylic fiber samples removed from the three acrylic sources as set out in Practical Exercise 19-1 Classification of Dyes. It is essential that all three sources used in this practical exercise contain fibers with the same dye class. This should not be a problem because the vast majority of acrylic fibers have dyes which classify as basic. If another dye class is identified, then a different source should be used for these experiments. Proceed through the following Parts 1, 2 and 3.

Part 1 - Testing of Eluents and Extractant

1. Use two TLC plates. Using a pencil, one is labeled in the top left-hand corner as Eluent A and the other Eluent B. The two plates are placed on a hot plate (70 ^{O}C approximately).

2. One spot of the appropriate dye solution is applied 1 cm from the lower edge of the TLC plates, using a finely drawn capillary. The spot should be no larger than 2 mm diameter. Six to eight applications of the formic acid/water 1:1 should be applied to form one spot alongside the standard dye. This would represent a blank sample. Each application should be allowed to dry before additional applications are applied.

3. Dry the plates in an oven at approximately 100 ^{O}C for 5 minutes.

4. Place each plate in approximately 10 ml of the appropriate eluent (the plate labeled A with standard dye A should be eluted in eluent A) in a 250 ml glass beaker, which is then covered with a glass petri dish. The plates are eluted for about 2 cm above the origin, removed and dried in a hot air stream. The eluent is discarded.

Part 1 - **Observations:**

If the standard dye has adequately separated, then the eluent is suitable for use. If the formic acid/water 1:1 solution is not showing any colored bands, then it is free from colored contaminants which may interfere with subsequent TLC.

If the standard dye does not separate adequately, then the eluent should be discarded and re-made, and the above procedure should be repeated. If colored bands appear on the formic acid/water track, then this should also be discarded and re-made, and the above procedure should be repeated.

Part 2 - TLC of Basic Dyed Acrylic Fiber Bulk Samples

1. Place a small tuft of fibers removed from each of the three acrylic sources into labeled micropipette tubes. Cover the sample with formic acid/water 1:1 and extract in an oven heated to 100 ^{O}C.

2. A TLC plate is marked for the appropriate eluent and labeled at equidistant points across the plate indicating which sample should be applied in which position. The TLC plate is then placed on the hot plate.

3. The appropriate standard dye (for Eluent A) is applied to the plate 1 cm from the lower edge of the plate. In turn each of the three acrylic fiber dye solutions are applied to form a row of spots across the plate. The number of applications for each sample depends on the strength of the solution. Sufficient applications are applied to produce a good colored spot.

4. Dry the plate in an oven heated to approximately 100 ^{O}C for 5 minutes.

5. Place the plate in approximately 10 ml of the appropriate eluent in a 250 ml glass beaker, which is then covered with a glass petri dish. The plate is eluted for about 2 cm above the origin, removed and dried in a hot air stream.

6. Repeat this process for Eluent B.

Part 2 - **Observations:**
The standard dye on each plate should have separated as previously observed when the dyes were tested. The test samples should have separated into their component colors. The degree of separation will depend on the dye(s) itself and the eluent system.

Each of the three samples has now been eluted in two different systems. Five parameters can now be considered to decide which is the best system for each of the samples:

- separation of component bands
- sharpness of bands
- movement from the origin
- components traveling at or close to the solvent front
- strength of dye extract from a single fiber that may need to be tested

Part 3 - TLC of Basic Dyed Acrylic Fibers of Differing Lengths

1. Select one of the three samples used in Part 2 and decide, by using the parameters listed above, which eluent system is the most appropriate. Remove a single fiber from the sample and cut it into lengths of approximately 1 mm, 2 mm, 4 mm, 6 mm and 8 mm.

2. Place each piece into a glass capillary tube about 2.5 cm in length, with an internal diameter of 1.5 mm, heat sealed at one end. A fine wire is used to push the fiber down the tube. Each tube should be carefully labeled.

3. Add approximately 10 µl of formic acid/water 1:1 (sufficient to cover the fiber) to each tube using a glass pipette or syringe. The tubes should then be heat sealed to avoid evaporation and incubated at 100 ^{O}C for 20 minutes.

4. A TLC plate should be labeled in pencil with the details of the samples about to be applied. This is then placed on a hot plate at approximately 70 ^{O}C. The appropriate standard dye is spotted onto the plate.

5. The tubes containing the test samples are then cut open using a glass scribe. The entire contents of each tube is then applied to form a spot on the TLC plate. In turn, a row of spots 1 cm from the base of the plate is formed. Ensure that each application is dry before the next is applied. The contents of each tube are then applied equidistant apart to correspond to the labeling. It is preferable that the samples are applied in increasing order of length for ease of comparison.

6. Dry the plate in an oven heated to approximately 100 ^{O}C for 5 minutes.

7. Place the plate in approximately 10 ml of the appropriate eluent in a 250 ml glass beaker which is then covered with a glass petri dish. The plate is eluted for 2 cm above the origin, removed and dried in a hot air stream. The eluent is discarded.

Part 3 - **Observations:**
The standard dye should have separated as when previously tested. The test samples should have separated into their component colors. It will also be obvious that the separation and movement of the component colors is affected by the concentration of the dye. If a pale color sample was initially selected, then the shorter length samples may not show colored bands.

Discussion:
Having completed the three parts of this exercise the trainee should be capable of extracting dyes from polyacrylonitrile fibers. They should also be able to obtain a thin layer chromatograph from bulk and single polyacrylonitrile fibers. Extracts from a bulk sample will contain a more concentrated dye solution which may yield more color components. They should also be able to decide what is a suitable eluent system and why. Although this experiment is based on polyacrylonitrile fibers the trainee should be able to adapt it to allow them to perform TLC on any fiber type containing extractable dyes.

Chapter 21 Practical Exercise 21-1

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 effect 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.

References:
Adolf FP. Physical fits between textiles. Proceedings of the 3rd meeting of the European Fibres Group, 1995; Linkoping, Sweden.

Green MA. Stab wound dynamic-a recording technique for use in medico-legal investigations. J For Sci Soc 1978; 18:161-163.

Knight B. The dynamics of stab wounds. J Forensic Sci 1975; 6:249-255.

Monahan DL, Harding HWJ. Damage to clothing-cuts and tears. J Forensic Sci 1990; 35(4):901-912.

Stowell L, Card KA. Use of scanning electron mircroscopy (SEM) to identify cuts and tears in a nylon fabric. J Forensic Sci 1990; 35 (4):947-950.

Taupin JM. Comparing the alleged weapon with damage to clothing-the value of multiple layers and fabrics. J Forensic Sci 1999; 44(1):205-207.

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
- various cutting and stabbing instruments including, at least, a single edge knife, double edge knife, razor blade, scissors, a flathead screwdriver and Phillips screwdriver
- various 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.

1. 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.

2. 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.

3. Repeat steps 1 and 2 for each instrument and each fabric sample.

4. 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.

5. 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.

6. Retain the damaged fabric sections for use in Practical Exercise 21-2.

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; and
- 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.

Chapter 21 Practical Exercise 21-2

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.

References:
Hearle JWS, Lomas B, Cooke WD. Atlas Of Fibre Fracture And Damage To Textiles, 2nd edition. Cambridge: Woodhead Publishing, 1998; 397-425.

Janaway RC. Degradation of clothing and other dress materials associated with buried bodies of both archaeological and forensic interest. In: Haglund WD, Sorg, MH, editors. Advances in Forensic Taphonomy: Method, Theory and Archaeological Perspectives. Boca Raton FL: CRC Press LLC, 2001; 379-402.

Pelton W. Distinguishing the cause of textile fiber damage using the scanning electron microscope (SEM). J Forensic Sci 1995; 40:874-882.

Pelton W, Ukpabi P. Using the scanning electron microscope to identify the cause of fibre damage. Part II: an exploratory study. Can Soc For Sci J 1995; 28:189-200.

Ukpabi P, Pelton W. Using the scanning electron microscope to identify the cause of fibre damage. Part I: a review of related literature. Can Soc For Sci J 1995; 28:181-187.

Was J. Identification of thermally changed fibers. For Sci Int 1997; 85(1):51-63.

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, and your ingenuity and imagination

Safety:

Familiarize yourself with the various properties and hazards associated with any of the chemicals used. Use caution when using mechanical devices during the experimentation process and when using the razor or scalpel blades.

Directions:

1. 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.

2. 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, H_2SO_4, acetic
        - < bases like NaOH, ammonia compounds (fertilizers)
        - < ignitable liquids like gasoline, kerosene
        - < bleach, hydrogen peroxide, other household chemicals

3. Perform some of the environmental testing, particularly burial or burning, on fabric samples from Practical Exercise 21-1. Examine severed edges from Practical Exercise 21-1 after the fabric has been soaked with blood.

4. Compare the exposed fabric to the comparison sample. Note any changes, if any, that 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.

Chapter 22 Practical Exercise 22-1

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.

References:
Laux DL. Identification of a rope by means of a physical match between the cut ends. J Forensic Sci 1984; 29(4):1246-1248.

Wiggins KG. Recognition, identification and comparison of rope and twine. Sci Justice 1995; 35:53-58.

Preparation:
Note that Practical Exercise 22-2 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

Safety:
Know the hazards associated with the use of sharp instruments and handle them according to the rules prescribed by your laboratory.

Directions:
Perform steps 1-9 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.

1. 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

2. 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.

3. 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.

4. 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

5. 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

6. 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.

7. 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.

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

9. For each type of cordage you are examining, attempt single and double edged force cuts, with and without tension stress. Attempt a physical match 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 match between a questioned and known item. In the absence of a physical match, 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.

Chapter 22 Practical Exercise 22-2

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.

References:
Kirk PL. Ropes, cordage and packaging material. In Thornton JL, editor. Crime Investigation, 2nd edition. Malabar, FL: Krieger Publishing Co., 1974; 136-142.

Van Nostrand's Scientific Encyclopedia, 6th editon, (see Rope). New York: Van Nostrand Reinhold Co., 1983.

Preparation:
Refer to completed Practical Exercise 22-1. 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

Safety:
Familiarize yourself with the various properties and hazards associated with any of the chemicals used. Be cautious when using mechanical devices during the experimentation and when using the razor or scalpel blades.

Directions:

1. Subject the cordage previously examined in Practical Exercise 22-1 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, H_2SO_4, acetic
        - < bases like NaOH, ammonia compounds (fertilizers)
        - < ignitable liquids like gasoline, kerosene
        - < bleach, hydrogen peroxide, other household chemicals

2. Re-examine the cordage using the same techniques learned from Practical Exercise 22-1. 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.