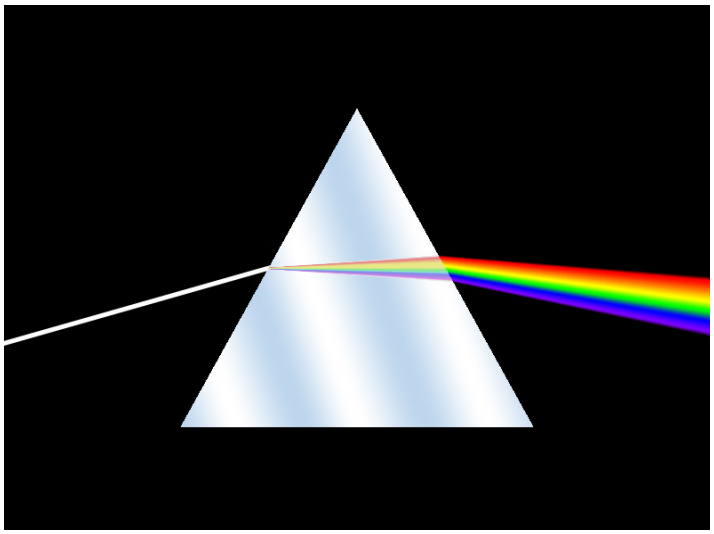
Washington State Patrol



Crime Laboratory Division

Materials Analysis
Primary Foundation Training Manual

December 2016

Contents

1 INTRODUCTION

1.1 PURPOSE AND SCOPE

This manual contains an outline for training and/or assessing a forensic scientist in the area of materials analysis. Each scientist will have a unique training program depending on the individual's strengths and weaknesses, previous background, the needs of the laboratory, and available personnel to provide the training. The sequence in which the various sections are presented should not necessarily be considered as a mandatory order of training. Exposure to legal aspects, testimony, and many other facets of forensic science will be continuous throughout the training.

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

1.2 ASSESSMENT OF TRAINEE'S BACKGROUND

Commencement of this training program does not assume prior experience in forensic materials analysis. It is assumed, however, that the trainee will have a solid understanding of basic organic and inorganic chemistry and instrumental analysis.

Trainees who have prior related training and experience may be able to progress through the training program at an accelerated pace or perform competency testing to demonstrate their ability in a particular materials analysis discipline or sub-discipline rather than performing redundant training. All trainees will be required to demonstrate competence in a particular area in which they will be performing analysis.

The instructors must be experienced in the area of materials analysis for which they are to instruct. The instructor's casework and courtroom experiences, both prior and present, provide a unique aspect to the trainee's learning process that is impossible to duplicate in this training program. It is expected that the instructor will share such experiences with the trainee. Although the trainee's primary interaction will be with the assigned instructor, this program promotes and encourages discussions with other experienced examiners. The trainee's instructor and/or supervisor, or other experienced scientist, will preliminarily assess the trainee's:

- Knowledge of:
  - Analytical chemistry
  - Organic chemistry
  - Inorganic chemistry
  - Physical chemistry
  - Microscopy
  - Instrumentation
  - Physics
- Laboratory experience
- Determination of strengths and deficiencies:
  - From this an initial plan developed for remedial readings, lectures, and schooling, if necessary.
  - Training will be customized to meet the needs of the trainee.

Once the course of training has been established and initiated, the trainee will maintain a notebook or multiple notebooks for the various segments throughout the duration of this training program and will record notes and observations for each study segment. The trainee notebook should be maintained in a

neat and current fashion and should be present during conversations with the trainer. Upon completion of training, the trainee will maintain the training notebook for the duration of their career.

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

In order to successfully complete this training program, the trainee must, after completion of each study segment, demonstrate their capability to apply learned knowledge and skills. Training checklists for this training are located in Appendix A and must be completed by the trainee and trainer. The trainer is responsible for writing an interoffice communication (IOC) to the trainee's supervisor when the trainee has successfully completed the Primary Foundation Training. The trainee's supervisor will maintain copies of training IOCs and authorizations in their files.

1.3 ORGANIZATION OF THE TRAINING MANUAL

The training manual consists of several study segments, each covering different aspects of chemical analysis.

Each study segment is comprised of six parts:

- The *Objectives* summarizes the purpose of each training segment.
- The *Topic Areas* designates topics to be included in the training segment.
- The *Safety* section indicates specific safety information relating to the training segment.
- The *Suggested Readings* section lists the reference material that may be useful to successfully complete the study segments. The reading assignments are cumulative; comprehension of prior readings may be required to successfully complete study/discussion questions and exercises of subsequent study segments. It may not be necessary or practical to read every reference listed. Trainers should specify references from this list which are most pertinent and suggest other references which may not be listed. The trainee will work with the trainer for specifics.
- The *Study Questions* have a number of purposes:
    - To assist reading comprehension by providing a focus on certain concepts prior to completing the Reading section;
    - To evaluate understanding of relevant concepts after completing the Readings; and
    - To promote active discussions between the trainer, trainee and trainee's co-workers using the questions as a starting point.
    - Written answers to these questions will be maintained in the training notebook as documentation of training.
- The *Practical Exercises* are designed to provide the trainee first-hand experience with the main concepts of each study segment. Data or written explanation for each Practical Exercise must be maintained in the training notebooks.

Module 1 of the Primary Foundation is the Introduction which includes:

- General Forensic Procedures
- Lab Safety
- Criminalistics 101
- Ethics/Professional Conduct
- Law Basics and Court Testimony

Module 2 of the Primary Foundation covers Instrumentation and Techniques, which includes:

- Balances and Weighing

- Thin Layer Chromatography
- Gas Chromatography/Flame Ionization Detector
- Mass Spectrometry & Pyrolysis
- Capillary Electrophoresis
- Infrared Spectroscopy
- Gas Chromatography/Fourier Transform Infrared Spectroscopy
- High Performance Liquid Chromatography
- Raman Spectroscopy
- Scanning Electron Spectroscopy/Energy Dispersive X-Ray Spectroscopy
- Spectroscope
- X-Ray Fluorescence Spectrometry

Module 3 of the Primary Foundation is Basic Practical Microscopy

Module 4 of the Primary Foundation covers:
- Imaging & Visualization
- Evidence Recovery

A comprehension examination will follow Modules 2, 3, and 4. Competency testing will follow Modules 3 and 4.

The instructor is responsible for ensuring that the trainee is prepared to testify as an expert witness. This can be done with mock trials, prearranged as well as impromptu question and answer sessions, and observation of courtroom testimony given by experienced forensic scientists. One or more mock trials will be scheduled during the training program.

2 LAB SAFETY

2.1 OBJECTIVES

- To familiarize the trainee with potential safety hazards in the laboratory.
- To instruct the trainee on how to properly deal with chemical spills.
- To make the trainee aware of safety equipment available in the laboratory.
- To familiarize the trainee with proper handling and disposal of chemicals.

2.2 TOPIC AREAS

1) Safety tour
2) Material Safety Data Sheets (MSDS)
3) Proper gas cylinder handling
4) Work station set up
5) Specific cautions
6) Fume hood use
7) Glass and sharps disposal
8) Chemical waste plan
9) Injury reports
10) Safety committee
11) Personal Protective Equipment (PPE)
    a) Gloves
    b) Goggles
    c) Lab coats
    d) Other
12) Chemical spills
13) Scalpel use and syringe techniques
14) Biohazards and disposal
15) Specific precautions
    a) Some controlled substances, like LSD (Lysergic Acid Diethylamide), can be absorbed into the skin.
16) Biohazards: Not all requests are labeled if this is the case. Evidence can be from a body cavity.
17) Sharps hazard: Though not normally accepted as evidence, syringes, broken glass, or razor blades can be found in evidence. Do not blindly place your hand into an evidence container. Repackaging may be necessary to reduce the risk of injury to others who may handle the evidence in the future.
18) Chemical hazards: The majority of chemicals used in this unit have one or more hazardous properties.

2.3 SUGGESTED READING

1) Crime Laboratory Division's "Safety Manual"
2) Crime Laboratory Division's "Operations Manual"
3) Crime Laboratory Division's "Quality Manual"
4) Forensic Services Guide
5) WSP Safety and Wellness Manual
6) WSP Bloodborne Pathogens Training for Civil Service Employees PowerPoint

2.4 STUDY QUESTIONS

1) What is the FLSB policy regarding accepting hypodermic needles, razor blades and other sharps?
2) What is the FLSB policy regarding accepting evidence retrieved from body cavities?
3) Research gloves. Which ones are best for chemical analysis and why?

4) Find the following MSDS and answer the questions: Chloroform, Methanol, Methylene Chloride, Pentane, Hydrochloric Acid, and Sodium Bicarbonate.
   a) What is the target organ?
   b) What is the first aid measure for eye exposure? Dermal exposure?
   c) What are Quickits? Where can they be found?
   d) What is the recommended PPE?
   e) What are the signs and symptoms of exposure?
   f) What is the HMIS (Hazardous Materials Identification System) flammability rating?

2.5 PRACTICAL EXERCISES

1) Complete a facility safety tour. At a minimum the following should be covered in this tour:
   a) Operation and location of emergency showers.
   b) Operation and location of emergency eyewashes.
   c) Operation and location of the spill clean-up kits.
   d) Location of fire alarms, emergency exits, and evacuation plan.
   e) Location and understanding of Material Safety Data Sheets.
   f) The rules concerning eating and drinking in the laboratory.
   g) Location and appropriate use of safety glasses.
   h) Appropriate use and disposal of laboratory coats.
   i) Location and appropriate use of hood(s) in your area.
   j) Location and explanation of the fire extinguishers.
   k) Location and contents of first aid stations and kits.
   l) Location and use of fire blankets.
   m) Proper disposal of scalpel blades, syringes, broken glass, and biohazards.
   n) How to deal with bomb threats.
   o) Explanation of the alarm and security system.
   p) Proper handling and storage of compressed gas cylinders.
2) Complete the New Employee/Transfer Safety Orientation form 3000-342-175.
3) Complete the Safety Orientation Checklist form CLD-SAF-15001.
4) Perform the eyewash inspection with the designated person.
5) Perform the fume hood inspection.
6) Review the mandatory online WSP Bloodborne Pathogens Training for Civil Service Employees PowerPoint.
7) In the fume hood and wearing the appropriate PPE, pour approximately 10 milliliters of water onto the counter. Go through the proper clean up procedure, using the spill kit, if the water was methanol. Repeat as if the water was sulfuric acid. Repeat for sodium hydroxide.
8) With the assistance of a colleague, blindfolded, make your way to the nearest shower or eyewash from your lab bench.
9) Determine if the following are compatible and therefore can be stored next to each other:
   a) aluminum hydroxide and aluminum oxide
   b) aluminum oxide and aluminum sulfate
   c) benzoyl chloride and benzylamine
   d) oxalic acid and nitric acid
   e) nitric acid and pine-sol
   f) methanol and heptanes

3 GENERAL FORENSIC CASE CONSIDERATIONS

3.1 OBJECTIVES

- To introduce the trainee to evidence handling, accountability and chain of custody policies.
- To instruct the trainee on proper preparation of case notes and reports.
- To make the trainee aware of forensic ethics and standards of professional conduct.
- To introduce the trainee to case approach and interpretation of data.
- To familiarize the trainee with special safety considerations involved in materials analysis casework.

3.2 TOPIC AREAS

1) Evidence Handling
   a) Preservation of evidence
      i) The integrity and security of the evidence shall be maintained.
      ii) Any significant change in the evidence whether intentional or accidental must be noted.
      iii) Care will be taken to ensure analytical techniques employed do not adversely affect future testing of the evidence by other functional areas, or to anticipate the possibility and plan the analysis scheme accordingly.
   b) Chain of custody
      i) Definition
      ii) Performing evidence transfers with user agencies
      iii) Performing evidence transfers within the lab
   c) Security measures
      i) Seals (tape, staples, markings)
      ii) Laboratory security and vault procedures
      iii) Personal evidence lockers
   d) Contamination Issues
      i) Potential for DNA and fingerprint contamination
      ii) Maintain a clean, organized work area and use clean utensils.
      iii) Work one case at a time.
      iv) Work one item at a time.
      v) Minimize exposures from large items (such as large quantities of powder or moldy vegetable material).
      vi) Keep evidence covered or sealed when not present.
   e) Safety
      i) Safety checks performed on guns and explosives
      ii) Do not blindly reach into evidence packaging especially potential drug kits or clothing pockets as they may contain sharps.
      iii) Be cognizant of potentially explosive or ignitable evidence.
      iv) Handle potential biohazard evidence using appropriate techniques.
   f) Initial Observation of Evidence
      i) Are all seals intact and properly marked?
      ii) Note signs of tampering, multiple seals.
      iii) Are evidence description and numbers consistent with the Request for Laboratory Examination?
   g) Labels and other devices/procedures to avoid confusion and/or enable future identification (such as in court):
      i) of the original evidence and packaging
      ii) of generated samples (e.g. GC/MS vials)
      iii) of data and notes
      iv) of packaging provided by the Lab in addition to the original packaging

v) of items that may need fingerprint examinations

h) Proper Seals
   i) Evidence tape must be used whenever practical when sealing evidence packaging.
   ii) Staples do not constitute proper seals.
   iii) Proper evidence seals must be initialed or otherwise marked to document the person sealing the evidence. Such markings on all taped seals must be made so that the initials flow from the tape to the package. Inclusion of the date of sealing and the Crime Lab number is also recommended.
   iv) Manufactured seams may be sufficiently secure or may be taped.
   v) Heavy packages may need a reinforcement tape, such as book tape, below the evidence tape in order to prevent the evidence from falling out of a resealed package.
   vi) For bottles, jars, cans and film canisters, make sure the lid is secure and that evidence tape extends across the top of the lid and down both sides of the lid to the container body.
   vii) Heat sealed packages must have initials or other identification across the heat seal to be properly sealed.
   viii) Evidence seals should ensure that any efforts of entry or attempted entry will be obvious.
   ix) Proper seals protect evidence from loss, cross-transfer and/or contamination.

i) Introduction to LIMS and how evidence is logged (See also the LIMS Manual)

2) Case Work Documentation

Case files must be complete, organized, legible, and must support all conclusions stated in the written report. Case notes and analytical documentation include but are not limited to the following:

a) Relevant case information/history/background; communications with investigators, attorneys, etc…
b) Chain of custody records
c) Description of evidence packaging, seals, and case identifiers (or lack of)
   i) Note and address discrepancies in evidence versus the Request for Laboratory Examination Form. Evidence shortages will be witnessed by a coworker and documented in the notes. Evidence surplus will also be documented in the notes. (See the Operations Manual Section 3.3 for details.)
   ii) Description of items of evidence (packaging, quantity, size, colors, physical appearance/conditions), both analyzed and not analyzed.
      (1) Use Submitting Agency exhibit numbers where possible.
      (2) Outer packaging descriptions are adequate for items not analyzed.
   iii) Refer to the Operations Manual, Section 3.1.2 for instructions regarding high value items, especially currency.
   iv) Describe photographs, diagrams, and/or photocopies if necessary.
   v) Weight or volume of examined items: methods used to derive weights or volumes are specifically outlined.
d) Examination procedures used and the parameters for those procedures
e) Analytical procedures
   i) Special handling of evidence (such as for prints)
   ii) Any trace evidence collection
   iii) Microscopic examinations
   iv) Preparation of controls or comparison samples
   v) Description of solvents used and extractions performed
   vi) Instrument parameters
   vii) Description of mathematical calculations
   viii) Identity and source of any standards or references used
   ix) Results of examinations
f) Data generated
   i) Spectra, chromatograms and other instrumental printouts
   ii) Photographs, digital images and diagrams
   iii) Observations and descriptions
   iv) Drawings, sketches or other illustrations

g) Records of inter/intralaboratory transfers
h) Notations showing the generation and disposition of generated evidence items (trace collections, sub-samples, controls, etc…) and any repackaging of evidence
i) Case notes and analytical documentation must be marked with unique laboratory case numbers, the handwritten initials of the examiner (and cosigner if applicable), dates, and page numbers.
j) Dates should be recorded in the documentation to indicate when work was performed.
k) Documentation of technical review on each page of the draft copy of the laboratory report will remain in the case notes.
l) Errors in Note taking
    i) Nothing in the case notes and analytical documentation may be erased or obliterated. Strikeouts to designate information removed will be documented by the examiner's initials. Changes, additions, or any other form of alteration must be initialed by the person making the alteration.

3) Case Reports
a) Refer to WSP CLD QM & OM sections concerning the protocol for case reports.
b) All reports should be able to stand alone without the necessity of explanation by supporting verbal testimony.
c) All conclusions must be accurate, thorough, clearly stated, and justified by data in notes.
d) All evidence examined must be listed in the report.
e) Items not examined must be documented in the report. The analyzed items are clearly differentiated from the non-analyzed items.
f) Conclusions, opinions and interpretations must be identified in the report.
g) Other report contents include:
    i) Complete and accurate case heading;
    ii) The controlled substances conclusively identified;
    iii) Weight/amount of analyzed exhibits;
    iv) Procedures must be clearly and completely outlined except for controlled substance reports.
    v) Test certifications on controlled substance case reports, and other court or RCW requirements;
    vi) Signature and date line.
h) Verbal reports
    i) May be issued when preapproved by the section supervisor.
    ii) A draft of the verbal report must be written.
    iii) The draft of the verbal report and the supporting data must be technically reviewed.
    iv) Documentation by the technical reviewer will be entered in LIMS.
    v) Analyst must document the date time and name of the person to whom the verbal report was released on the written version of the verbal report.
i) Amended reports
    i) Issued to modify the content of previously issued laboratory reports.
    ii) The report will be titled "Amended Report" and will contain a brief explanation describing the need for the amended report.
    iii) All amended reports must be documented in the Case Information field in LIMS. The reason for the amended reported will be noted in the case file.
    iv) Review of the amended report is required.
j) Use of LIMS for report generation
    i) Edit findings
    ii) Milestones
    iii) Activities
    iv) NFLIS
    v) Analytical techniques

Refer to the Crime Laboratory Division Operating Manual and Quality Manual for additional details on case notes, administrative documentation and case reports.

4) Case Approach and Analysis
a) Prioritization of casework

i) Immediate Response
   (1) Immediate investigative need to identify, arrest, hold/release a suspect in custody;
   (2) Lab work needed to avert danger to someone's life;
   (3) Immediate need to preserve physical evidence for future work.
ii) Court Deadlines in crimes against persons
   (1) Information from lab work needed to prosecute crimes against persons and drug crimes
   (2) Trial date set
iii) 30 Day Turnaround Goal
   (1) Consider number of items to be examined including one item per suspect. (See the Forensic Laboratory Services Guide for submitting agency guidelines and general analysis policy.)

b) Prior to opening evidence:
   i) Consult user agencies as necessary.
   ii) Consider any inter/intralaboratory case coordination.
   iii) Preparation of work area
   iv) Preparation of various commonly used chemical solutions/reagents
c) Evaluate evidence to plan analytical strategy.
   i) Documentation of evidence prior to manipulation
      (1) description, photos, sketches
   ii) Choose appropriate analytical methods
   iii) Examine using least destructive methods first and minimize sample consumption.
   iv) Obtain appropriate reference material or control samples as necessary.
      (1) verification and documentation of reference materials
   v) Collection of evidence for analysis by other functional areas
   vi) Packaging/labeling of items to be examined by other functional areas
   vii) Consider travel to other facilities to use instrumentation not available locally. (See the Quality Manual Section 6.2.3.6 for details on use of outside instrumentation.)
d) Interpretation of Data
   i) Make use of reference materials, libraries, consultations, manufacturers or specialists, and/or other forensic scientists.
   ii) Specificity of applied techniques
   iii) Prevention of false-positives
   iv) Two-prong analysis: if possible, each analysis utilizes independent theory, reagents, etc…
      (1) It is desirable that different portions be taken from a sample for analysis by different techniques; however, evidence items containing a small amount of material may not be sufficient to allow multiple samples to be taken. When only one sampling of an item is practical, particular care should be taken.
   v) Use of blanks/controls
   vi) Formulation of Conclusion
      (1) Objective vs. subjective (identification and qualified conclusions)
      (2) Inconclusive/lack of conclusion
      (3) Insufficient sample
      (4) Residues
      (5) All conclusions justified by data

5) Case Review
   a) Analyst review
      i) Analysts will conduct a thorough case review of their own work prior to technical review.
      ii) The analyst review must cover all elements of the technical and administrative review.
   b) Technical review
      i) The technical reviewer must be technically proficient in the functional area and the specific sub-discipline to participate in technical review.
      ii) An approved Technical Review Checklist will be completed for all cases.
      iii) Covers the elements listed in the CLD QM for technical review.

iv) Reviewer will initial and date each page of the final draft report upon completion of the review and will mark the technical review milestone in LIMS. For cases involving inter-laboratory review, each page of the draft will be signed and dated by the technical reviewer.
v) Keys to successful technical review
(1) Listen courteously.
(2) Always be respectful.
(3) Be open and receptive to suggestions.
(4) Keep an open mind and behave professionally.
(5) Address each of the suggested changes/improvements.
(6) Work with the reviewer to resolve all issues at the peer level, as much as possible. For situations where the technical review issues cannot be resolved, see the Quality Manual for guidance.

c) Inter-Lab Technical Review
i) One case per quarter from each analyst will be sent to another lab for technical review.
ii) Original case file will be retained in the lab and a copy of the file will be sent out for technical review by the section supervisor or designee.

d) Administrative review
i) Conducted on the case file and final report prior to the release of the written report.
ii) Covers the elements listed in the CLD QM for administrative review.
iii) Administrative reviewer does not need to be technically proficient in the functional area.
iv) The administrative review is documented in LIMS and may be documented in the case file as well.
v) The administrative review may be combined with the technical review.

3.3 SUGGESTED READING

1) WSP Forensic Services Guide
2) WSP CLD Quality Manual
3) WSP CLD Operations Manual
4) WSP CLD LIMS Manual
5) WSP CLD Materials Analysis Technical Manual
6) Forensic Sciences Handbook, Volume I, Chapter 1; Volume II, Chapters 2 and 3; Volume III, pages 1-23, Saferstein, R.
7) DeForest PR, Gaensslen RE, Lee HC. 1983. Forensic Science: An Introduction to Criminalistics, Chapters 1-3. New York:Mc-Graw Hill. p. 1-94.

3.4 STUDY QUESTIONS

1) Discuss the methods and purpose of evidence security and chain of custody.
2) Discuss the marking of evidence. What are the minimum markings required on evidence packaging?
3) Discuss the required documentation used for analytical examinations.
4) What types of “new evidence” may be created during the course of analysis? How would you document this new evidence in your notes, in your report and in LIMS?
5) You are analyzing evidence and you are paged to go to the phone. Describe your activities.
6) You receive a phone call from an officer with a syringe. What do you tell him/her?
7) Its lunchtime and you have an open case on your desk. Describe your actions. It's quitting time and the same case is on your desk. Describe your actions.
8) What must be considered when a piece of evidence has multiple exam codes listed on the RFLE?
9) How are cases received and logged into the Lab?
10) What are some elements that a report should contain?

3.5 PRACTICAL EXERCISES

1) Observe your instructor or other qualified employee during the analysis of a case.

2) Review a minimum of 10 case folders from several experienced forensic scientists.
3) Observe the entire process that a case follows while in the laboratory.
4) Log a case into the Lab and into the LIMS system.

4 CRIMINALISTICS 101

4.1 OBJECTIVES

This two day training program will cover the fundamental aspects of criminalistics including the interdisciplinary chain of evidence examination in the crime laboratory. The trainee will learn what types of evidence are examined in each functional area. The trainee will learn how to safely handle and evaluate a broad range of evidence including firearms, smears and stains, blood patterns, cuts and tears and small particle characterization. Trainees will receive basic information to begin to develop strategies to document, recover evidence, prevent contamination and preserve evidence (e.g. A DNA examiner will understand the potential significance of smokeless powder grains, metal turnings or vomit on a shirt).

4.2 TOPIC AREAS

1) INTRODUCTION TO CRIMINALISTICS
    a) Goals of the workshop
    b) PowerPoint lectures and labs - types of physical evidence encountered.
    c) Team building approach-we all have value
    d) Interdisciplinary study of physical evidence
    e) Contamination and evidence preservation
        i) Overview of each section of the laboratory
        ii) Specific evidence types encountered
    f) Documentation, collection, and packaging of evidence
        i) References and useful documents such as the ABC study guide
    g) Cognitive bias
2) LAW and ETHICS
3) CSRT
    a) Blood Stain Pattern Analysis
    b) Trajectory analysis
    c) Evidence recovery
4) LATENT PRINTS
    a) Overview of work performed
    b) Evidence preservation, contamination prevention, and packaging
    c) Examples of impressions on materials such as handguns and duct tape
5) MATERIALS ANALYSIS
    a) Overview of work performed
    b) Trace evidence recovery
    c) Use of microscopes
    d) Evidence sub-disciplines
        i) Fabric damage
        ii) Physical match
        iii) Impressions/smears/stains
        iv) Fibers/fabric/cordage
        v) Hairs
        vi) Paint
        vii) Explosives/residue
        viii) Soil
        ix) Botanicals
        x) Tape
        xi) Lamps
        xii) Glass
        xiii) Controlled substances
        xiv) Fire debris

xv) Clandestine laboratories
xvi) Unknowns

6) DNA/SEROLOGY/CODIS
   a) Overview of work performed
   b) Blood and body fluids
   c) Blood testing methods
   d) DNA
   e) Contamination prevention methods
7) FIREARMS/TOOLMARKS
   a) Overview of work performed
   b) Safety concerns
   c) Types of Weapons
   d) Ammunition - cartridges/cartridge cases/bullets
   e) Distance determinations
   f) Bullet holes in clothing
   g) Gunpowder particles
8) DOCUMENTS EXAMINATION
   a) Overview of work performed
   b) Evidence preservation, contamination prevention, and packaging
9) LABORATORY EXAMINATIONS AND DEMONSTRATIONS
   a) Examination/identification of particle types (hairs/fibers, paint chips, glass, building materials, insect parts, botanicals, cosmetics, mud/soil, vomit, etc)
   b) Clothing damage (cuts and tears)
   c) Impressions, smears and stains
   d) Handgun safety, prior packaging and handling
   e) Awareness of hazardous evidence submissions
   f) Bloodstain patterns on clothing, tools/weapons, porous and non-porous surfaces

5 ETHICS

5.1 OBJECTIVES

- To make the trainee aware of forensic ethics and standards of professional conduct.
- To make the trainee familiar with State policies on use of resources and acceptance of gifts.

5.2 TOPIC AREAS

The following were compiled verbatim from SWGDRUG (Scientific Working Group for the Analysis of Seized Drugs) and the American Board of Criminalistics' Rules of Professional Conduct in Forensic Science. They are considered to meet general acceptance by peers in this profession.

1) Analysts should:
   a) act with honesty, integrity and objectivity;
   b) maintain an attitude of independence and impartiality in order to ensure an unbiased analysis of the evidence;
   c) work only within the bounds of their professional competence;
   d) take reasonable steps to maintain their competence;
   e) recognize that their overriding duty is to criminal justice;
   f) declare to their employer any prior contact or personal involvement, which may give rise to conflict of interest, real or perceived;
   g) declare to their employer or other appropriate authority any pressure intended to influence the result of an examination;
   h) carry out the duties of the profession in such a manner so as to inspire the confidence of the public.
2) Regarding casework, analysts should:
   a) ensure and be able to demonstrate that the integrity and security of evidential materials and the information derived from their analysis have been maintained while in their possession;
   b) ensure that they have a clear understanding of what the customer needs and all the necessary information, relevant evidential materials and facilities available to reach a meaningful conclusion in an appropriate timeframe;
   c) employ an appropriate analytical approach, using the facilities available;
   d) make and retain full, contemporaneous, clear and accurate records of all examinations and tests conducted, and conclusions drawn, in sufficient detail to allow meaningful review and assessment of the conclusions by an independent person competent in the field;
   e) accept responsibility for all casework done by themselves and under their direction;
   f) conduct all professional activities in a way that protects the health and safety of themselves, co-workers, the public and the environment.
3) Regarding reporting, analysts should:
   a) present advice and testimony, whether written or oral, in a clear and objective manner;
   b) be prepared to reconsider and, if necessary, change their conclusions, advice or testimony in light of new information or developments, and take the initiative in informing their employer and customers promptly of any such changes that need to be made;
   c) take appropriate action if there is potential for, or there has been, a miscarriage of justice due to new circumstances that have come to light, incompetent practice or malpractice;
   d) preserve customer confidentiality unless officially authorized to do otherwise;
   e) render opinions and conclusions strictly in accordance with the evidence in the case (hypothetical or real) and only to the extent justified by that evidence;
   f) testify in a clear, straightforward manner and refuse to extend oneself beyond one's field of competence, phrasing one's testimony in such a manner so that the results are not misinterpreted;
   g) not exaggerate, embellish or otherwise misrepresent qualifications when testifying;

h) consent to, if it is requested and allowed, interviews with counsel for both sides prior to trial;
i) make efforts to inform the court of the nature and implications of pertinent evidence if reasonably assured that this information will not be disclosed to the court;
j) find it appropriate to report to their supervisor any violation of these professional rules of conduct by another scientist.

5.3 SUGGESTED READINGS

1) Budowle B. et al. 2009. A Perspective on Errors, Bias and Interpretation in the Forensic Sciences and Direction for Continuing Advancement. J Forensic Sci. 54(4):798-809.
2) Dror IE. 2008. Biased Brains. Police Review. 116:23-23.
3) Dror IE, Charlton D, Péron AE. 2006. Contextual Information Renders Experts Vulnerable to Making Erroneous Identifications. J For Sci Int. 156:74-78.
4) Dror IE, Péron AE, Hind SL, Charlton D. 2005. When Emotions Get the Better of Us: The Effect of Contextual Top-down Processing on Matching Fingerprints. Appl. Cognit. Psychol. 19:799-809.
5) Dror IE, Charlton D. 2006. Why Experts Make Errors. J For Ident. 56(4):600-616.
6) Lucas DM. 1989. The Ethical Responsibilities of the Forensic Scientist: Exploring the Limits. J For Sci. 34(4):719-729.
7) Peterson JL, Murdock JE. 1989. Forensic Science Ethics: Developing an Integrated System of Support and Enforcement. 24(3):749-762.
8) Revised Code of Washington (RCW) 42.52.
9) Saks MJ. 1989. Prevalence and Impact of Ethical Problems in Forensic Science. J For Sci. 34(3):772-793.
10) Schroeder OC. 1984. Ethical and Moral Dilemmas Confronting Forensic Scientists. J For Sci. 29(4):966-986.
11) Starrs JE. 1971. The Ethical Obligations of the Forensic Scientist in the Criminal Justice System. J AOAC. 54(4):906-914.
12) Thompson WC. 1995. Subjective Interpretation, Laboratory Error and the Value of Forensic DNS Evidence: Three Case Studies. Genetica. 96:153-168.

The following documents can be located on the Portal FLSB Home Page under the Ethics area:

13) ASCLD/LAB Guiding Principles of Professional Responsibility for Crime Laboratories and Forensic Scientists.
14) Guidance on Gifts
15) Guidance on Use of State Resources
16) Use of State Resources

5.4 PRACTICAL EXERCISES

1) Review the Ethics Training for WSP and Ethics in Forensics PowerPoint presentations located on the Portal FLSB Home Page under the Ethics area. Discuss the presentations and scenarios with the trainer.
2) Review the Ethics Scenarios and Answers on the Portal FLSB Home Page under the Ethics area. Discuss these scenarios with the trainer.
3) Review the ASCLD/LAB: Guiding Principles. Discuss this information with the trainer.
4) RTI International provides free online forensic training. These courses are optional and may be recommended by your trainer.

6 COGNITIVE BIAS

6.1 OBJECTIVES

- Understand what cognitive bias is.
- Understand the importance and potential impact to the trainee's work and forensic science in general.
- Understand the various tactics that can be used to minimize the influence of cognitive bias.

6.2 TOPIC AREAS

Introduction

Cognitive bias can play a role in all aspects of investigations, from the evidence that is collected (or not collected) at the scene, what is submitted to the lab, what is chosen to be examined, how the exam is conducted, how the data is interpreted, what conclusions are reached, how they are reported, and to how they are presented in a court of law. It is critical as scientist to: 1) remain as objective and unbiased as possible from start to finish; 2) not dilute the science with task-irrelevant information; and 3) remain free of influence from the adversarial nature of our court system. While it may be impossible to shield the scientist from all external influences, there are some ways to minimize cognitive bias. Training and understanding is the first step. Just as we take great effort to protect the evidence from physical contamination, so we must take effort to minimize cognitive contamination.

Definitions

Cognitive Bias is a pattern of deviation in judgment, whereby inferences about other people and situations may be drawn in an illogical fashion.

There are several types of cognitive bias. Two forms that may have the most likely influence in forensic science are contextual and confirmation bias:

Contextual bias occurs when irrelevant contextual information about an event, or the way in which some information is presented, influences reasoning; when people are affected by information which has nothing to do with the actual decision or task at hand.[1]

Confirmation bias occurs when people interpret information, or look for new evidence, in a way that conforms to their pre-existing beliefs or assumptions. People more easily see and give more weight to information which is consistent with what they already believe, and are less likely to see and give less weight to information which is not consistent with what they already believe.[2] Confirmation bias is a tendency to search for, interpret, or recall information that confirms one's belief; seeing what you expect to see.

Task relevant information is information that should be considered when performing a particular task and depends, in part, on the propositions being asked to assess. Forensic scientists should draw conclusions about the propositions in question solely from the physical evidence that they are asked to evaluate (along with any task-relevant context), and not from any other evidence in the case. Conclusions should be based on methods that are accepted as valid within their categories of testing and that they are trained and qualified to use.

The 2009 National Academy of Sciences/National Resource Council Strengthening Forensic Science in the U.S. report indicated that forensic science experts are vulnerable to cognitive and contextual bias. In the view of the National Commission on Forensic Science:

1. Forensic scientists should rely solely on task-relevant information when performing forensic analyses;
2. The standards and guidelines for forensic practice being developed by the Organization of Scientific Area Committees should specify what types of information are task-relevant and task-irrelevant for common forensic tasks.
3. Forensic laboratories should take appropriate steps to avoid exposing analysts to task-irrelevant information through the use of context management procedures.

Cognitive bias can influence the collection, selection, perception and interpretation of evidence during the entire course of an investigation. When evaluations and data interpretation involve subjective elements, and/or when there are ambiguities in the underlying data, forensic scientists may be influenced by information that is irrelevant to their task. It is important that all staff understand the potential to be unknowingly and inadvertently influenced by information that is irrelevant to a task or examination. Task-irrelevant information can bias the work of forensic scientists even when they earnestly and honestly believe they are operating with utmost objectivity, honesty and professional commitment.

There are several sources and layers of external factors that impart task-irrelevant information to the scientist. These include organizational/cultural factors (adversarial legal system), base-rate expectations (what a scientist expects to see based on experience), case information (from the RFLE, detective, prosecutor), reference or comparison materials (comparison of known to unknown vs. comparison of unknown to known leading to backward or circular reasoning), and even the evidence itself.

From the customer's perspective, report wording, conclusions, and statistics can all influence how a reader interprets a laboratory report. Reports must be as unambiguous, objective and unbiased as the testimony they may provide.

In court, the trier of fact must accurately understand, interpret and infer forensic expert testimony. How conclusions are presented can be more important than what is presented. For example, the confidence with which an expert testifies, ambiguity of conclusions, use of statistics, and range of conclusions could all impact how a juror receives and interprets information. At least one study has shown that the adversarial nature of our court system can cause experts to be influenced by the side asking them to testify.[3] The research demonstrated that when the same evidence was presented to experts who understood they were working either for the prosecution or the defense, their forensic work and conclusions were influenced by the side they represented and were inclined to support the side they were on.

The risk of contextual bias is minimized by:

- Having a methodical approach with defined standards and guidelines built on principles that have been tested and validated;
- Insuring scientists are well trained, experienced and continuously meet acceptable standards of competence.

Some contextual information will often be necessary to understand and interpret physical evidence. But by limiting non-relevant task information, one can minimize the likelihood of being influenced by information that is not relevant to the physical evidence designated for examination.

There are several tactics for managing and reducing the possibility of cognitive bias and each functional area or category of testing may use one or more tactics depending on their work. Many of the quality control and assurance procedures we have in place serve to minimize the impact of not only cognitive bias, but also other potential sources of error. These include:

- technical and administrative review of all cases
- interlab technical review
- verifications of physical matches, and impressions identification
- proficiency testing
- corrective action procedures
- technical leads
- internal quality audits
- independent, external assessments

Other measures to minimize or manage cognitive bias are included in the suggested readings.

6.3 SUGGESTED READINGS

There are several articles on the FLSB Portal under the Cognitive Bias section that provide a wealth of information. Some are more pertinent to certain functional areas or categories of testing than others. Below is a list of suggested readings:

Cognitive Bias, PowerPoint presentation

Forensic Science Error Management, various links to NIST website

[1] Cognitive forensics: human cognition, contextual information and bias, Dror, I. and Stoel, R. 2014, in the Encyclopedia of Criminology and Criminal Justice, pp. 353-363, Springer

[2] Confirmation Bias: A Ubiquitous Phenomenon in Many Guises, Nickerson, R., 1998, Review of General Psychology, 2:2, 175-220

[3] Are forensic experts biased by the side that retained them?. Murrie D, Boccaccini M, Guarnera L, Rufino K. 2013, Psychol. Sci. 24, 1889–1897. (doi:10.1177/0956797613481812)

National Commission on Forensic Science: Ensuring That Forensic Analysis is Based Upon Task-Relevant Information

Cognitive Bias Effects Relevant to Forensic Science Examinations, Forensic Science Regulator Guidance, FSR-G-217, Issue 1 © Crown copyright 2015

Contextual bias and cross-contamination in the forensic sciences: the corrosive implications for investigations, plea bargains, trials and appeals, Edmond, G. et al., Law, Probability and Risk (2015) 14, 1–25

Unintentional Bias in Forensic Investigation, Sophie Stammers and Sarah Bunn, Houses of Parliament, Parliamentary Office of Science and Technology, POSTbrief No. 15, October 2015

6.4 STUDY QUESTIONS/PRACTICAL EXERCISES

1. Describe three ways that cognitive bias can be or is minimized in your casework.
2. Select at least one of the articles from the reading list and discuss with your trainer or section.

6.5 ASSESSMENT

Training in cognitive bias will be completed by all new employees. The material should also be reviewed by experienced staff training in this area to ensure knowledge is current.

No practical or written examination, or competency, is provided for this training section. The trainer will assess through discussion of the trainee's knowledge of the subject matter and document using the trainer's evaluation form.

7 LAW BASICS AND COURT TESTIMONY

7.1 OBJECTIVES

- Demonstrate a basic understanding of terms, legal decisions and issues relevant to the forensic scientist.
- Demonstrate a basic understanding of the judicial process and how cases are tried in various courts of law.
- Provide an overview of the legal system and its participants.
- Present a brief summary of criminal procedure.
- Understand the forensic significance of the Bill of Rights and other amendments to the US Constitution.
- Understand the importance of and how to prepare for testimony.
- Understand the demeanor and delivery of an expert witness testimony.
- Demonstrate how to effectively employ visual displays to aid in testimony.

7.2 TOPIC AREAS

1) The Legal System Overview
    a) The basis of law
    b) Law foundations
        i) The Constitution
        ii) Statutes
        iii) Administrative regulations
        iv) Judicial decisions
    c) Adversarial system
    d) Presumption of innocence
        i) Beyond a reasonable doubt
        ii) Preponderance of the evidence
    e) Rights of the accused
        i) Due process
        ii) Bill of Rights
    f) Civil versus criminal law
2) Dual Court System
    a) Jurisdiction
    b) Federal Courts
        i) US District Courts
        ii) US Court of Appeals
        iii) US Supreme Court
        iv) Specialized Courts
            (1) Military Justice
            (2) Tribal Courts
    c) State Courts
        i) Courts of Limited Jurisdiction: Lower Courts
            (1) District
            (2) City
            (3) Municipal
        ii) Courts of General Jurisdiction: Major Trial Courts
            (1) Superior
            (2) Criminal and civil cases
        iii) Intermediate Courts of Appeals
        iv) State Supreme Court
3) Participants in the Court System

a) Law Enforcement
    i) Security Staff
    ii) Sheriff's Deputies
    iii) Bailiff
b) Courts
    i) Legal
        (1) Prosecutor
        (2) Public defender
        (3) Private defense attorney
        (4) Judge
        (5) Law clerk
    ii) Court Support Staff
        (1) Court clerk
        (2) Court reporter
        (3) Court administrator
        (4) Translator
c) Corrections
    i) Probation officer
    ii) Pretrial services
    iii) Drug rehabilitation program
d) Public
    i) Defendant
    ii) Victim
    iii) Witness
    iv) Jurors
    v) Bail agent
    vi) Victim witness assistance program
    vii) Rape crisis center
    viii) Child advocate
    ix) Court watchers
    x) Media

4) Steps of Criminal Procedure
    a) Crime
    b) Arrest
    c) Initial appearance
        i) Accused is told of the charges, advised of their rights, bail is set and a date for the preliminary hearing is set.
        ii) Suspect may enter a plea.
    d) Charging
        i) Information
        ii) Complaint
        iii) Arrest warrant
    e) Preliminary hearing
        i) Hearing to determine if there is probable cause to proceed with the case.
    f) Grand jury
        i) Required in twenty-two states and federal courts
        ii) Indictment
    g) Arraignment
        i) The defendant is formally charged and must enter a plea.
        ii) Types of pleas
            (1) Guilty
                (a) Regular guilty plea
                (b) Nolo contendere (no contest)
                (c) Alford plea

(d) Conditional guilty plea
(2) Not guilty
(3) Insanity plea
h) Bail
i) Disclosing and suppressing evidence
j) Negotiated Justice
i) Plea agreements
(1) Charge bargaining
(2) Count bargaining
(3) Sentence bargaining
k) Trials
l) Sentencing
m) Appeals
5) Constitutional Rights of Forensic Significance
a) Fourth Amendment
b) Fifth Amendment
c) Sixth Amendment
d) Fourteenth Amendment
6) The Uniform Rules of Evidence
a) Rule 401
b) Rule 402
c) Rule 702
d) Rule 703
e) Rule 705
f) Rule 1002
7) Court Decisions of Forensic Significance
a) Jencks v. United States
b) Brady v. Maryland
c) Giglio v. United States
d) Frye v. United States
e) Daubert v. Merrell Dow Pharmaceuticals
f) General Electric Co. v. Joiner
g) Kuhmo Tire Co. v. Carmichael
h) Arizona v. Youngblood
i) Crawford v. Washington
j) Melendez-Diaz v. Massachusetts
k) Bullcoming v. New Mexico
l) State v. Nation
m) United States v. Plaza
n) Williams v. Illinois
o) Washington v. Manion
p) Washington v. Lui
8) Testimony
a) Preparation for testimony
i) pretrial discussions and/or meetings with attorneys
ii) review of case notes and report
iii) Curriculum Vitae (CV)
iv) notification of supervisor
b) Courtroom appearance and dress
i) professional appearance
ii) appropriate attire
iii) posture
c) Courtroom demeanor
i) exclusion from the courtroom

ii) outside courtroom considerations
iii) eye contact
iv) tone of voice
v) volume
vi) courtesy
vii) impartiality
viii) ethics

d) Presenting Testimony
i) taking the oath
ii) taking the witness stand
iii) qualifying as an expert
(1) voir dire

e) handling evidence on the stand
i) recognition
ii) safety
iii) gloves
iv) refrain from displaying evidence (“publishing”) to jury without prior permission

f) direct examination
i) communication skills
(1) verbal: avoid slang, professional jargon, profanity, unexplained abbreviations and acronyms
(2) nonverbal
(3) pace
ii) use of analogies
iii) credibility
(1) recognizing limits of knowledge or expertise
iv) admitting mistakes, limitations and problems
v) inability to remember
vi) objections
vii) cross-examination
(1) open-ended questions
(2) leading questions
(3) unclear questions
(4) compound questions
(5) hypothetical questions
(6) yes or no questions
(7) listening carefully
viii) re-direct
ix) re-cross-examination
x) leaving the witness stand
xi) being finally excused

g) Defense tactics
i) attacking credibility and qualifications
ii) attacking chain of custody
iii) attacking procedures
iv) attacking conclusions
v) weight of testimony
vi) proffering “authoritative” texts
vii) hostile demeanor

h) Use of visual displays and other presentation aids
i) prior discussion with attorney
ii) easily seen and understood
iii) photographs and glare
iv) digital images

v) use of colors on charts, graphs
vi) retention as court exhibit
vii) demonstrations

i) Testimony review
   i) required annually
   ii) performed by supervisor, another analyst or officer of the court
   iii) documented on testimony review form
   iv) evaluation criteria

j) Discovery
   i) WAC 446-10-050
   ii) WSP policies

7.3 SUGGESTED READINGS

1) Cleary EW. 1972. McCormick's Handbook of the Law of Evidence. 2nd ed.West Publishing Co.
2) Farley M. 1993. Legal Standards for the Admissibility of Novel Scientific Evidence. In: Saferstein, R. Editor. Forensic Science Handbook. Vol. 3. Englewood Cliffs (NJ): Prentice-Hall.
3) Kogan, J.D. 1978. On Being a Good Expert Witness in a Criminal Case", J For Sci. 23:190-200.
4) Matson JV. 2003. Effective Expert Witnessing, 4th ed. CRC Press.
5) Neubauer DW. Fradella HF. 2010. America's Courts and the Criminal Justice System. 10th Ed. Blemont (CA): Wadsworth. *or equivalent book.*
6) Sapir GJ. 2002. Legal Aspects of Forensic Science. In: Saferstein R. Editor. Forensic Science Handbook. Vol. 1. 2nd ed. Englewood Cliffs (NJ): Prentice-Hall.
7) Training Seminar: Ron Smith & Associates, Inc., "Courtroom Testimony Techniques "Success Instead of Survival".
8) The Uniform Rules of Evidence
9) Court opinions for the listed decisions

7.4 STUDY QUESTIONS

1) Briefly describe the difference between the Frye Standard and the Daubert Standard? Which standard is the law in Washington?
2) What is the Confrontation Clause of the Sixth Amendment? How does this impact forensic science? Which legal decisions are based on the Confrontation Clause?
3) What is "Relevant Evidence"? Where is relevant evidence legally defined?
4) What is a *subpoena duces tecum?* What should you do if you receive this type of subpoena?
5) You have spent months analyzing evidence in a case and on the eve of the trial you learn the evidence was excluded under the "fruit of the poisonous tree doctrine". What does this mean?
6) What is a bench trial? How does this differ from a jury trial?
7) It is estimated that less than ten percent of cases go to trial. What processes could contribute to so few of cases going to trial?
8) What is an Omnibus hearing? What is a 3.5-3.6 hearing?
9) Discuss appropriate court demeanor and dress.
10) What is the difference between a regular witness and an expert witness?
11) As you are delivering testimony, one of the attorneys exclaims, "Objection". What do you do?
12) How can you avoid technical jargon when testifying?
13) In a jury trial, whom should the witness address?
14) How would you answer in court when asked what is your error rate?
15) Define the following terms:
    a) Continuance
    b) Deposition
    c) Disclosure
    d) Discovery
    e) Omnibus hearing

f) Spoliation
g) Stipulation
h) Subpoena duces tecum
i) Voir dire

16) What does WAC 446-10-050 say regarding availability of public records? What is the difference between a disclosure request and a discovery request? How is each handled?
17) How would you address a question regarding why you didn't examine an item for fingerprints? For DNA?
18) Why is testimony reviewed annually? What criteria are included in the annual review?

7.5 PRACTICAL EXERCISES

1) Develop a Curriculum Vitae (CV).
2) Observe court testimony by other members of the laboratory staff.

8 BALANCES AND WEIGHING

8.1 OBJECTIVES

- To familiarize the trainee with the appropriate use of electronic balances.
- To familiarize the trainee with the common terms associated with weighing and electronic balances.
- To familiarize the trainee with balance quality assurance and techniques that will minimize error.

8.2 TOPIC AREAS

1) Basic concepts
2) Mass versus weight
   a) Scales or balances measure force (weight) caused by mass that is influenced by gravity.
3) Mechanical balance scales
   a) An unknown mass is balanced by a known counter mass. This direct mass to mass comparison is equally influenced by gravity and therefore calibration is not required when moving the scale.
4) Electronic scales
   a) Load cell transducers measure force that occurs when gravity acts on a mass. As gravity changes, it is necessary to calibrate the scale to accommodate for the change in gravity.
   b) When an object is placed on the load cell transducer in the electronic scale, the mechanical force is converted to an electric property. This electric property is amplified and processed to be displayed as the weight of the object.
5) International System of Units
   a) Kilogram
   b) Traceability
6) Types of electronic balances /Readability
   a) Microbalance
   b) Analytical balance
   c) Precision balance
7) Terms used in weighing
   a) Accuracy
   b) Adjustment
   c) Calibration
   d) Corner load
   e) Hysteresis
   f) Linearity
   g) Precision
   h) Readability
   i) Repeatability
   j) Sensitivity
8) Optimizing the performance of the balance

***The following are conditions that will improve the precision and reproducibility of weighing especially for analytical and micro-balances. Not all of the conditions can be achieved in each laboratory due to facilities/ environmental conditions or are necessary for weighing with precision balances.

   a) Location
      i) Balance placement
      ii) Temperature
      iii) Humidity
      iv) Light
      v) Air
   b) Balance operation
      i) Leaving the balance on at all times

ii) Leveling the balance
iii) Balance adjustment
iv) Reading
v) Weighing pan
vi) Weighing vessel
vii) Draft shield
viii) Care of the balance

8.3 SAFETY

As with all electronic equipment, caution should be taken to avoid exposing the balance to liquids.

8.4 SUGGESTED READING

1) Correct Use and Handling of Analytical and Microbalances. Sartorius. Available as a .pdf at www.sartonews.com
2) The Fundamentals of Weighing Technology: Terms, Methods of Measurement, Errors in Weighing. Sartorius, 1996. Available as a .pdf at www.sartonews.com.
3) Norden KE. 1993.Electronic weighing: Fundamentals and applications. Butterworth-Heinemann, Ltd. Oxford.
4) Proper Weighing with Laboratory Balances. Mettler Toledo, 2008. Available as a .pdf at www.mt.com.
5) Quantitative Chemical Analysis text of your choosing.

8.5 STUDY QUESTIONS

1) Define the following terms
   a) Accuracy
   b) Corner load
   c) Hysteresis
   d) Linearity
   e) Precision
   f) Readability
   g) Repeatability
   h) Sensitivity
2) How do calibration and adjustment differ in regards to electronic balances?
3) What is the difference between microbalances, analytical balances, and precision balances?
4) Discuss how the location in which a balance is placed can affect weighing precision and reproducibility.
5) Why should the balance be left on at all times? How long does it take to warm-up analytical and precision balances?
6) What factors should be considered in the selection and handling of a weighing vessel?
7) Which of the following physical influences can impact the weight determination of routine controlled substances cases? What can be done to minimize the physical influences that impact weighing items of controlled substances?
   a) Temperature
   b) Moisture gain/evaporation
   c) Electrostatics
   d) Magnetism
   e) Static buoyancy
   f) Gravitation
8) What is “traceability” in regards to the quality assurance of balances? What standard are balances traced to?

9) What is the difference between internal and external calibration? Should internal calibration be performed on our balances? Why or why not?
10) How would you describe to a jury the concept of a gram? What is the mathematical conversion between grams and ounces?

8.6 PRACTICAL EXERCISES

1) Check the performance of the balances in your laboratory following the quality assurance plan.
2) Weigh the following:
   a) Weighing paper – various sizes
   b) Weigh boats – various sizes
   c) Contents of a “Sweet ‘n Low” or “Equal” package.
   d) Ten marihuana seeds, if available.
   e) A piece of rolling paper, if available.

9 THIN LAYER CHROMATOGRAPHY

9.1 OBJECTIVES

- To familiarize the trainee with the theory and application of thin layer chromatography in drug analysis.
- To familiarize the trainee with the advantages and limitations of using thin layer chromatography in materials analysis.

9.2 TOPIC AREAS

1) Theory
   a) Thin-layer chromatography (TLC) is a method for separating chemical mixtures. Compounds are separated from each other based on differences in their interactions between a stationary phase and a mobile phase.
2) Materials
   a) Stationary phase
   b) Mobile phase
   c) Developing chamber
   d) Visualizing agents
3) TLC Separation Concepts
   a) Partitioning/adsorption
   b) Mobile phase selection
   c) Stationary phase selection
   d) Retention factor (Rf)
      i) Sample concentration effects
      ii) Mobile phase composition
      iii) Mobile phase/stationary phase equilibrium
      iv) Edge effects
      v) Stationary phase uniformity
      vi) Temperature
   e) Developing methods
      i) Closed chamber
      ii) Open chamber
   f) Visualization methods
      i) UV
      ii) Fluorescence
      iii) Color reagent sprays
   g) Preparative TLC
      i) Sample application
      ii) Development
      iii) Visualization
      iv) Sample collection
      v) Sample recovery
4) TLC Procedures
   a) Sample preparation
   b) Sample application
      i) Spot size
      ii) Spot location
   c) QA/QC
      i) Blanks
      ii) Standards
      iii) Documentation
   d) Safety

   i) Developing agents
   ii) Visualizing agents
   iii) Stationary phase
5) TLC Information Generated
   a) Number of components
      i) Visualized
      ii) Not visualized
   b) Rf values
   c) Color of visualized spots
   d) Value of information
      i) Category B test
      ii) Comparative
      iii) Indicative
      iv) Semi-quantitative
6) The following TLC systems and visualizing agents are examples that may be used and are not to be considered all inclusive or the only appropriate method for conducting the analysis of the example compounds. Ultraviolet light and reagents used for color tests may also be used as TLC location aids.

Barbiturates	Solvent -- chloroform:acetone (9:1) Visualized – potassium permanganate; mercuric nitrate, oversprayed with diphenylcarbazone
Cocaine	Solvent -- chloroform:methanol (4:1) Visualized -- acidified iodoplatinate
Diazepam	Solvent -- methanol:concentrated ammonia (100:1.5) Visualized -- acidified iodoplatinate
Ephedrine	Solvent -- chloroform:methanol (4:1) Visualized -- acidified iodoplatinate
Heroin	Solvent – diethyl ether:diethylamine (9:1) chloroform:methanol (4:1) Visualized -- acidified iodoplatinate
LSD	Solvent -- Acetone (or acetone:chloroform 1:4) Spot sample versus known LAMPA, LSD and mix. Plate can be irradiated with long wavelength UV and replaced in developing reservoir. Visualize with UV. Can be over sprayed with Erhlich's. Also can be visualized with acidified iodoplatinate.
Marihuana	Solvent – hexane:diethyl ether (4:1); toluene Visualized -- Fast Blue B (carcinogenic), or Fast Blue BB
Methamphetamine	Solvent -- chloroform:methanol (4:1) Visualized -- acidified iodoplatinate
Pseudoephedrine	Solvent -- chloroform:methanol (4:1) Visualized -- acidified iodoplatinate
Psilocyn/Psilocybin	Solvent -- methanol:concentrated ammonia (20:1) Visualized -- Fast Blue B, overspray with conc. HCl -- Erhlichs: Psilocyn, Psilocybin

Steroids	Solvent -- chloroform:acetone (8:2) Visualized -- sulfuric acid/ethanol reagent

7) Visualizing Agents

Mercuric chloride-Diphenylcarbazone	19mg in 200mL (50% acetone/water). Used for barbiturates.
Ceric sulfate	5g Ce(SO4)2 in 500mL water and 14 mL sulfuric acid. Used as an overspray to intensify the reaction with iodoplatinate.
Dragendorff	1.3g of bismuth subnitrate in 60Ml water with 15mL acetic acid. Add this to 12g KI in 30 mL water. Dilute with 100mL of water and 25mL acetic acid. General spray, good for diazepam, alkaloids, and nitrogenous compounds.
Ehrlich's	2g p-dimethylaminobenzaldehyde in 50mL 95% ethanol and 50mL concentrated HCl. Visualizes LSD, reacts with indole nucleus of alkaloids. Heat plate to intensify color.
Fast Blue B	Approx. 0.5% to 1% solution of Fast Blue B in water. Used for marihuana. Δ9-THC — red, cannabidiol — orange, cannabinol — purple
Fluorescamine	20mcg in 100mL acetone. Visualize amino acids, amines and amino sugars. Heat after spraying, check under long wavelength UV light.
Furfuraldehyde	Furfuraldehyde in ethanol, HCl. May heat plate after spraying. For non-aromatic carbamates; black spots.
HCl - 6N	Used to acidify plates (e.g., with Fast Blue B for psilocyn/psilocybin).
Iodoplatinate	1g chloroplatinic acid in 10mL conc. HCl, plus 20g of KI in 400mL water. Used for nitrogenous compounds.
Ninhydrin	Ninhydrin in various solvents. For amino acids, amines and amine sugars. Heat after spraying, view under long wave UV.
Potassium Permanganate	Potassium permanganate in water. Unsaturated hydrocarbons — yellow on purple.
Sulfuric Acid/Ethanol	Gradually add 10mL conc. sulfuric acid to 90mL of ethanol. Used for steroids.

9.3 SAFETY

1) UV radiation can be harmful to the eyes and care should be exercised to avoid direct exposure to UV radiation.
2) Visualizing reagent sprays may be hazardous.
3) Good chemical safety practices should be employed when working with reagents. TLC should be performed in a functional fume hood. When the TLC plate has been reviewed and observations recorded, the plate should be disposed of properly and should not be kept as part of the case record.

9.4 SUGGESTED READING

1) Gough TA. 1991. The Analysis of Drugs of Abuse: Separation Science Series. Chichester, England:Johan Wiley and Sons, p. 3-22.
2) Hamilton R. Hamilton S. 1978. Thin Layer Chromatography: Analytical Chemistry by Open Learning. London:Johan Wiley and Sons.
3) Moffat AC. Clarke's Isolation and Identification of Drugs. London:The Pharmaceutical press.
4) Saferstein R. 2011. Organic Analysis. In: Criminalistics - An Introduction to Forensic Science, 10th ed. Englewood Cliffs (NJ): Prentice-Hall.

9.5 STUDY QUESTIONS

1) Describe the safety hazards associated with TLC and how these hazards can be mitigated.
2) What materials are widely used as stationary phases and what applications are each best used for?
3) What "samples" should be spotted onto a TLC plate when analyzing case exhibits?
4) How are TLC plates documented in the case file?
5) What is retention factor and how can it be affected by the following:
   a) Sample concentration
   b) Mobile phase
   c) Mobile phase/stationary phase equilibrium
   d) Edge effects
   e) Stationary phase uniformity
   f) Temperature
6) Describe the technique of TLC as you would to a jury.

9.6 PRACTICAL EXERCISES

1) Separate a mixture of cocaine, lidocaine, nicotinamide and lactose. Compare to the individual standards.
2) Separate a mixture of psilocin, psilocybin and bufotenine.
3) Separate a mixture of cannabinoids using an extraction from marihuana. Develop with Fast Blue B.
4) Choose an analyte (e.g., cocaine) and place spots of increasing concentration onto a single TLC plate and observe overloading effects.
5) Demonstrate edge effects.
6) Experiment using different visualizing agents with different analytes. Determine the advantages and disadvantages.
7) Run plates consecutively using the same mobile phase to evaluate how often the mobile phase can be used without replacement.

10 GAS CHROMATOGRAPHY

10.1 OBJECTIVES

- To familiarize the trainee with the theory and application of gas chromatography (GC) in materials analysis.
- To familiarize the trainee with the GC instrumentation and software used in the laboratory.
- To have the trainee understand the advantages and disadvantages of gas chromatography.
- To have the trainee demonstrate familiarity with gas chromatography terminology.
- To have the trainee demonstrate how to properly interpret gas chromatographic data.

10.2 TOPIC AREAS

1. Theory
    a. GC involves the partitioning of gaseous solutes between an inert gas mobile phase and a stationary solid or liquid phase. It is an instrumental separation technique based on the difference in the distribution or partition coefficients of substances having appreciable volatility at temperatures below approximately 350 - 400ºC.
    b. History of chromatography
    c. Gas/liquid phase equilibrium
        i. distribution coefficient
2. Instrument Design
    a. General: oven, carrier gas, injection port, analytical column, detector, recorder
    b. Injection systems
        i. Syringe types/methods, septa sweep
        ii. Packed and capillary, on-column, split/splitless injections
        iii. Solvent effects
        iv. Background, maintenance/trouble-shooting strategies
    c. Types of columns
        i. Packed columns – filled with granular packing that is kept in place by gas-permeable plugs at both ends.
        ii. Capillary columns
            1. Wall-coated open tubular (WCOT) – have the liquid phase coated directly on the inside, relatively smooth wall of the column tubing.
            2. Porous-layer open-tubular (PLOT) – have a solid porous layer present on the tube wall but still maintain the unobstructed central gas-flow channel. This porous solid layer can either act as an adsorbent or a support which in turn is coated with a thin film of the liquid phase, or both. The solid layer can either be deposited on the inside tube wall or formed by chemical means from the wall.
            3. Support-coated open tubular (SCOT) – those PLOT columns where the solid layer consists of the particles of a solid support which were deposited on the inside tube wall.
        iii. Capillary columns (current)
            1. Advantages of modern capillary columns; cross linked and bonded liquid phases.
            2. Capillary column nomenclature
            3. Common stationary phases used in our labs
                a. HP/DB-1 nonpolar 100% Dimethylpolysiloxane
                b. HP/DB-5 nonpolar (5%-Phenyl)-methylpolysiloxane
                c. RTX/DB-200 midpolarity 35% Trifluoropropyl)-methylpolysiloxane
            4. Column construction

5. Liquid phases/selection/temperature limits
    a. column bleed
6. Solid supports
7. Adsorbents
8. Column diameters (narrow, wide megabore)
9. Film thickness and column capacity
10. Compatibility, practical operating tips
11. Installation of columns
    a. cutting columns
    b. gas flow adjustments
    c. column conditioning
    d. troubleshooting
12. Linear velocity through capillary column
13. Causes and prevention of column damage
    a. physical damage
    b. oxygen damage – most common reason for a column to fail
    c. thermal damage – lack of carrier gas to the column while heating is probably the most common cause of thermal damage
    d. chemical damage
        i. organic solvents and water – generally not damaging although phase stripping will occur over time
        ii. bases – inorganic bases are particularly damaging, while organic bases are not
        iii. acids – in general, mineral acids will damage stationary phases
    e. guard or pre-columns

3. Separation Concepts
    a. Retention time and retention volume
    b. Dead volume
    c. Adjusted retention time
    d. Flow rate and average linear velocity
    e. Partition coefficients
    f. Column efficiency
        i. Theoretical plates and HETP
        ii. van Deemter equation and plots
        iii. Choice of carrier gas and liquid phase
    g. Resolution
    h. Temperature effects
4. Optimization of Operating Conditions
    a. Temperature programming
    b. Isothermal programs
    c. Electronic pressure control
5. Kovats indices and McReynolds constants
6. Detection
    a. Flame ionization
        i. principle of operation
        ii. gases
        iii. column position
        iv. linear range and sensitivity
        v. common problems
    b. Thermal conductivity
    c. Electron capture
    d. Others (NPD, HEC, PID, AED, MSD, IR, AA)
    e. Strengths and weakness of each detector

7. Quality Assurance/Quality Control
    a. System check-out
    b. Documentation
    c. Log books
    d. Calibrations
    e. Blanks and reference materials (instrument libraries)
8. Instrument Maintenance
    a. Routine maintenance
        i. Changing septa
        ii. Changing injection port liners and O-rings
        iii. Carrier gas and detector gases
        iv. FID flame jet and collector cleaning
    b. Leak detection
    c. Calibration
    d. Oxygen traps and carrier gas considerations
    e. Column installation
    f. Cleaning injection ports and detectors
    g. Troubleshooting
        i. baseline disturbances
            1. spiking
            2. noise
            3. wander
            4. drift
            5. offset
        ii. ghost peaks
        iii. irregular peak shape or size
            1. reduced peak size
            2. tailing peaks
            3. fronting peaks
            4. rounded or flat-top peaks
            5. split peaks
        iv. retention time shifts
        v. loss of separation or resolution
        vi. rapid column deterioration

9. Special Techniques
    a. Derivatives (see Chemistry Principles in the Controlled Substances Training Manual)
    b. Pattern recognition in analyzing complex mixtures
    c. Quantitation (overview)

10.3 SAFETY

All systems should be turned off and cooled prior to performing maintenance. Refer to the GC technical procedures for specific safety information regarding the handling of compressed gases.

10.4 SUGGESTED READING

1. ASTM E 355 (Current version) - Standard Practice for Gas Chromatography Terms and Relationships
2. Barry EF, Grob RL. 2004. Modern Practice of Gas Chromatography. New York:John Wiley & Sons.
3. Fowlis IA. 1995. Gas Chromatography, Analytical Chemistry by Open Learning. 2nd ed. West Sussex (England):John Wiley & Sons.
4. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.

5. Poole CF, Schuette SA. 1984. Contemporary Practice of Chromatography. New York: Elsevier.
6. Regis Chemical Company. 1976. A User's Guide to Chromatography. Illinois: Innovative Products and Technical Support.
7. Rood D. 1999. A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatographic Systems, Systems. 3rd ed. New York:Wiley-VCH.
8. Stafford DT. 1988. Forensic Capillary Gas Chromatography. In: Saferstein R, editor. Forensic Science Handbook. Vol II. Englewood Cliffs (N J):Prentice Hall. p.38-67.
9. Stafford DT. 1992. Forensic Gas Chromatography. In: Tebbett I. Gas Chromatography in Forensic Science. West Sussex (England):Ellis Horwood.
10. Willard HH, Merritt LL Jr., Dean JA, Settle FA Jr. 1988. Instrumental Methods of Analysis. 7th ed. New York:Van Nostrand.
11. Instrument manufacturer manuals.

NOTE: Most any general text on chromatography may substitute for one or more of the above references. The material in this outline may be covered by a basic GC course sponsored by a local vendor.

10.5 STUDY QUESTIONS

1. What is gas chromatography?
2. What types of information are obtained using a GC-FID system?
3. Draw a schematic diagram for a GC and describe the purpose of each component.
4. Describe the differences between the solid support used in packed columns and that used in a capillary column GC system.
5. What general criteria should all stationary phases possess? How do they differ between packed and capillary systems?
6. What general criteria should all mobile phases possess?
7. Besides the stationary phase, what factors influence column selection for a given GC application?
8. What determines the appropriate column diameter for a given GC system? The appropriate length? Why are packed column lengths usually less than 3 meters?
9. Describe how the following concepts affect GC separation between components:
    a. Solubility
    b. Boiling point
    c. Intermolecular forces
10. How are packed columns or liners deactivated after installation? How does it work?
11. What is column bleed?
12. When and why are columns conditioned? Describe the process.
13. Define:
    a. Retention time (TR or tR),
    b. Relative retention time (RRT),
    c. Retention volume,
    d. Unretained retention time (tm)
    e. Phase ratio (β)
    f. Selectivity (α)
14. Define the following:
    a. Theoretical plate (n)?
    b. Effective theoretical plate (N)?
    c. Theoretical plate height /height equivalent to a theoretical plate (H or HETP)
    d. Height equivalent to an effective theoretical plate (H or HEETP)
    e. Average linear gas velocity (μ)
    f. What is a good value for the HETP? And why?
    g. How is the # of N related to column efficiency?
15. Define Resolution (R).
    a. What is chromatographic resolution a function of?
    b. Why is resolution not the best measure of column efficiency and column performance?

16. Diagram and explain the van Deemter plot. Why does the lab use helium or hydrogen as a carrier gas for its drug instruments?
17. What is the Kovats retention index (I)? What does it mean if I = 650?
18. What affect do the following have on retention time?
    a. Concentration
    b. Other compounds in the sample
    c. Free base/acid form vs. salt form
19. Discuss the sample introduction of gases and vapors, volatile liquids and solids into a GC.
20. What factors govern the amount of sample to be injected? How much sample/component can the average capillary column hold? What factors influence this?
21. What temperature should the injection port be under normal circumstances and why?
22. What type of septa are recommended for GC work and why?
23. What are the differences and purposes of split, splitless, pulsed split, pulsed splitless, on-column, and direct on-column injection?
24. What is an injection port liner? What is it made of? Why is it used? Describe the packing process including the materials used.
25. What is a "split ratio" and how is it calculated?
    a. What factors govern the use of a particular split ratio (100:1 vs. 50:1)?
26. Why is it necessary to regulate the carrier gas flow?
    a. How is this done?
    b. What factors influence the optimum flow rate for a given carrier gas?
    c. If the carrier gas is too fast or too slow how will it affect the peak shapes of your sample components?
27. What is "make-up" gas?
    a. How and why is it used?
    b. What determines which gas will be used as a make-up gas?
28. What are some of the common causes and remedies for the following GC system problems?
    a. No peaks
    b. Solvent peak only
    c. Baseline drift or unstable baseline
    d. Ghost peaks
    e. Tailing peaks
    f. Leading peaks
    g. Split peaks
    h. Retention time shift
29. Explain how derivatization is performed, including why it is used sometimes for analysis.
30. If two drug compounds were to co-elute on the GC, what could be done to resolve the peaks?
31. Explain as to a jury how a GC operates.

10.6 PRACTICAL EXERCISES

(Note: Not all laboratories have a GC/FID. Some of these exercises may be done with either a GC/FID, GC/MSD, or both. These exercises may be combined with exercises in the GC/MSD training module. Obtain instructor approval before manipulating any parameters.)

1. Familiarize yourself with instrument software.
2. Run a 50/50 mix of ephedrine and pseudoephedrine base on a GC/FID or GC/MS having a DB-1, DB-5 and/or DB-200 column. How many peaks do you see? Now rerun the sample with acetic anhydride (derivatizing agent). Under which conditions are the substances separated?
3. Run a primary, secondary and tertiary amine sample on a GC/FID or GC/MS. How do the spectral patterns differ? How can you account for the differences that are present in the spectra?
4. Run a mix of methamphetamine, phentermine and N, N-dimethyl amphetamine. Determine which spectrum belongs to its respective compound.

5. Choose a known dilution of a substance in solution and run on the GC/FID or GC/MS. Repeat the injection with successive dilutions. What are the sensitivity/levels of detection of your instrument? Is the GC more sensitive than the GC/MS when run splitless and at a given split ratio?

11 MASS SPECTROMETRY & PYROLYSIS

11.1 OBJECTIVES

- To familiarize the trainee with the theory and application of mass spectrometry in materials analysis.
- To familiarize the trainee with the MS instrumentation and software used in the laboratory.
- To have the trainee demonstrate a basic understanding of how a mass spectrometer operates.
- To have the trainee demonstrate how to properly interpret mass spectral data.
- To familiarize the trainee with the theory and application of pyrolysis.
- To familiarize the trainee with the pyrolysis instrumentation and software.
- To have the trainee demonstrate a basic understanding of how the pyrolysis instrument operates.

11.2 TOPIC AREAS

1. Theory
    a. Mass spectrometry
    b. Pyrolysis
2. Instrumentation
    a. Mass spectrometry
        i. Inlet systems
            1. Chromatographic column
            2. Direct inlet (DIP)
        ii. Ionization
            1. Electron impact (EI)
            2. Chemical ionization
            3. ICP
            4. Laser ablation
        iii. Mass analysis
            1. Magnetic sector
            2. Time of flight
            3. Quadrupole
            4. Ion trap
        iv. Detection
    b. Pyrolysis
        i. Furnace pyrolyzers
        ii. Inductively heated (Curie-point) filament pyrolyzers
        iii. Resistively heated filament pyrolyzers
            1. CDS 5150 autosampler
            2. Sample introduction
            3. Valve oven
            4. Transfer line and interface with GC column
3. Routine instrument maintenance
4. Calibration and tuning
5. Sample preparation
6. Interpretation of mass spectra & pyrograms
    a. Molecular formula
        i. Isotopic abundances
        ii. Rings and double bonds
        iii. Nitrogen rule
    b. Molecular ion

c. Fragmentation
    i. Ionization energies
    ii. Ion stability
    iii. Common fragments
    iv. Common fragmentation mechanisms
        1. Sigma-bond dissociation
        2. alpha cleavage
        3. Inductive cleavage
            a. Rearrangements
            b. Radical site
            c. Charge site

11.3 SAFETY

1. Components of the pyrolysis system are very hot. All systems should be turned off and cooled prior to performing maintenance. Refer to the GC/MS technical procedures for specific safety information regarding the handling of compressed gases.

2. Scientists should be aware that there are two sources of exhaust on the GC/MSD system: the foreline pump and the GC split vent. The foreline pump outputs gas removed from the vacuum manifold by the high vacuum pumps. The foreline pump exhaust will also contain traces of solvent and sample.

3. Caution should be exhibited when working with vacuum pumps. Waste oil should be treated as hazardous and should be handled and disposed of appropriately.

11.4 SUGGESTED READING

1. Allen AC et al. 1981. The Cocaine Diastereomers. J For Sci. 26(1):12-26.
2. Challinor JM. 2006. Chapter 8: Examination of Forensic Evidence. In Wampler TP, editor. Applied Pyrolysis Handbook, 2nd Edition. CRC Press: 175-200.
3. Maddock CJ, Ottley TW. 2006. Chapter 3: Pyrolysis Mass Spectrometry: Instrumentation, Techniques, and Applications. In : Wampler TP, editor. Applied Pyrolysis Handbook, 2nd Edition. CRC Press. p.47-64.
4. McLafferty FW, Turecek F. 1993 Interpretation of Mass Spectra. 4th ed. Sausalito (CA):University Science Books.
5. McLafferty FW, Venkataraghavan R. 1982. Mass Spectral Correlations. 2nd ed. Washington (DC):American Chemical Society.
6. Message GM. 1984. Practical Aspects of Gas Chromatography/Mass Spectrometry. New York:John Wiley & Sons, New York.
7. Mills T. Roberson JC. 2005. Instrumental Data for Drug Analysis, 3nd ed. New York:Elsevier.
8. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
9. Moldoveanu SC. 1998. Chapter 1. Introduction and nomenclature. In: Analytical Pyrolysis of Natural Organic Polymers. Elsevier. p. 3 - 7.
10. Moldoveanu SC. 1998. Chapter 2. The chemistry of the pyrolytic process. In: Analytical Pyrolysis of Natural Organic Polymers. Elsevier. p. 9 - 32.
11. Moldoveanu SC. 1998. Chapter 3. Physico-Chemical aspects of the pyrolytic process. In: Analytical Pyrolysis of Natural Organic Polymers. Elsevier. p. 33 - 69.
12. Moldoveanu SC. 1998. Chapter 5. Analytical techniques used with pyrolysis. In: Analytical Pyrolysis of Natural Organic Polymers. Elsevier. p. 97 - 199.
13. Saferstein R.. 1982. Forensic Applications of Mass Spectrometry. In Saferstein R. editor. Forensic Science Handbook. Vol I. Englewood Cliffs (N J):Prentice Hall 1982. p. 92-138.
14. Saferstein, R. 1982. Forensic Science Handbook, Vol. 1, , pp. 28-91.

15. Silverstein RM, Bassler GC. 1991. Spectrometric Identification of Organic Compounds. 5th ed, New York:John Wiley & Sons.
16. Watson J T. 1997. Introduction to Mass Spectrometry. 3rd ed. New York: Lippincott.
17. Wampler TP. Chapter 1: Analytical Pyrolysis: An Overview. In: Wampler TP, editor. Applied Pyrolysis Handbook, 2nd Edition. CRC Press. p.1-26.
18. Wampler TP. Chapter 2: Instrumentation and Analysis. In: Wampler TP, editor. Applied Pyrolysis Handbook, 2nd Edition. CRC Press. p.27-46.
19. Yinon J. 1987. Forensic Mass Spectrometry. Boca Raton:CRC Press, Inc.
20. Computer-based NIST library of organic compounds (NIST98.l or higher)
21. GC/MS instrument manuals.
22. Instrument manufacturer computer-based tutorials.

NOTE: Most any general text on chromatography may substitute for one or more of the above references. The material in this outline may be covered by a basic GCMS course sponsored by a local vendor.

11.5 STUDY QUESTIONS

Discuss the importance and relevance of questions below with your instructor.

1. What is mass spectrometry?
2. Describe the theory behind its use as an identification technique.
3. What types of information are obtained from a GC/MS?
4. Draw a schematic diagram of a GC/MS. What is the purpose of each component?
5. Define the following terms:
    a. Relative abundance
    b. Base peak
    c. Molecular ion
    d. Quasimolecular ion
    e. Parent/Precursor ion
    f. Daughter/Product ion
    g. Mass/charge ratio
    h. Mass spectrum
    i. Resolution
    j. Unit mass resolution
    k. Normalization
    l. PFTBA Normalization
    m. Cleavage
    n. AMU
    o. Isobaric
    p. Radical
    q. Doubly charged ion
    r. Calibration compound
    s. Torr
    t. Atmosphere
    u. Total Ion Current
6. What is a “metastable peak”? When and where does it occur?
7. What is the sensitivity of a GC/MS?
8. What is the difference between spectrometry and spectroscopy?
9. Describe any method considerations in using a MS detector instead of an FID detector for the GC.
10. Why can column bleed cause a problem in GC/MS and how is it corrected? Septum bleed?
11. What types of septa are required?
12. How can non-volatile compounds be introduced into a mass spectrometer?
13. What things must an interface between a GC and a MS accomplish?

14. What is the most common mode of ionization?
15. Diagram the EI source for the MS systems in your laboratory.
    a. Are the ions formed positive or negative?
16. Do they have an even or odd number of electrons?
17. What is the ionization efficiency of this technique?
18. What governs the relative abundance of the ions formed?
19. What governs the number and energy of the electrons emitted by the filaments?
20. From what are the filaments made?
21. What is an "ionization appearance potential" curve?
    a. What is the usual electron energy used in an EI source for complete ionization and why?
    b. What effect does variation in this energy have on ion abundance?
    c. If a molecule is ionized with energy just at its appearance potential, what information may be obtained?
22. What vacuum conditions are necessary in the ionization source and the analyzing regions of a MS and why?
    a. Describe how a rough pump works.
    b. Describe how a diffusion pump works.
    c. Describe how a turbomolecular pump works.
    d. Is it necessary that the vacuum remain constant?
23. What temperature conditions must be maintained in the ion source?
24. Describe how the ions are accelerated once they are formed.
25. Describe how a quadrupole mass analyzer works.
    a. What factors influence the practical limits of the quadrupole as a mass filter?
    b. What determines whether an ion will have a stable trajectory through the quadrupoles?
26. Describe how an electron multiplier works.
27. Why is the electron multiplier the detector of choice? What are the limiting factors as to how well an electron multiplier can detect incoming ions?
28. What reference spectra collections are available for your use?
    a. Do they consist of "normalized" data?
    b. Do they consist of DFTPP ion abundance calibrated data?
    c. Do they contain verified data?
    d. If not, are they still viable references for spectral comparisons?
29. Can optical isomers and diastereomers be differentiated via MS?
30. What is the nitrogen rule?
31. Describe how fragmentation patterns are influenced by:
    a. Branched carbon atoms
    b. Double bonds
    c. Rings
    d. Hetero-atoms
    e. Carbonyl groups
32. What requirements are necessary for an ion to be considered a molecular ion?
    a. What does increasing saturation and number of rings result in with respect to the abundance of a molecular ion?
    b. What effect does chain branching have?
33. In what types of compounds is a molecular ion peak frequently not detectable?
34. In what types of compounds are molecular ion peaks most likely to occur?
35. What do the peaks occurring at higher mass numbers than the molecular ion represent?
36. Describe the isotope pattern for Cl and Br.
37. What ions can be associated with the following m/e ratios?
    a. 43
    b. 58
    c. 77
    d. 91

38. Define the following terms and describe how these terms relate mass spectrometry to chromatography?
    a. scan rate
    b. scan cycle time
    c. reset time
    d. a/d conversion rate
    e. spectral tilting
    f. mass peak detect threshold
    g. GC peak detect threshold
39. What macros are used on the GC/MS in your laboratory and how do they work?
40. What is SIM and what is it used for?
41. Explain as to a jury how a mass spectrometer operates.
42. Explain the key advantages of resistively heated filament pyrolysis instruments over furnace and Curie-point instruments.
43. Explain the advantages and disadvantages of Curie-point pyrolysis instruments.
44. Explain how sample size and shape can affect reproducibility of pyrolysis analysis.
45. Discuss the importance of sample homogeneity and how analysts can deal with samples that are non-homogeneous.
46. Define pyrolysis as an analytical method.
47. Explain relative bond strengths and how bond strengths determine macromolecular degradation in pyrolysis.
48. Explain random scission, side group scission, and monomer reversion mechanisms.

11.6 PRACTICAL EXERCISES

(Note: Obtain instructor approval before manipulating any instrument parameters. These exercises may be combined with exercises from the GC/FID training module.)

1. Perform a standard spectra autotune on the GC/MS and describe what each value on the report represents. What types of parameter values may indicate a problem with the instrument?
2. Perform an autotune on the GC/MS and compare the results to the standard spectra autotune. Run the QA mix of drugs using both types of tunes and discuss the differences in spectra observed.
3. Obtain an unknown spectrum from your instructor. Using interpretive methods, give as much information about the unknown compound as possible.
4. Obtain mass spectra for groups of similar compounds (e.g., phenethylamines, homologous series of alcohols, etc) and compare data from each compound.
5. Derivatize certain compounds, including ephedrine and pseudoephedrine, and observe the resulting data. Compare to data from the underivatized compound.
6. Obtain pyrograms for several polymers, including polyethylene, polypropylene, polystyrene, polymethyl methacrylate, and nylon. Determine degradation mechanisms for each, and identify major degradation products.
7. Test reproducibility of pyrolyisis by running a polymer such as polypropylene numerous times over a period of several days. How much variation in retention times and peak height ratios is observed?

12 CAPILLARY ELECTROPHORESIS

12.1 OBJECTIVES

- To familiarize the trainee with the theory and application of capillary electrophoresis (CE) in materials analysis.
- To familiarize the trainee with the CE instrumentation and software used in the laboratory.
- To have the trainee understand the advantages and disadvantages of capillary electrophoresis.
- To have the trainee demonstrate how to properly interpret capillary electrophoresis data.
- To familiarize the trainee with quality control and maintenance associated with capillary electrophoresis.
- To familiarize the trainee with safety regarding the use and maintenance associated with capillary electrophoresis.

12.2 TOPIC AREAS

1. History
    a. 1800's
        i. Early 1800's - Faraday – Electrophoresis
        ii. 1886 – Lodge – H+ migration
    b. 1900's
        i. 1930 – Tiselius –moving boundary
        ii. 1967 – Hjerten –electrophoresis in tube
        iii. 1979 – Mikkers –CZE with Teflon tube
        iv. 1981 – Jorgenson –CZE with silica tube
2. Theory
    a. Basic electrophoretic principles
        i. Size and charge
        ii. Electrophoresis
        iii. Electroösmosis
            1. Electroösmosis factors
                a. Dielectric constant
                    i. Solvent (i.e. water, acetonitrile, etc.)
                b. Zeta potential
                    i. Effected by buffer counter ions
                    ii. pH of buffer
                    iii. Concentration of buffer
                c. Viscosity
                    i. Concentration dependant
                    ii. Temperature dependant
                d. Other interactions
                    i. Analyte Velocity
                    ii. Migration time
                    iii. Column length
                        1. Equations use total column length = L
                        2. Migration is for analyte to travel L.
        iv. Equations of merit
            1. Elecrophoretic mobility

$$\mu_{ep} = \frac{q}{6\pi\eta r}$$

where q = ion charge

η = solution viscosity
r = ion radius

2. Electroömostic mobility

$$\mu_{eo} = \frac{\varepsilon\zeta}{4\pi\eta}$$

where ε = dielectric constant
ζ = zeta potential
η = solution viscosity

3. Analyte velocity
v = (μep + μoe) V/L
where v = analyte velocity
V = applied voltage
L = length of column

4. Migration time
Tm = L/v
where Tm = migration time
L = column length
v = analyte velocity

b. Flow profile
   i. Efficiency
      1. Inversely proportional to peak width
      2. Effected by:
         a. Eddy diffusion – minimal in CE
         b. Longitudinal diffusion – biggest factor
         c. Mass transfer – minimal in normal CE
            i. Stationary phase
            ii. Mobile phase
      3. $$N = \frac{\mu El}{2D}$$
      where μ = mobility
      E = electric field
      l = effective length
      D = diffusion constant
      4. Efficiency comparison

	N / meter	N / typical column
Packed GC	2,500	15,000 / 6 meter
Capillary GC	4,000	120,000 / 30 meter
HPLC	60,000	15,000 / 0.25 meter
CE – normal	1,000,000	6-800,000 / 0.8 meter
CE – gel filled	5,000,000	2,500,000 / 0.5 meter

      5. Resolution
         a. Improves proportional to efficiency
         b. As resolution increases, peak capacity increase
         c. $$R = \tfrac{1}{4}\sqrt{N}\,\frac{\Delta\mu}{\overline{\mu}}$$

         where Δμ = μ2 - μ1

$$\overline{\mu} = \frac{\mu_2 + \mu_1}{2}$$

c. Quanititation
   i. Migration dependant

ii. Peak areas
iii. Internal standards
iv. Calibration curves

d. What does it all mean?
i. Liquid phase separation method
ii. Electroösmotic pump
1. no laminar flow
2. flat flow profile
iii. Size and charge separations
iv. High efficiency – large peak capacity

3. Instrumentation
a. Power supply
i. Typically supply 0-30 kV
ii. Special applications >30 kV
b. Polarity is reversible
c. Modes of operation
i. constant voltage
ii. constant current
iii. constant power
d. Safety cabinet
i. Protects from high voltage
ii. Protects from detector radiation
iii. Allows for temperature control
1. capillary
a. Liquid
b. Heat block
c. Peltier
d. Forced air
2. samples
a. Liquid
b. Forced air
3. buffers
a. Liquid
b. Forced air
iv. Automation of injection
e. Buffer / Sample reservoir
i. Same for both sample and buffer
ii. Different for both sample and buffer
iii. May be thermostated
iv. Glass or plastic
v. Various sizes
vi. Single or multiple
vii. Manual or automated
f. Buffers
i. Aqueous based
1. Conductive
2. May include small amounts of organic modifier
3. Detection may dictate composition
ii. Non-aqueous based
1. conductive
2. e.g. Dimethylformamide based
g. Capillary
i. Types
1. Open tubular

a. Fused silica
    i. Uncoated inside
    ii. Coated inside
    iii. Various outside coatings
b. Teflon

2. Packed

ii. Sizes
1. 25 – 150 microns i.d.
2. <10 cm to about 1 meter long

h. Injection
i. Hydrodynamic
1. pressure
2. vacuum
3. siphoning
4. Advantages/Disadvantages
a. Reproducible
b. No sample bias
c. Introduces laminar flow for sample zone

$$Volume = \frac{\Delta P \cdot d^4 \pi t}{128 \eta L}$$

where ΔP = pressure differences across capillary [Pa]
d = capillary inside diameter [m]
t = time [s]
π = buffer viscosity [Pa·s]
L = total capillary length [m]

For siphoning ΔP = 2.8x108·Δh, where Δh is the height differential

ii. Electrokinetic
1. Voltage
a. Normal
b. Stacking
2. Advantages/disadvantages
a. Reproducible
b. Injection bias for charged analytes
c. No laminar flow

$$Quantity = \frac{vC \cdot r^2 \pi t}{L}$$

where v = analyte velocity
C = analyte concentration
r = capillary inside radius
t = time
L = total capillary length

i. Detection
i. UV
1. Direct
2. Indirect
3. Diode-array, fast scanning, variable, fixed
ii. Fluorescence
1. Direct
2. Indirect
3. Laser-induced, non-laser
iii. Amperometry

iv. Conductivity
v. Mass Spectrometry
vi. Others
1. Radioactivity
2. Refractive index
3. Raman
4. Thermal Lens
5. NMR

j. Data Collection
i. Instrument control
1. onboard
2. microprocessor
ii. Data collection
1. large complex files
iii. Data handing
1. calibrations
2. normalizations
iv. Data output
1. electronic
2. hardcopy

4. Techniques
a. Methods
i. Anion analysis
1. Uses a dynamic coating method and a commercially available buffer kit.
2. This method allows for separation of a wide variety of inorganic anions and organic acids.
3. Detection is achieved by indirect UV detection.
4. See reference 17 listed below.
ii. Cation analysis
1. Uses a commercially available buffer kit.
2. This method is a free zone electrophoresis method that uses indirect UV detection.
3. See reference 33 listed below.
iii. Organic species analysis
1. Adapted from reference 29 listed below.
2. Micellar electrokinetic separation method that allows for the separation of neutral species based on partitioning of analytes with a micellar pseudophase.
3. This method uses direct UV detection.
iv. General drug screen
1. This method is similar to the organic species method and is based on the procedures outlined in reference 40 listed below.
2. This is a modified micellar electrokinetic method that includes a small fraction of an organic solvent in the buffer to broaden the dynamic elution range for organic species.
v. Other analysis methods
1. Analysis of other species (such as chiral species) can be accomplished by following numerous published procedures.
2. Methods for non-aqueous CE may be used for analytes that do not readily dissolve in polar solvents.
b. Sample preparation
i. Dissolving sample in appropriate solvent.
ii. CE grade water is most appropriate
iii. A polar solvent used for species that are not water soluble

iv. The best results are obtained when the ionic strength of the sample is 100 fold lower than the ionic strength of the run buffer.
    1. Typical analyte concentrations are in the millimolar range.
v. Method may require the use of an internal standard.

c. Instrument maintenance
    i. Maintenance should follow the manufacturer's recommended procedures.
    ii. Maintenance tasks include:
        1. changing the capillary
        2. cutting and preparing new capillaries
        3. detector lamp replacement
        4. detector calibration
        5. electrode cleaning and replacement
        6. vacuum system maintenance
        7. buffer preparation

d. Case approach
    i. Selection of the appropriate analysis method is dependent upon the form of the sample and the types of analytes that may be suspected.
    ii. In general the analysis is conducted by running a blank, the sample, and then the sample plus the appropriate standard(s).
    iii. Alternatively the sample can be run with the appropriate internal standard(s).

12.3 SAFETY

A high voltage power supply is required to drive the separation in the CE. A safety interlock is in place to shield the user from the high voltage. This safety mechanism should not be tampered with.
Exposure to high powered UV can cause blindness and thermal burns. The interior top cover in the detector chamber should not be opened while the UV lamp is turned on.

12.4 SUGGESTED READING

1. Required reading
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k. Terabe S, et al. 1984. Electrokinetic separations with micellar solutions and open-tubular capillaries. Anal. Chem. 56:111-113.
l. Weinberger R, Lurie IS. 1991. Micellar Electrokinetic Capillary Chromatography of Illicit Drug Substances. Anal. Chem. 63(10):823-826.

2. Additional reading
   a. Bruno AE. et al, 1991. On-column laser-based refractive index detector for capillary electrophoresis. Anal. Chem. 63(23):2689-2697.
   b. Burger, F. et al, 2005. Forensic Analysis of Condom and Personal Lubricants by Capillary Electrophoresis. Talanta, 67(2):368-376.
   c. Camilleri, P. 1998Capillary Electrophoresis Theory and Practice. 2nd Ed. CRC Press.
   d. Curcuruto, O., et al, 1995. Capillary Zone Electrophoresis/Electrospray Ionization Mass Spectrometry for the Characterization of Drugs of Forensic Interest. Rapid Commun. Mass Spectrom. 9:1487-1491.
   e. Fujiwara S, Honda S. 1987. Determination of ingredients of antipyretic analgesic preparations by micellar electrokinetic capillary chromatography. Anal. Chem. 59(23):2773-2776.
   f. Flurer CL, Wolnik, KA. 1994. Chemical Profiling of Pharmaceuticals by Capillary Electrophoresis in the Determination of Drug Origin. J. Chromatography A. 674:153-163.
   g. Gassman E. et al, 1985. Electrokinetic Separation of Chiral Compounds. Science. 230:813-814.
   h. Guzman N. 1993. Capillary Electrophoresis Technology. CRC Press, Inc.
   i. Hjerten S. 1967. Chromatography Reviews. 9:122-219.
   j. Issaq HJ. et al. 1990. Factors that influence mobility, resolution, and selectivity in capillary zone electrophoresis. I, Sodium phosphate vs potassium phosphate. J. of Liquid Chromatography. 13:1247-1259.
   k. Jorgenson J. 1987. ACS Symposium Series. 335:182-188.
   l. Knox JH, Grant IH. 1991. Electrochromatography in Packed Tubes Using 1.5 to 50 µm Silica Gels and ODS Bonded Silica Gels. Chromatographia. 32(7/8):317-328.
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   n. Lee TT, Yeung ED. 1991. Facilitating data transfer and improving precision in capillary zone electrophoresis with migration indexes. Anal. Chem. 63(24):2842-2848.
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   s. Lurie IS, et al. 1994. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC. J. For. Sci. 39:74-85.
   t. Lurie IS. 1992. Micellar Electrokinetic Capillary Chromatography of the Enantiomers of Amphetamine, Methamphetamine and Their Hydroxyphenethylamine Precursors. J. Chromatogr. 605:269-275.
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   v. Pentoney, et al. 1989. On-Line Radioisotope Detection for Capillary Electrophoresis. Anal. Chem. 61: 1642-1647.
   w. Pumera M. 2006. Analysis of Explosives Via Microchip Electrophoresis and Conventional Capillary Electrophoresis: A review. Electrophoresis. 27: 244-256.
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z. Tagliaro F, et al. 1996. Capillary Electrophoresis: Principles and Applications in Illicit Drug Analysis. Forensic Sci. Int. 77:211-229.
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dd. Xu X, et al. 2001. Analysis of Single Textile Fibers by Sample-Induced Isotachophoresis – Micellar Electrokinetic Capillary Chromatography," Sci. Justice. 41(2):93-105.
ee. Yu M, Dovichi NJ. 1989. Attomole amino acid analysis by capillary zone ... with thermo-optical absorbance detection. Anal. Chem. 61:37–40.

12.5 STUDY QUESTIONS

1. List the main components of a capillary electrophoresis system and briefly describe their function.
2. What are the two main injection methods for CE? Briefly describe how each method works.
3. What is Joule heating? What limitations does it place on CE?
4. Why is the efficiency of CE separations so much greater than HPLC?
5. How is it possible to use much higher separation voltages in CE than in traditional electrophoresis?
6. What is capillary zone electrophoresis?
7. What is micellar electrokinetic capillary chromatography?
8. Which analytical capabilities make CE particularly useful for controlled substances?

12.6 PRACTICAL EXERCISES

1. Spend time with an experienced CE user learning basic operation of the CE unit in your lab and discussing specific techniques and theories.
2. Install a new capillary and prepare the instrument to run a method.
3. Obtain three cation unknowns and use the cation method to identify the unknowns.
4. Obtain three anion unknowns and use the anion method to identify the unknowns.
5. Obtain three organic unknowns and use the micellar method to identify the unknowns.
6. Obtain three drug unknowns and use the drug screen method to identify the unknowns.

13 FOURIER TRANSFORM INFRARED SPECTROSCOPY

13.1 OBJECTIVES

- To familiarize the trainee with the theory and application of FT-IR spectroscopy in materials analysis.
- To familiarize the trainee with the FT-IR instrumentation and software used in the laboratory.
- To have the trainee demonstrate a basic understanding of how a FT-IR operates.
- To have the trainee demonstrate how to properly interpret FT-IR spectra.

13.2 TOPIC AREAS

1. Theory
    a. Classical model of vibrational motion
        i. Harmonic oscillator (one mass on a spring)
            1. Force constant
            2. Potential energy function
            3. Frequency of vibration
        ii. Two masses joined by a spring
        iii. Three or more masses
    b. Quantum mechanical model.
        i. Vibrational energy levels for a harmonic oscillator
        ii. Diatomic molecules
            1. Homonuclear
            2. Heteronuclear
    c. Selection rules for infrared / Raman absorption
        i. Allowed energy level changes
        ii. Change in dipole moment
        iii. Deviations from selection rules
    d. Polyatomic molecules
        i. CO2
        ii. H2O
    e. Overtone, combination, and difference bands
2. Derivation of Beers' Law
3. The infrared region of electromagnetic spectrum
4. Instrumentation
    a. Dispersive IR
        i. Instrument design (overview)
        ii. Limitations
    b. Fourier Transform IR
        i. Principles of operation
            1. Michelson interferometer
            2. Interferogram
            3. Fourier transform
        ii. Detectors
            1. DTGS
            2. MCT
        iii. Signal to noise ratio
            1. Resolution
            2. Acquisition time
    c. Routine maintenance and calibrations

5. Sampling

   a. DRIFTS
   b. Diamond anvil cell
   c. Salt plates
   d. Salt pellets
   e. IR microscope
   f. Vapor cells
   g. ATR
6. Operating parameters
   a. Spectral resolution
   b. Acquisition time
   c. Gain
   d. Apodization
   e. Zero filling
7. Analysis of drugs using IR
   a. Advantages and disadvantages
   b. Specificity of IR for identification
   c. Choosing the correct sample holder
   d. Interpretation
8. Hydrocarbons
   a. Characteristic functional group frequencies
   b. Inorganics
   c. Comparing to known standard
   d. Handling mixtures
   e. Identifying specific frequencies in a spectrum
   f. Detectors
      i. Stretching Frequencies
      ii. Bending Frequencies
      iii. Scissoring Frequencies
      iv. Prominent Frequencies

13.3 SAFETY

Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar on instruments equipped with an MCT detector. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves should be used when handling liquid nitrogen.

13.4 SUGGESTED READING

1. Cooper JW. 1980. Spectroscopic Techniques for Organic Chemists. New York:John Wiley & Sons. p. 22-52.
2. Suzuki EM. 2009. Forensic Applications of Infrared Spectroscopy. In: Saferstein R. Forensic Science Handbook. Vol 3. 2nd ed. p. 71-195.
3. Suzuki EM. Infrared Spectroscopy. training guide.
4. Willard HH, Merritt LL Jr., Dean JA, Settle FA Jr. 1988. Instrumental Methods of Analysis. 7th ed. New York:Van Nostrand.
5. Any instrumental analysis book, which discusses infrared spectroscopy.
6. Any organic chemistry book, which discusses infrared spectroscopy.

13.5 STUDY QUESTIONS

1. What is Infrared Spectroscopy?
2. Describe the theory behind its use as an identification technique.
   a. What wavelengths in the electromagnetic spectrum are used in FT-IR?
   b. Describe the vibrations in a molecule from an FT-IR.

3. Draw a schematic diagram of a FT-IR. What is the purpose of each component?
4. How is this design different from a dispersive IR?
    a. What items can be used as dispersive elements?
5. How does a Michelson interferometer work?
6. What is a Fourier transform? What does it do?
7. Discuss the following sampling techniques, their drawbacks and benefits:
    a. Diffuse reflectance
    b. Diamond anvil cell
    c. Infrared microscope
    d. Gas chromatography
    e. Attenuated total reflectance
    f. Vapor Cell
8. What differences can be observed in spectra from inorganic versus organic samples?
9. Discuss the limitations of IR.
    a. Why must we take a background spectrum?
    b. What happens if the sample is not pure?
    c. What sort of samples give little or no IR data?
    d. What sort of different chemicals produce very similar IR's?
    e. When should a H_2O/CO_2 algorithm and/or purging be used?

13.6 PRACTICAL EXERCISES

(Note: Obtain instructor approval before manipulating any instrument parameters.)

1. Perform a standard calibration of the FT-IR. Why do we do this? What is a failure and what does it mean?
2. Open up the FT-IR and identify all major components.
3. Analyze methamphetamine, pseudoephedrine and ephedrine on the FT-IR; how are they different from each other? Are there substances that this technique can differentiate that GC/MS cannot? Why?
4. Analyze and compare the spectra of cocaine, heroin and methamphetamine, using each of the available sampling techniques. How do the sampling techniques change the spectra? Are these differences significant?
5. Analyze a set of inorganic materials given to you by your instructor. Why do some chemicals have an infrared spectrum and others do not?
6. Analyze ethanol, chloroform, pentane, petroleum ether, methanol and acetone using a vapor cell. If you have an ATR analyze them with that as well. How do the spectra differ between the two sample holders?
7. You will be given an unknown spectrum by your instructor, attempt to identify the substance.
8. Analyze a series of unknowns given to you by your instructor.

14 GAS CHROMATOGRAPHY/FOURIER TRANSFORM INFRARED SPECTROSCOPY

14.1 OBJECTIVES

• To familiarize the trainee with the theory and application of GC/FT-IR spectroscopy in materials analysis.
• To familiarize the trainee with the GC/FT-IR instrumentation and software used in the laboratory.
• To have the trainee demonstrate a basic understanding of how a GC/FT-IR instrument operates.
• To have the trainee demonstrate how to properly interpret infrared spectra, and in particular, to be able to explain how and why such spectra differ from those obtained for condensed phase samples and high resolution vapor spectra of small molecules.

14.2 TOPIC AREAS

1. Theory
    a. Review of GC theory (Section 9.2)
    b. Review of infrared spectroscopy theory (Section 12.2)
    c. Concepts specific to GC/FT-IR analyses: Dependence of infrared spectra on the phase of the analyte and characteristics of vapor phase infrared absorptions
        i. Characteristics of infrared absorptions of solids
            1. Crystalline solids
                (a) Narrow bands, often with fine structures, lattice splittings.
                (b) Causes: Molecules in a crystal lattice cannot rotate, hence narrow bands; lattice splittings often result from the presence of two or more molecules in the crystal unit cell (the smallest configuration of molecules that is repeated throughout the crystal lattice). A particular vibration of the molecule may differ for two or more molecules that occupy different positions in this unit cell.
            2. Non-crystalline solids (Glasses)
                (a) Broad bands lacking fine structures (these may sharpen if the sample anneals (crystallizes) from the heat of the infrared analysis beam).
                (a) (b) Causes: Random orientations of molecules produce differences in the frequencies of specific absorptions, as these depend on interactions with neighboring molecules.
                (c) Spectra of non-crystalline glasses are very similar to spectra of the same material analyzed as a liquid.
        ii. Characteristics of infrared absorptions of liquids
            1. Broad bands lacking fine structures and similar to those of non-crystalline solids.
            2. Causes: Random orientations of molecules (that are constantly changing).
        iii. Characteristics of infrared absorptions of vapors
            1. These absorptions represent a series of vibrational rotational transitions. Each of these transitions produces a very narrow line.
            2. Molecular rotational energies, like vibrational and electronic energies, are quantized.
            3. The spacings between adjacent rotational energy levels are much smaller than those between adjacent vibrational energy levels. They are typically fractions of a wavenumber, although in a few cases, these may be several wavenumbers, or 10 wavenumbers or greater.
            4. The dimensions of the spacings depend on the size of the molecule:
                (a) Size in this case is *not* molecule weight.
                (b) The spacings depend on the moment of inertia of the molecule, which represent the combined effects of atomic masses and their distances away from the center of mass of the molecule.
                (c) Except for some very symmetric molecules (such as CO_2, methane, acetylene, CO HCl, etc.), there is no simple formula that expresses the spacings between adjacent

rotational levels. In all cases, however, this spacing decreases as the moment of inertia of the molecule increases.

(d) For molecules having small moments of inertia, individual vibrational rotational transitions can be resolved using high spectral resolutions (0.5 or 0.125 wavenumbers, for example). The difference between adjacent vibrational rotational lines for CH_3OH and CO_2 is approximately 2 wavenumbers, whereas it is approximately 20 wavenumbers for HCl.

(e) The greater the spacings between the adjacent vibrational-rotational lines, the greater is the breadth of the entire vibrational band. This is the reason the vapor bands of H_2O, NH_3, HCl and some other small molecules are quite broad compared to those observed in GC/FT-IR spectra of most molecules.

5. The overall contour of vibrational-rotational transitions depends on which rotational transitions are allowed. This in turn depends on the symmetry of the vibrational mode involved.

(a) The details of the selection rules that govern these transitions are beyond the scope of this discussion, but analysts should note the differences in the vibrational rotational lines and band contours for the two absorptions of CO_2 vapor, that is, the antisymmetric C—O stretch centered at 2340 wavenumbers and the out-of-plane bending mode centered at 667 wavenumbers. At 4 wavenumber resolution, the former has two distinct bands (known as branches) whereas the bending mode has two branches and a sharp spike between them (the individual vibrational-rotational lines of the branches are observed using 0.5 wavenumber resolution).

(b) Although individual vibrational rotational lines may not be resolved for some moderate sized molecules, similar contours are often observed (for example, in the vapor spectra of acetone and ethanol obtained at 0.5 wavenumber resolution).

(c) The molecules that are normally analyzed using GC/FT-IR are larger in size and the spacings between adjacent vibrational-rotational lines are so small (0.01 wavenumber or less) that even the branches cannot be resolved, much less the individual vibrational-rotational lines. Consequently, only a single broad band lacking structures is normally observed.

2. Instrumentation and Data

a. Differences from GC analyses used for GC-FID and GC/MS instruments

i. Wide bore capillary columns are used having thicker stationary phases.

ii. Splitless injections are used and broad assymetric (“bearding”) Gram Schmidt chromatographic peaks usually result.

iii. Chromatographic separation efficiency is less of an issue than with GC/MS, as infrared spectra of most isomers are distinguishable.

b. Differences from FT-IR bench accessories

i. Narrowband MCT detectors are used because of their increased sensitivity and faster scan rates.

ii. The spectral range of the narrowband MCT is less than that for most bench accessories, with a low frequency cut-off region near 700 wavenumbers.

iii. Because signal-averaging is limited in a GC/FT-IR analysis and the concentrations of analytes passing through the light pipe are relatively low, higher noise levels are typically observed for GC/FT-IR infrared spectra compared to those obtained for bench instruments.

iv. A spectral resolution of 8 wavenumbers is used in order for the interferometer to scan faster. However, this has little or no effect on the infrared spectra that result, as the absorptions of most vapor phase samples are quite broad.

3. Applications

GC/MS and GC/FT-IR are complementary techniques. However, because of the sensitivity differences between GC/MS and GC/FT-IR analyses, when dilute samples requiring splitless GC/MS

injections are needed for identification, it is usually not possible to obtain GC/FT-IR infrared data having sufficient signal to noise ratios for confirmation using these same sample vials.

14.3 SAFETY

Appropriate safety precautions should be employed when refilling the liquid nitrogen Dewar.

14.4 SUGGESTED READING

1. White, R. 1990. Chromatogrpahy/Fourier Transform Infrared Spectroscopy and Its Applications. New York: Marcel Dekker, Inc.
2. Kempfert, K. 1988. Forensic Drug Analysis by GC/FT-IR. Applied Spectroscopy, 42. P. 845-849.
3. Griffiths PR, de Haseth JA. 2007. Fourier Transform Infrared Spectroscopy, 2nd ed., Section V. GC-IR. NY: Wiley InterScience, p. 268-282.

14.5 STUDY QUESTIONS

1. In what respects does the GC analysis used for GC/FT-IR differ from that used for GC-FID and GC/MS?
2. Why is this GC analysis different?
3. Why is chromatographic separation efficiency less of a concern for a GC/FT-IR analysis compared to a GC/MS analysis?
4. Describe the optical configuration of a GC/FT-IR instrument.
5. Why is a narrowband MCT detector, with its more limited spectral range, used with a GC/FT-IR instrument?
6. Why is a spectral resolution of 8 wavenumbers, instead of 4 wavenumbers (which is used for most analyses on FT-IR spectrometer bench accessories) used for a GC/FT-IR analysis?
7. What effect does this lower spectral resolution have on the resulting infrared data?
8. How does a typical GC/FT-IR infrared spectrum differ from the spectrum of the same analyte analyzed as a crystalline solid?
9. What causes this difference?
10. Why is a higher resolution (0.5 wavenumber) used for the analysis of ammonia or HCl vapors (such as may be encountered in a clandestine laboratory analysis or in an assault case)?
11. Considering your answer to Question 10, why don't we see similar results when acetone vapor is analyzed under the same resolution?

14.6 PRACTICAL EXERCISES

(Note: Obtain instructor approval before manipulating any instrument parameters.)

1. Familiarize yourself with the three software programs used to collect GC/FT-IR data.
2. Run the GC/FT-IR standard drug mix (phentermine, methamphetamine, cocaine, diazepam, and zolpidem) and analyze the data as you would a case sample (that is, print out the Gram Schmidt chromatogram labeled with retention times, and for each component, the minuend and subtrahend spectra if a background subtraction is needed and the final spectrum).
3. Run a sample of methamphetamine base in methylene chloride on the GC/FT-IR instrument. Compare the infrared spectrum that you obtain to that of methamphetamine base oil and to that of methamphetamine HCl salt both run in transmission on a FT-IR spectrometer bench accessory.
4. Using existing spectral libraries, compare the mass spectra of methamphetamine and phentermine, then compare their infrared spectra obtained using GC/FT-IR.
5. Do this same exercise for pseudoephedrine and ephedrine.
6. Using existing spectral libraries, compare the spectra of ammonia and HCl vapors run at 0.5 wavenumber resolution to that of acetone run at this same resolution. Explain the differences.
7. Why are the band contours for the ammonia and HCl vapor absorptions broader than those of acetone?

8. A number of infrared spectra of common drugs acquired on the GC/FT-IR instrument were compared to literature spectra of these same compounds from Mills *et al*. Compare these for yourself. What conclusions can you draw regarding the reproducibility of infrared data obtained on a GC/FT-IR instrument?

15 FOURIER TRANSFORM RAMAN SPECTROSCPY

15.1 OBJECTIVES

- To familiarize the trainee with the theory and application of FT-Raman spectroscopy in materials analysis.
- To familiarize the trainee with the FT-Raman instrumentation and software used in the laboratory.
- To have the trainee demonstrate a basic understanding of how a FT-Raman operates.
- To have the trainee demonstrate how to properly interpret FT-Raman spectra.

15.2 TOPIC AREAS

1. Theory
    a. Inelastic scattering of monochromatic light, usually from a laser source.
        i. The frequency of photons of monochromatic light changes upon interaction with a sample.
        ii. Photons from the laser light are absorbed by the sample and reemitted.
        iii. The frequency of the reemitted photons is shifted up or down in comparison with the original monochromatic frequency (Raman effect).
    b. Raman effect is based on molecular deformations determined by molecular polarizability.
    c. Nuclear displacement is the amplitude of vibration. Three different types exist:
        i. Rayleigh scattering
        ii. Stokes frequency
        iii. Anti-Stokes frequency
    d. Comparison of Infrared and Raman

Infrared	Raman
Absorption	Emission of scattered laser light
Requires a dipole moment change (O-H, N-H, C=O)	Requires polarizability change (C=C, aromatics)
Sample preparation of accessory usually necessary	Little or no sample preparation necessary
Short optical pathlength required	Measure through transparent packaging
Non-aqueous samples	Aqueous samples

2. Instrumentation
    a. Dispersive
        i. Technology Basics
            1. Spectral analysis performed by grating
            2. Visible lasers
                a. Diode laser
                b. Ar+ and Kr+ ion lasers
            3. Detectors
                a. CCD arrays
        ii. High sensitivity
        iii. Resolution/coverage trade-off
        iv. Variable resolution across spectrum
        v. Potential fluorescence interference
    b. Fourier-Transform
        i. Technology Basics
            1. Spectral analysis performed by interferometer
            2. Near infrared lasers
            3. Detectors
        ii. Avoids fluorescence interference
        iii. Advantages
            1. Inherent wavelength calibration

2. Higher resolution possible – limited by laser line width and optical path difference of the interferometer
3. Full spectrum coverage at high resolution

c. A typical Raman System consists of:
   i. Beam Splitter
   ii. Excitation Laser
   iii. Detector
   iv. Sample compartment

3. Analysis
   a. Choosing the right sample holder
   b. Adjusting the sample position
   c. Setting laser power
      i. Is a filter needed for the sample?
   d. Dealing with problems
      i. Is the sample fluorescing?
      ii. Is the sample heating up too much?
4. Results
   a. Compared to known standard
   b. Dealing with spectral drift
   c. Handling mixtures

15.3 SAFETY

Analysts need to be aware of two aspects of using Raman spectroscopy that may pose potential personal safety hazards: laser exposure and possible ignition of samples. Refer to the Raman spectroscopy technical procedures for specific safety information.

15.4 SUGGESTED READING

1. Bowie BT, Chase B, Griffiths PR. 2000. Factors affecting the performance of bench-top Raman spectrometers. Part I: instrumental effects. Appl Spectrosc. 54:164A-73A.
2. Hodges CM, Akhavan J. 1990. The use of Fourier transform Raman spectroscopy in the forensic identification of illicit drugs and explosives. Spectrochim Acta. 46A:303-7.
3. Hodges CM, Hendra PJ, Willis HA, Farley T. 1989. Fourier transform Raman spectroscopy of illicit drugs. J. Raman Spectrosc. 20:745-9.
4. Kuptsov AH.1994. Applications of Fourier transform Raman spectroscopy in forensic science. J. Forensic Sci. 39:305-18.
5. Neville GA, Shurvell HF. 1990. Fourier transform Raman and infrared vibrational study of diazepam and four closely related 1,4-benzodiazepines. J. Raman Spectrosc. 21:9-19.
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7. Ryder AG, O'Connor GM, Glynn TJ. 1999. Identifications and quantitative measurements of narcotics in solid mixtures using near_IR Raman spectroscopy and multivariate analysis. J Forensic Sci. 44:1013-9.
8. Sands HS, Hayward IP, Kirkbride TE, Bennett R, Lacey RJ, Batchelder DN. 1998. UV-excited resonance Raman spectroscopy of narcotics and explosives. J. Forensic Sci. 43:509-13.
9. Tsuchihashi H, Katagi M, Nishikawa M, Tatsuno M, Nishioka H, Nara A, Nishio E, Petty C. 1997. Determination of methamphetamine and its related compounds using Fourier transform Raman spectroscopy. Appl Spectrosc. 51:1796-9.

15.5 STUDY QUESTIONS

1. What is Raman Spectroscopy?
2. Describe the theory behind its use as an identification technique.

3. How is this technique different than FT-IR?
4. Draw a schematic diagram of a FT-Raman. What is the purpose of each component?
5. Define the following terms:
    a. Stokes scattering
    b. Anti-stokes scattering
    c. Fluorescence

15.6 PRACTICAL EXERCISES

(Note: Obtain instructor approval before manipulating any parameters.)

1. Perform a standard calibration of the FT-Raman. Read the manufacture's explanation of how the calibration works, and what the pass/fail limits are.
2. Analyze methamphetamine, pseudoephedrine and ephedrine on the FT-Raman, how are they different from each other? How are they different from their FT-IR spectra?
3. Analyze and compare the spectra of cocaine, heroin and methamphetamine, using each of the available sample holders. How do these spectra differ from their FT-IR spectra? Which sample holder is best for each sample?
4. Analyze lithium carbonate, sodium carbonate, and potassium carbonate. Compare their spectra and explain why it is important to calibrate your instrument regularly, and how it relates to these chemicals specifically.
5. Analyze red phosphorus and iodine. Compare their spectra. Explain why you might need to use a filter to analyze red phosphorus.
6. Analyze chloroform, ethanol, methanol, and pentane. Compare their spectra.
7. Combine lithium carbonate, sodium carbonate and potassium carbonate in a ration of 3:2:1. If needed, re-run the chemicals to have current spectra, then run the combination and practice subtracting out the extraneous peaks to identify each individual substance.
8. Analyze a series of unknowns given to you by your trainer. The will include pure chemicals and mixtures. Identify each significant chemical present in each sample.

16 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

16.1 OBJECTIVES

- To familiarize the trainee with the theory and application of high performance liquid chromatography (HPLC) in materials analysis.
- To familiarize the trainee with the HPLC instrumentation and software used in the laboratory.
- To have the trainee understand the advantages and disadvantages of HPLC.
- To have the trainee demonstrate familiarity with HPLC terminology.
- To have the trainee demonstrate how to properly interpret HPLC data.

16.2 TOPIC AREAS

1. Theory
    a. HPLC involves the partitioning of solutes between a liquid phase and a stationary solid phase. It is an instrumental separation technique based on the difference in the distribution or partition coefficients of substances having solubility in the mobile phase.
    b. History of chromatography
    c. Chromatographic principles
        i. distribution coefficient
        ii. Chromatographic mechanisms
        iii. Retention factor, retention time, retention volume
        iv. Effect of temperature on retention time
        v. Column efficiency
        vi. Resolution
        vii. Peak Shape
        viii. Chemical Bonding and Polarity
        ix. Intermolecular forces
        x. Polarity of compounds and solubility
        xi. Van Deemter equation
            1. Maximizing theoretical plates
2. Instrument Design
    a. General overview
        i. Mobile phase reservoir
        ii. Pumps
        iii. Injectors: septum, stop flow
        iv. Thermostats
        v. Column switches
        vi. Detectors
        vii. Data systems
        viii. Pre-columns/columns
        ix. Fraction collection
    b. Types of columns
        i. Types: preparative, normal, mini-bore, micro-bore, capillary,
        ii. Packing materials: silica based, zirconia, polymer, monolithic
    c. Mobile phase considerations
        i. Isocratic
        ii. Gradient
        iii. Buffers
        iv. Filtration
        v. Degassing
        vi. Mixing

d. Separation Types
    i. Normal phase
    ii. Reverse phase
    iii. Ion exchange
    iv. Derivatization
    v. Chiral separations
    vi. High-speed/high-temperature HPLC
e. Injector Systems
    i. Manual system considerations
    ii. Automatic injector considerations
    iii. Sample loop size consideration
    iv. Effects on quantitive analysis
f. Detection systems
    i. UV/visible
    ii. Diode array
    iii. Electrochemical
    iv. Mass spectrometry/Infrared/NMR
    v. Fluorescence
    vi. Refractive index
    vii. Strengths and weakness of each detector
g. Method development
    i. Normal phase
    ii. Reverse phase
    iii. Method Validation
    iv. Qualitative/Quantitative method consideration
    v. Considerations when transferring a method between instruments
        1. Software packages
h. Quantitative Analysis
    i. Peak height vs. peak area in calculations
    ii. Dynamic range considerations
    iii. Calibration Tables
    iv. Specialized software packages

3. Quality Assurance/Quality Control
    a. System check-out
    b. Documentation
    c. Log books
    d. Calibrations
    e. Blanks and reference materials
4. Instrument Maintenance
    a. Routine maintenance
    b. Column installation
    c. Troubleshooting
        i. Baseline disturbances
            1. spiking
            2. noise
            3. wander
            4. drift
            5. offset
        ii. Peaks
            1. Tailing
            2. Fronting
            3. Broad peaks
            4. Splitting peaks
        iii. Column Care

1. Storage
2. Deterioration/Damage
3. Regeneration

5. Forensic Applications
    a. Drug analysis
    b. Toxicology
    c. Color analysis
    d. Explosives
    e. Food and environmental samples
    f. Poisons and toxins
    g. Dyes
    h. Other

16.3 SAFETY

All systems should be turned off prior to performing maintenance. Special consideration needs to be taken when working with solvent preparation and handling around the instrument due to the hazards of working with large quantities of hazardous solvents. Disposal of the solvents generated by the HPLC should follow the local laboratory's established procedures for solvent disposal. Also, the UV sources used in the detection system pose potential eye and skin hazards. Refer to the HPLC technical procedures and the manufacturers guidelines for specific safety and maintenance information. Also consult the CLD safety manual.

16.4 SUGGESTED READING

1. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press, Chapter 29.
2. R.N. Smith 1988. Forensic Applications of High-Performance Liquid Chromatography . In: Saferstein R, editor. Forensic Science Handbook. Vol II. Englewood Cliffs (N J):Prentice Hall. p.28-91.
3. Bayne, Shirley; Carlin, Michelle. Forensic Applications of High Performance Liquid Chromatography, CRC Press, 2010.
4. Waters Corporation. How does high-performance liquid chromatography work? (www.waters.com)
5. Waters Corporation. What is HPLC (high-performance liquid chromatography)? (www.waters.com)
6. Willard H.H., Merritt L.L. Jr., Dean J.A., Settle F.A. Jr. 1988. Instrumental Methods of Analysis. 7th ed. New York: Van Nostrand.
7. Instrument manufacturer's manuals.
8. van Deemter JJ, Zuiderweg FJ and Klinkenberg A (1956). "Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography". *Chem. Eng. Sc.* **5**: 271–289

NOTE: Most any general text on chromatography may substitute for one or more of the above references. The material in this outline may be covered by a basic HPLC course sponsored by a local vendor.

16.5 STUDY QUESTIONS

1. What is high performance liquid chromatography? What information is obtained using an HPLC system?
2. Draw a schematic diagram for an HPLC and describe the purpose of each component.
3. What is the difference between an isocratic and gradient method? Why might each be used?
4. How do polarity, molecular size and electrical charge impact separation? Discuss columns and mobile phases as they relate to polarity, molecular size and electrical charge.

5. How are samples introduced into an HPLC system? What are the advantages and disadvantages of the different types of autosampler systems?
6. What is the capacity factor? How is it related to the chemistry of the system?
7. Why is it important to select the appropriate wavelength for the analyte when using a DAD?
8. How will excess tubing in the system influence chromatography?
9. Define:
    a. Retention time (TR or tR),
    b. Relative retention time (RRT),
    c. Retention volume,
    d. Unretained retention time (tm)
    e. Phase ratio (β)
    f. Selectivity (α)
    g. Average linear gas velocity (μ)
    h. Theoretical plate (n)?
    i. Effective theoretical plate (N)?
    j. Theoretical plate height /height equivalent to a theoretical plate (H or HETP)
    k. Height equivalent to an effective theoretical plate (H or HEETP)
10. What is a good value for the HETP? And why?
11. How is the # of N related to column efficiency?
12. Define Resolution (R).
    a. What is chromatographic resolution a function of?
    b. Why is resolution not the best measure of column efficiency and column performance?
13. What are some of the common causes and remedies for the following system problems?
    a. No peaks
    b. Baseline drift or unstable baseline
    c. Ghost peaks
    d. Tailing peaks
    e. Leading peaks
    f. Split peaks
    g. Retention time shift
14. Explain how derivatization is performed, including why it may be used for analysis.
15. Explain as to a jury how an HPLC operates.

16.6 PRACTICAL EXERCISES

(Note: Not all laboratories have an HPLC. Obtain instructor approval before manipulating any parameters.)

1. Familiarize yourself with instrument software.
2. You will be given a method by your trainer to modify to determine how method changes affect resolution and method efficiency.
3. Calculate the column volume for the following columns:
    a. 150mm, 4.6 mm i.d.
    b. 100mm, 2.1 mm i.d.
    c. 75 mm, 3.0 mm i.d.
4. At a flow rate of 1.25 ml/min how long does it take to replace the column solvent once in 3a, 3b, and 3c?
5. Measure your dwell volume using the acetone and methanol method. Why is a lower dwell volume helpful in separating components on the HPLC?. Describe any way to lower your dwell volume.
6. Create a 90:10 mix of water:methanol. Measure 90 mL of water in a graduated cylinder. Measure 10 mL of methanol and add to the cylinder of water. Mix, measure the volume and explain the results.

17 SCANNING ELECTRON MICROSCOPY/ENERGY DISPERSIVE X-RAY SPECTROSCOPY

17.1 OBJECTIVES

- To familiarize the trainee with the theory and application of scanning electron microscopy and elemental analysis.
- To familiarize the trainee with the SEM/EDX instrumentation and software used in the laboratory.
- To have the trainee demonstrate a basic understanding of how a SEM/EDX operates.
- To have the trainee demonstrate how to properly interpret data generated by SEM/EDX.

17.2 TOPIC AREAS

1. Theory
    a. General
        i. The SEM/EDX can be used like a traditional light microscope to give visual information, but can also provide data regarding the elemental composition a specimen.
        ii. “Electron gun” is the source of electrons for illumination.
        iii. Electrons are focused by electromagnetic lenses.
        i. Beam and specimen interaction produces signals which are collected and processed by various detectors.
    b. Magnification is the result of the ratio between the scanning area of the beam to the scanning area of the display monitor.
    c. Image Formation
        i. Primary electron beam
            1. Primary electrons
        ii. Interaction volume
            1. A variety of signals (including secondary electrons, backscattered electrons, and X-rays) are produced from this zone.
            2. The size and shape of this zone ultimately determines the maximum resolution of a given SEM with a particular specimen.
            3. Imaging typically utilizes either the secondary electrons or the backscattered electrons.
            4. The X-rays produced in the beam interaction with the specimen will provide the data for elemental analysis (described later under Elemental Analysis).
        iii. Secondary electrons.
            1. Have an energy of less than 50 eV.
            2. Most common type of image produced by modern SEMs. It is most useful for examining surface structure and gives the best resolution image of any of the scanning signals.
            3. Depending on the initial size of the primary beam and various other conditions (composition of sample, accelerating voltage, position of specimen relative to the detector) a secondary electron signal can resolve surface structures down to the order of 10 nm or better.
            4. The topographical image is dependent on how many of the secondary electrons actually reach the detector.
            5. Secondary electrons that are prevented from reaching the detector will not contribute to the final image and these areas will appear as shadows or darker in contrast than those regions that have a clear electron path to the detector.

iv. A backscatter electron is defined as one which has undergone a single or multiple scattering events and escapes with an energy greater than 50 eV.
    1. The result of elastic collisions with the atoms of the sample and usually retain about 80% of their original energy.
    2. The number of backscattered electrons produced increases with increasing atomic number of the specimen. For this reason, a sample that is composed of two or more different elements which differ significantly in atomic number will produce an image that shows differential contrast of the elements despite a uniform topography. Elements that are of a higher atomic number will produce more backscattered electrons and will therefore appear brighter than neighboring elements of lower atomic number.
    3. The region of the specimen from which backscattered electrons are produced is considerably larger than it is for secondary electrons. For this reason the resolution of a backscattered electron image is considerably less (1.0 um) than it is for a secondary electron image (10 nm).
    4. Because of their greater energy, backscattered electrons can escape from much deeper regions of the sample than can secondary electrons, hence the larger region of excitation. By colliding with surrounding atoms of the specimen, some backscattered electrons can also produce X-rays, auger electrons, cathodoluminescence, and even additional secondary electrons.

d. Elemental Analysis (X-rays)
    i. X-ray formation
    ii. Each X-ray is characteristic of the atom from which it originated.
    iii. X-ray maps
        1. Resolution is usually greater than 1 micron.
    iv. Bremsstrählung X-radiation (braking radiation)

2. Applications in Casework
    a. Wide variety of applications
    b. Not useful when samples are so small that they do not accurately represent the bulk material from which it originated.
    c. Samples with large inclusions can be problematic.
    d. Generally not used for quantitative analysis in our system.
3. Advantages and Limitations
    a. SEM
        i. Advantages
            1. Provides greater resolution, magnification, and depth of field
            2. Low energy electrons, or secondary electrons, can be used to observe fine surface details at either low or high magnifications.
            3. The high energy electrons (energy greater than 50 eV, or backscattered electrons) show a strong correlation with atomic number and can be used in the contrast mode to examine relative composition
            4. Non-destructive method
        ii. Limitations
            1. Image is monochromatic
            2. The sample may be subjected to charging, possibly creating damage to the specimen. This can sometimes be avoided or minimized through additional sample preparation such as carbon coating before analysis (if allowable).
    b. EDX
        i. Advantages

1. Elements can be quickly determined according to the various energies of x-rays produced.
2. Data may be obtained from a bulk sample, or individual particles within a specimen may be analyzed by spot focusing the electron beam rather than scanning.

ii. Limitations

1. Inability to detect elements in trace concentrations or below atomic number 6 (carbon)
2. Need for a conductive coating of some samples
3. Inability to remove a sample from most embedding materials after analysis
4. Discoloration of materials by irradiation
5. Energy dispersive X-ray spectrometry resolution is generally no better than approximately 140eV. As a result, there may be an overlap of peaks in the energy dispersive X-ray spectrometry spectrum of materials containing several elements.

17.3 SAFETY

1. Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar. When filling the dewar, eye level should be above the funnel. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves should be used when handling liquid nitrogen.
2. Caution should be exhibited when working with vacuum pumps. Waste oil should be treated as hazardous and should be handled and disposed of appropriately.
3. The SEM/EDX is a high voltage system. After powering off the system, allow any stored energy to discharge prior to performing any maintenance. All maintenance should be undertaken with caution.

17.4 SUGGESTED READING

1. ASTM E766 (current version) – Standard Practice for Calibrating the Magnification of a Scanning Electron microscope.
2. ASTM E1508 (current version) - Standard Guide for Quantitative Analysis by Energy-Dispersive Spectroscopy.
3. Goldstein JI, Newbury DE, Echlin P et al. 1992. Scanning Electron Microscopy and X-Ray Microanalysis: A Text for Biologists, Materials Scientists, and Geologists. 2nd Ed. New York: Plenum Press.
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5. Haffner B. Energy Dispersive Spectroscopy on the SEM: A Primer. http://www.charfac.umn.edu/education.html.
6. JEOL USA. www.jeolusa.com. RESOURCES/ELECTRONOPTICS/DocumentsDownloads.
7. Johnson R. Environmental Scanning Electron Microscopy: An Introduction to ESEM. http://www.cb.uu.se/~ewert/SEM.pdf
8. Microbeam Analysis – Instrumental Specification for Energy Dispersive X-Ray Spectrometers with Semiconductor Detectors. Geneva, Switzerland: International Organization for Standardization; 2002. Standard Number IOS 15632:2002.
9. Newbury D. 2005. Misidentification of major constituents by automatic qualitative energy dispersive x-ray microanalysis: a problem that threatens the credibility of the analytical community. Microsc. Microanal. 11:545-561.
10. Oxford Instruments NanoAnalysis. 2006. An introduction to energy-dispersive and wavelength-dispersive x-ray microanalysis. Microscopy and Analysis. 20(4):S5-S8.
11. Posteck MT, Howard KS, Johnson AH, McMichael KL. 1980. Scanning Electron Microscopy: A Student's Handbook. Ladd Research Industries.

12. Ward D. Sample preparation for scanning electron microscopy in forensic science. Quantico, VA: Federal Bureau of Investigation Laboratory; 2006. Notes from course Forensic Analysis of Pressure Sensitive Tapes held June 5-9, 2006.
13. Watt IM. 1985. The Principles and Practice of Electron microscopy. New York: Cambridge University Press.
14. www.mse.iastate.edu/microscopy/choice.html
15. User's manual for the SEM-EDX available in the analyst's laboratory.

17.5 STUDY QUESTIONS

1. How is the SE image produced from an electron beam?
2. How is elemental data produced from an electron beam?
3. Briefly describe the Böhr model of an atom. How it is used to name characteristic X-rays?
4. What are you adjusting in the SEM to focus an image? How is this different from light microscopy?
5. What is astigmatism? How do you correct astigmatism in the SEM?
6. What are the various types of resolution in the SEM and in the EDX? (There are three.) Describe how to optimize each.
7. How do you increase "dead time" in the EDX? Why would you want to do this? What happens if dead time becomes excessively high?
8. What are sum peaks, escape peaks and system peaks?
9. Why do peak overlaps occur in EDX spectra? How can you resolve them?
10. Why is the backscatter electron detector positioned in the sample chamber as it is?
11. What is critical excitation energy?
12. When would you choose to use low KeV vs. high KeV accelerating voltage? What are the effects on the analytical results (imaging and elemental analysis)?
13. What is Bremsstrählung radiation?
14. How does working distance affect imaging results and X ray results?
15. How do you change depth of field in the SEM? What other effects should you consider when adjusting depth of field?
16. How do you change the size of the final aperture? What advantage or disadvantage results from this?
17. What is sample charging? Why and how should you avoid it?
18. What information is gained from the backscatter image that is not in the SE image?
19. Can you detect Lithium in the SEM/EDX? Why or why not?
20. How is magnification achieved in the SEM image?

17.6 PRACTICAL EXERCISES

Obtain the approval of the trainer before manipulating any parameters on the instrument. The trainer will determine which of the following exercises will be completed or if additional exercises are more appropriate.

1. X-checker Instrument Verification
    a. Test SEM resolution using a test sample having high contrast, sharp edges and closely spaced features such as a gold, tin, or aluminum-tungsten resolution test specimen or any other comparable specimen. Examine the specimen at progressively higher magnifications from 100x to 20,000x or higher. Capture and save highly resolved images at a variety of magnifications. Include the instrument parameters that were used to capture the image.
    b. Obtain a commercially available measurement grid and compare the results from the software measurement tool against the known dimensions of the grid. It is recommended

to check both the resolution of the image and the measurement tool accuracy at different magnifications.

c. Using the X-Checker Standard ™ and the directions supplied, perform the following tests:
    i. Spectrum calibration using copper
    ii. Resolution of x-ray detector using Mn Kα peak &1/2 height of peak
    iii. Check for detector window contamination by using Mn Kα and Cu Kα peak ratios.
    iv. Check artifact peaks using the carbon sample. Also using the adhesive carbon tabs used in casework mounted on aluminum stubs, check for what elements are observed in the background.

d. Using commercially available reference standards such as the Micro-Analysis Consultants Limited reference block, perform semi-quantitative analysis on a variety of standards and compare to the supplied results.

2. Bulk Analysis
    a. Cut a newer penny, dated from the 1980s or later, in half. (Newer pennies are coated. Older pennies are alloyed. These exercises may be repeated with an older - 1970s or earlier - penny to compare the results.)
    b. Mount one half of the penny on an SEM sample stub using double-sided sticky tape or carbon tape so that the exposed cross section is facing up.
    c. Mount the other half of the penny lying flat on another SEM sample stub. (This sample will be used later in this exercise.)
    d. Place the sample stubs with the penny halves in the SEM chamber.
    e. Evacuate the chamber using the high vacuum mode.
    f. Check the tuning of the instrument.
    g. Set the scan parameters to obtain a secondary electron image of the penny cross section at an accelerating voltage of 20KeV. Include all layers of the cross section in the field of view.
    h. Set the X-ray parameters to obtain approximately 1000 counts per second and 30 to 40 percent dead time.
    i. Collect an X-ray spectrum scanning across the entire cross section for 300 live seconds.
    j. Identify the peaks in the spectrum.

3. Spot Analysis
    a. Using the same beam parameters, choose one layer of the penny cross section and set the SEM for spot (non-rastered) analysis on an area of that layer (be sure the magnification is high enough so that the beam area is smaller than the layer thickness).
    b. Collect an X-ray spectrum for 300 live seconds.
    c. Identify the peaks in the spectrum.
    d. Repeat the procedure for a different layer of the penny.

4. X-Ray Mapping a Secondary Electron Image
    a. Set the scan parameters to again include all layers of the cross section of the penny. Using the same conditions as for the bulk analysis above, collect a quick spectrum over several seconds for preliminary identification of the peaks in the sample.
    b. Place element labels on the significant peaks and “select” the peaks that will be mapped in the final image.
    c. Set the EDX monitor to display multiple windows in live scan mode.
    d. Collect maps for 1000 live seconds or until sufficient detail is obtained to manually stop the collection.
    e. Maps can be overlaid to demonstrate that two or more elements are in the same layer, such as a metal alloy layer containing both copper and nickel.

5. Effects of Beam Penetration in Layered Samples
    a. Move the sample stub with the penny lying flat into position for examination in the sample chamber.

b. Set the scan parameters to obtain a secondary electron image of the penny at an accelerating voltage of 30KeV.
c. Set the X-ray parameters to obtain approximately 1000 counts per second and 30 to 40 percent dead time.
d. Collect an X-ray spectrum for 300 live seconds.
e. Identify the peaks in the spectrum and compare these to the bulk analysis result in part A of this exercise.
f. Decrease the accelerating voltage to 25KeV and adjust the beam parameters to again obtain approximately 1000 counts per second and 30 to 40 percent dead time.
g. Collect an X-ray spectrum for 300 live seconds.
h. Identify the peaks in the spectrum and compare the peaks and relative peak ratios to the bulk analysis result in part A of this exercise.
i. Repeat this procedure stepping down by increments of 5 KeV to a final accelerating voltage of 5 KeV.
j. Compare the peaks and relative peak ratios as the accelerating voltage (beam penetration) is decreased.

6. Imaging With Back Scattered Electrons
    a. Place the cross sectioned penny back into position for examination in the sample chamber.
    b. Set the scan parameters to include all layers of the penny cross section. Using the same conditions as for the bulk analysis above, select the backscatter detector and adjust the beam parameters to obtain a backscattered electron image.
    c. Noting areas that appear darker or lighter in the image, collect x-ray spectra of these various areas in non-rastered mode. Note the elemental composition of each of these areas.
    d. Place the “X-Checker” stub into position for examination in the sample chamber.
    e. Set the scan parameters to include several squares of the grid. Using the same conditions as for the penny analysis above, select the backscatter detector and adjust the beam parameters to obtain a backscattered electron image.
    f. Noting areas that appear darker or lighter in the image, collect x-ray spectra of these various areas in non-rastered mode. Note the elemental composition of each of these areas.
    g. Try the various settings for the backscatter detector and observe any changes in contrast in the image.

18 SPECTROSCOPE

18.1 OBJECTIVES

- To familiarize the trainee with the theory and application of emission spectroscopy in chemical analysis.
- To familiarize the trainee with the spectroscope used in the laboratory.
- To have the trainee understand the advantages and disadvantages of spectroscope use.
- To have the trainee demonstrate familiarity with spectroscope terminology.
- To have the trainee demonstrate how to properly interpret spectroscope results.

18.2 TOPIC AREAS

1. Theory
    a. When a material is heated to incandescence, it emits light that is characteristic of the atomic makeup of the material. A flame test can be performed by presenting a sample to a lightly colored or uncolored flame. Samples containing certain elements will change the color of the flame, the color of which may indicate what elements are present in the sample. Elements emit specific wavelengths of light described as their emission, bright line, or line spectra. These individual emission lines contribute to the observed flame color. A spectroscope separates different wavelengths of light using a diffraction grating or prism and can be used to measure emission spectra.
2. Instrument Design
    a. Prisms and diffraction gratings
        i. transmission grating
        ii. reflection grating
        iii. replica grating
        iv. master grating
        v. holographic grating
    b. Spectroscope internal wavelength scale
        i. units of measure
        ii. measuring emission and absorption spectra

18.3 SAFETY

Care should be taken when working with an open flame. The spectroscope should be used in a functional and operating fume hood as the examination of some samples may result in the evolution of gases. Analysts should be familiar with the material attempted to be detected in the sample and should know any hazards associated with those materials.

18.4 SUGGESTED READING

Most any analytical textbook will offer information on this technique.

18.5 STUDY QUESTIONS

1. Define the term emission and explain the significance to this technique.
2. Explain how a diffraction grating works.
3. What advantages does a spectroscope offer over visual observation of flame color?
4. What disadvantages are there to using a spectroscope for detection of atoms?
5. How are absorption spectra measured with a spectroscope?

18.6 PRACTICAL EXERCISES

1. Observe the flame colors and line spectra for the following salts:
    a. Barium chloride
    b. Calcium chloride
    c. Lithium chloride
    d. Sodium chloride
    e. Strontium chloride
    f. A mixture of lithium and sodium salts
    g. Analyze increasingly dilute solutions of lithium chloride and determine a lower detection limit.

19 X-RAY FLUORESCENCE

19.1 OBJECTIVES

- To have trainees become familiar with the theory, instrumentation, sample preparation, spectral interpretation, advantages and limitations, and applications of X-ray fluorescence (XRF) spectrometry for the analysis and comparison of a wide variety of materials.
- To familiarize trainees with the particular XRF instrument and software used in their laboratories and how they should properly care for and maintain this instrument.
- To have trainees demonstrate a basic understanding of how a XRF instrument operates to include optimization of analysis parameters to achieve maximum sensitivity for a particular element or group of elements, and how these operational parameters affect the spectral results obtained.
- To have trainees demonstrate that they have a working knowledge of how to properly interpret XRF spectral data.

19.2 TOPIC AREAS

1. Caveats
    a. Critical areas of concern regarding instrument damage
    b. Perils of the “black box” approach
        i. Why is there argon in my powder? - Know your periodic table!
        ii. Fe or Tb?
2. History
3. Overview: Features, Applications and Limitations of XRF Spectrometry
    a. Advantages
    b. Limitations
    c. Sensitivity curve of XRF for various atomic numbers
    d. Fluorescence-what it means
    e. A comparison of XRF and X-ray analysis using SEM/EDX
4. Theory of XRF Spectrometry
    a. The electromagnetic spectrum
    b. Rayleigh and Compton scattering
    c. Bremsstrählung
    d. Absorption of matter by X-rays
    e. K, L and M series
5. XRF Instrumentation
    a. EDXRF versus WDXRF
    b. Direct excitation
    c. X-ray tubes
    d. Secondary target excitation
    e. Detection system
        i. Si(Li) detector
        ii. Pulse generation/discrimination
        iii. A to D converter
        iv. Multichannel analyzer
        v. Dead time
    f. Detector artifacts
        i. Compton escape
        ii. Silicon escape peaks
        iii. Sum peaks
            1. Causes/prevention
            2. Predicting relative intensities

g. Other artifacts
    1. Diffraction peaks
h. Analysis configuration

6. XRF Analysis Considerations
    a. Direct excitation
    b. Secondary target excitation
    c. Use of vacuums or helium purges
    d. Practical considerations
7. Spectral Interpretation
    a. Penetration depth
    b. Comparisons of samples based on relative peak intensities
        i. Elements having similar atomic numbers
        ii. Elements having moderately different atomic numbers
    c. Relative intensities observed using secondary targets
    d. Interferences
        i. Chlorine using direct rhodium excitation, sulfur using direct molybdenum excitation, etc.
        ii. Overlapping lines for lighter elements
        iii. Examples
            1. Ti, Ba and V in an automotive paint
            2. Molybdate orange (S, Mo, Pb)
        iv. Lead traces from instrument shielding
        v. Diffraction lines

19.3 SAFETY

1. XRF instruments are surrounded with lead shielding and equipped with safety switches which prevent the X-ray tube from being turned on if one of the access panels is removed. However, as a precaution, it is recommended that each instrument have a radiation monitoring badge kept in the vicinity of the user to insure that analysts are not getting exposed to X-rays.
2. Appropriate safety precautions should be employed when refilling the liquid nitrogen dewar. Personal protective equipment including safety goggles, face shields, insulating gloves and long sleeves should be used when handling liquid nitrogen.

19.4 SUGGESTED READING

1. Bertin EP. 1975. Principles and Practice of X-Ray Spectrometric Analysi. 2nd ed. New York:Plenum Press.
2. Burke VE, Jenkins R, Smith DK. 1998. A Practical Guide For The Preparation of Specimens for X-Ray Fluorescence and X-Ray Diffraction Analysis. New York:John Wiley & Sons, Inc.
3. Jenkins R. 1999. X-Ray Fluorescence Spectrometry. New York:John Wiley & Sons, Inc.
4. Vaughan D. 1989. Energy Dispersive X-Ray Microanalysis: An Introduction. San Carlos (CA):Kevex Corp.
5. www.learnxrf.com
6. en.wikipedia.org/wiki/X-ray_fluorescence

19.5 STUDY QUESTIONS

1. What is fluorescence? Why is this term applied to an XRF analysis and not to the process that produces X-rays in a SEM instrument?
2. Describe what happens at the molecular/atomic level to produce a XRF spectrum.
3. Describe how an XRF analysis differs from an X-ray analysis using an SEM/EDX instrument. What are the advantages and disadvantages of each of these instruments?
4. What is a secondary target and what are the advantages of having a secondary target?

5. Describe the differences between conventional XRF instruments (with secondary targets or filters) and micro-XRF systems. What are the advantages and disadvantages of each of these instruments?
6. Describe the components of an XRF instrument, what each does, and how a spectrum is produced.
7. Describe briefly how a lithium-drifted silicon (Si[Li]) works. Why do most Si(Li) detectors always have to be kept at liquid nitrogen temperatures?
8. What happens to the Si(Li) detector if a high voltage is applied (that is, the detector is turned on) and the detector is not cold?
9. How do you clean the window of the Si(Li) detector?
10. What is a K series? L series? M series?
11. Where in the XRF spectrum of helium would you expect the L series to occur?
12. For which element or elements are all three series observed between 1 and 40 KeV?
13. Describe what type of pattern each of the three series produces. For a K or L series, why might this pattern change somewhat as the atomic number of an element changes?
14. What are Kα, Kβ and Kab?
15. Why must a vacuum or a helium purge be used to analyze the lightest detectable elements?
16. You are trying to determine if there is magnesium in a powder suspected of being residue from a magnesium flare. How would you prepare your sample for analysis (using an instrument with a sample tray)?
17. Argon is often seen in a XRF spectrum. Where is it coming from?
18. What is Bremsstrählung? In what component (if any) of an XRF instrument is Bremsstrählung produced? What produces Bremsstrählung in an SEM/EDX?
19. Describe the spectrum of X-rays produced by an X-ray tube. What effect does changing the voltage and current have on the X-rays produced?
20. What are Compton and Rayleigh scattering? Give a general rule (or rules) that predicts the relative amounts of these two types of scattering for X-rays.
21. What factor (or factors) determines the analysis depth of XRF spectrometry?
22. What are the two things that should never be done when working with an XRF instrument (and if care is not taken in this regard, will lead to a very costly repair bill)?
23. What is a silicon escape peak? How is it recognized?
24. What is a sum peak? How is it recognized?
25. What is dead time? How do you control dead time? What happens when the dead time is too high? What is the optimal range for dead time?
26. You are trying to determine the presence of a small amount of a moderately heavy element using an XRF instrument with an X-ray source of your choosing (you are working in a facility that has a cyclotron that can generate either a monochromatic beam of X-rays of any energy and intensity, or any other X-ray distribution that you desire). Keeping in mind that a source that does not produce optimal excitation will only cause a large scattering background and raise your dead time, what type of X-ray excitation would you pick for this element?
27. You are analyzing a laminate that is situated in the instrument with a flat orientation. You know that the second layer of the laminate is comprised of PVC (poly [vinyl chloride]) and you suspect that a barium catalyst may have been used in this PVC layer. There is no barium or chlorine in the first layer (which is situated closest to the detector). You detect K lines of barium but no lines of chlorine (keeping in mind that there is probably much more chlorine in the second layer than barium). Explain.
28. You are analyzing an unknown metal by XRF and detect aluminum, iron, copper and zinc. You have used a tin secondary target and analyzed the sample in air. The intensities of the iron, copper and zinc Kα lines are 10 to 20 times as intense as the Kα line of aluminum. How would you report the composition of this metal?

19.6 PRACTICAL EXERCISES

1. Before any samples are run, examine where the various components of the XRF instrument are located and, in particular, determine the exact location of the detector. When any material is analyzed without a substrate, insure that no part of the object will ever come near the detector. Take special precautions if a narrow object, such as a wire or thin tube, is analyzed to insure that the object is never able to penetrate the detector aperture and reach the window. An automatic sequence analysis should not be made that includes such objects, as they may shift position when the sample tray is rotated or moved.
2. Become familiar with how the level of liquid nitrogen in the Dewar is monitored and be aware of any safeguards that are built into the instrument to warn users when the nitrogen drops below a certain level (such an audible alarm or a flashing light).
3. You are strongly encouraged to run as many samples of everyday items as possible to become familiar with the instrument response for a very wide variety of materials, and how to use optimal analysis conditions for each sample.
4. Determine where the escape peaks are for every sample that you analyze. How accurate is the instrument software in predicting the intensity of a particular escape peak?
5. Pick a steel object, such as a wrench, and analyze it without a substrate in air. Using direct excitation, use three different primary excitation conditions: 6, 8 and 10keV with a currents high enough to give dead times of around 20% (or use the maximum current if this dead time is not reached). Check your X-ray “slide rule” that came with the instrument and determine what the Kab is for iron. Based on the distribution of X-ray energies from your source (how did you arrive at this determination?), estimate roughly what percent of the source energy for each voltage was actually able to excite the iron atoms in your sample.
6. Analyze this same steel object with direct excitation using 20KV and a high enough current so that you can produce a dead time of 80% or higher (if necessary, increase the voltage). Identify the sum peaks in the spectrum. If this were an unknown material from a case, would you have been able to identify these peaks as sum peaks? Rerun the same sample with a dead time of 20% and compare the intensities of the sum peaks for both spectra.
7. Obtain the spectrum of a potassium chloride powder in a sample cup and compare it to the spectrum of the empty cup run under similar conditions using the overlay software. Use a primary excitation condition that you think might be optimal for analyzing these two elements. If you have an instrument with a secondary target, rerun the sample with a secondary target: pick a secondary target that will produce optimal excitation for the heavier of these two elements. If you are using a micro-XRF system and use a diamond anvil cell (DAC) as a substrate for XRF analyses, run a blank of the DAC. Note the relative intensities of the two elements that you have run. (Observation of a similar intensity ratio in a sample of suspected potassium chlorate or potassium perchlorate—both oxidants used in explosives, pyrotechnic devices and fusees—or in residues where these oxidants were suspected of being used, is significant. Why?) If using the DAC, orient the DAC in different positions and note and explain any differences in the spectrum.
8. Obtain XRF spectra of sodium chloride. Analyze the powder in both a sample cup with a Mylar window and mounted under a strip of tape, in both air and under a vacuum. If you are using an instrument with secondary targets, use both primary excitation and an appropriate secondary target of your choosing. Which analysis method is preferred for detecting sodium? For a condition where lines of both elements can be observed, compare the ratios of the sodium and chlorine K lines and compare these to the ratios observed below.
9. Obtain XRF spectra of potassium chloride and silver chloride, again using various excitation conditions. Compare the relative intensities of the cation and the chloride anion for sodium chloride, potassium chloride, and silver chloride. These two samples can be run in air and you don’t have to worry about obtaining background spectra.
10. Pick two elements of your choosing having atomic numbers of at least 20 (calcium) that are adjacent members of the periodic table and prepare an equimolar mixture of the two. If possible, pick a salt where the companion cation or anion is not detected using an XRF instrument since this exercise is designed to give you an idea of how the instrument sensitivity is similar for

adjacent elements regardless of their chemical natures. . Run the sample using primary excitation and secondary targets. A vacuum does not need to be used. Compare the intensities of the lines of the two elements.

11. Prepare an equimolar mixture of salts that contain potassium (atomic number 19) , some intermediate element having atomic number from 26 iron, to 38 strontium, and silver (atomic number 47), tin (atomic number 50), or antimony (atomic number 51). Analyze using different conditions. This range of elements was chosen since K lines are readily observed for all of these and the relative sensitivity of the instrument for a wide range of atomic numbers can be observed using both primary excitation and secondary targets.
12. If using a microXRF, select a sample and run the sample using different time constants. Observe the difference in peak shape and resolution.

20 BASIC PRACTICAL MICROSCOPY

20.1 OBJECTIVES

- To instruct the trainee on how to properly use a variety of microscopes in the laboratory.
- To instruct the trainee on polarized light microscopy and to properly set-up a microscope for Kohler illumination.
- Demonstrate the most appropriate techniques to make basic observations of the physical and optical properties of common evidential materials.

20.2 TOPIC AREAS

1. Particle characterization concepts
2. Historical Perspective
3. Components and function of stereobinocular microscopes
4. Components and function of polarized light microscopes
5. Mechanical alignment of the polarized light microscope for Köhler Illumination
6. Micrometry and calibration of the eyepiece micrometer
7. Small particle manipulation
8. Lens optics
9. Electromagnetic spectrum
10. Physical laws, theories and terminology
    a. Snell's Law
    b. Reflection, refraction
    c. Abbe's theory of resolution
    d. Numerical aperture, resolving power, resolution
11. Introductory Crystallography
    a. Symmetry
    b. Crystal habits
    c. Crystal axes, systems, and classes
    d. Crystal forms
12. Polarization of light
13. Particle Identification –Observations using ordinary light
    a. Morphology
    b. Size
    c. Absorption color
    d. Magnetism
    e. Brittleness/elastomericity
14. Particle Identification- Observations in Plane Polarized Light
    a. Pleochroism
    b. Refractive Index
15. Particle Identification using Crossed Polarized Light
    a. Birefringence
    b. Retardation
    c. Michel-Levy Chart
    d. Sign of elongation
    e. Extinction angles
    f. Interference figures
16. Use of Comparison microscopes
17. Maintenance of microscopes

20.3 SAFETY

1. Glass microscope slides and cover slips should be considered a sharp and should be handled with care.
2. Blades used for cross-sectioning samples are extremely sharp and must be handled carefully.
3. Never look directly into a light source.
4. Halogen lights should not be touched with bare hands as surface contaminants such as oils can create hot spots on the quartz envelope. This localized damage to the quartz envelope can lead to weakening and explosion of the bulb. Areas of contamination on a bulb should be thoroughly cleaned with alcohol and dried prior to use.

20.4 SUGGESTED READING

1. Abramowitz M. 1985. Microscope Basics and Beyond. Success(NY):Olympus Corporation Lake, Precision Instrument Division.
2. Bisbing RE, Schneck WM. 2006. Particle Analysis in Forensic Science, Forensic Science Review. 18(2):119-144.Delly JG. 2011. Essentials of Polarized Light Microscopy. Westmont(IL):Hooke College of Applied Sciences.
3. Goldberg O. 1980. Kohler Illumination. Microscope. 28:15-21.
4. McCrone WC. 1995. Polarized Light Microscopy. Ann Arbor(MI):Ann Arbor Science Publishers, Inc.
5. McCrone WC. 1982. Particle Characterization by PLM Part 1: No Polars. Microscope 3(3):185-196.
6. McCrone WC. 1982. Particle Characterization by PLM Part 2: Single Polar. Microscope 30(4):315-331.
7. McCrone WC. 1983. Particle Characterization by PLM Part 3: Crossed Polars. Microscope 31(2):187-206.
8. McCrone WC. 1985. Unit Operations. Microscope 33:121.
9. Palenik S. 1997. Forensic Microscopy. USA Microscopy and Analysis.
10. Petraco N, Kubic T. 2004. Color Atlas And Manual Of Microscopy For Criminalists, Chemists, And Conservators. Boca Raton (FL):CRC Press.
11. Pluta M. 1990. R.M.S. Microscopy Handbook No. 15: R.M.S. Dictionary of Light Microscopy. Journal of Microscopy. 157: 255–256.
12. Teetsov A. 1999. Preparation And Use Of Needles And Micropipettes For Handling Very Small Particles. Microscope. 47:63-70.
13. Teetsov, A. 2002. An Organized Approach To Isolating And Mounting Small Particles For Polarized Light Microscopy. Microscope. 50:159-68.
14. Weaver R. 2003. The Life Work of Dr. Walter C. McCrone Jr., Bibliography and Selected Historical Facts. Microscope 51(1):31-44.
15. Microscope manuals supplied with the microscopes in your laboratory.
16. The following web sites offer interactive tutorials on microscopy:
    a. www.olympusmicro.com/
    b. www.microscopyu.com/
    c. micro.magnet.fsu.edu/primer/
    d. www.olympusmicro.com/primer/lightandcolor/lenseshome
    e. www.olumpusmicro.com/primer/lightandcolor/mirrorhome
17. The following PowerPoint presentation located on the Portal:
    a. Basic Practical Microscopy
    b. Basic Microscopy Lecture 1, Principles of Optics
    c. Basic Microscopy Lecture 2, Determination of Optical Properties and Isotropic Refractive Index
    d. Basic Microscopy Lecture 3, Determining the Refractive Index of Anisotropic Materials

20.5 STUDY QUESTIONS

1. Name and describe the components of a stereomicroscope and explain how they work.
2. Name and describe the components of a polarized light microscope and explain how they work and what they do.
3. A ray of light strikes the surface of crown glass at an incident angle of 50º. Determine the direction of the refracted ray. The refractive index of the crown glass is 1.50.
4. The refractive index of diamond is 2.42. What is the critical angle, i_C of light passing from diamond into air?
5. A beam of light strikes the surface of water at an incident angle of 60º. Determine the direction of the refracted ray. The index of refraction of water is 1.33.
6. What is chromatic aberration in lenses?
7. What are two ways to correct chromatic aberrations in lenses?
8. What is the highest theoretical Numerical Aperture for a dry objective?
9. How can we increase Numerical Aperture?
10. Describe the 'Bracketing Method', and give examples of when it is useful.
11. What is a Becke Line? How is this optical phenomena formed and what are its practical implications?
12. Perform the following calculations on the effects of temperature on refractive index:
    a. A Cargille liquid has a nD of 1.520 ± 0.0002 at 25ºC.
    b. Its coefficient for temperature change is (-) dnD/dt = 3.96 X 10-4/ºC
    c. Calculate the refractive index for this liquid at 20ºC:_____
    d. Calculate the refractive index for this liquid at 30ºC:_____
13. What is the Michael-Levy Chart and how is it used?
14. What is extinction as it is applied to optical microscopy? Name and describe at least 5 different types of extinction.
15. What is pleochroism and is it any different than dichroism?
16. What is the sign of elongation?
17. How do you measure extinction angles?
18. What is conoscopy and how do you set-up the PLM to achieve this?
19. What is an interference figure?
20. You have measured a fiber and found that the diameter is 20 micrometers and you have determined that it exhibits a retardation of approximately 334 nm between crossed polars. Using the Michel-Levy chart provided, what is the approximate birefringence for the fiber?

20.6 PRACTICAL EXERCISES

1. Set-up a stereomicroscope for reflected and transmitted light observation.
2. Calibrate the eyepiece reticle of a stereomicroscope.
3. Set up two different models/brands of polarized light microscopes for Köhler illumination.
4. Set-up and color balance a comparison light microscope.
5. Measure the diameter of the supplied particles using the stereomicroscope and the PLM.
6. Sharpen several tungsten needles.
7. Particle pick and separate small particles supplied by the trainer using forceps and tungsten needles.
8. Estimate the refractive indices of the supplied fibers in both the parallel and perpendicular position as compared to the mounting media (Dd~1.525). Use the following notation: <<(much less than), <(less than), ~(approximately equal to), >(greater than), >>(much greater than).
9. Determine if the supplied particles are isotropic or anisotropic.
10. Estimate the relative retardation of the supplied fibers as almost zero, low, medium, high, very high.
11. Calculate the numerical birefringence of the supplied fibers.
12. Name the type of extinction exhibited in the supplied particles.

13. Estimate the extinction angle of the minerals hornblende and tremolite.
14. Examine the supplied particles and determine if any show pleochroism.
15. Record the sign of elongation as positive, negative or none using the supplied fibers.
16. Determine the refractive index of three unknowns supplied by your instructor using the bracketing method.
17. Determine the relative retardation (almost zero, low, med, high, very high), sign of elongation, birefringence, and actual retardation of the fibers supplied by your trainer.
18. Examine both uniaxial and biaxial interference figures of the minerals supplied.
19. Characterize the following 10 unknowns using the supplied table containing, morphology, color, pleochroism, relative refractive indices, retardation, sign of elongation, diameter, identity.
20. Additional exercises can be added by the trainer as necessary.

21 IMAGING AND VISUALIZATION

21.1 OBJECTIVES

- To obtain an understanding of different types of imaging and visualization techniques available in the Crime Laboratory Division.
- To familiarize the trainee with image processing software and the advantages and limitations of this software in casework.

21.2 TOPIC AREAS

1. Introduction
    a. The ability to record observations is a vital part of the forensic examination of evidence. The scientist must be trained in various imaging and visualization techniques.
    b. It is highly recommended that the trainee attend a photography and/or Adobe Photoshop® course taught at a local community college or camera shop. The trainee is also encouraged to attend classes taught by other law enforcement agencies such as the FBI.
    c. Since each laboratory in the Division:
        i. Has different cameras and imaging systems, the trainer for this module in each lab will describe the main features of each camera that is used, the reason for using one camera over another in a particular situation, and how the cameras are attached to the microscope.
        ii. Has different imaging processing software the trainer for this module in each lab will describe the imaging processing software used in that laboratory and go over the basic points of image duplication, adjusting size, adjusting brightness, contrast, color, hue and saturation, affixing scales or magnification descriptors to images.
2. Visualization Techniques
    a. Oblique lighting
        i. It works by scattering light off of particles thus causing contrast between an object and the substrate. This is particularly useful in searching for reflective material on clothing such as glass or photographing two-dimensional objects in which there is only a light deposit or removal of dust on a substrate
    b. Filters
        i. Various filters are available which can enhance a certain color or contrast, to polarize the light waves before they enter the camera's lens thus reducing glare, or neutral density filters to control the amount of light getting to the lens. There are too many filters and their uses to mention here but sometimes the best one to use can be found by trial and error.
    c. Hand-held ultra-violet (long and short wave) – often referred to as a 'black light'
        i. These can be sued to search for stains such as vomit, examine the ultra-violet properties of rocks and minerals, and used to observe if optical brightners are present on fibers such as seen in duct tape.
    d. Alternate light source
        i. An ALS delivers a high intensity light of adjustable wavelength. Different types of physical evidence such as semen and fibers may fluoresce when exposed to this light.
    e. Infrared (IR)
        i. Certain substances will either absorb or reflect different wavelengths that are outside the visible region. For example, blood and gunshot residue will both absorb infrared and x-rays. This can be useful when searching for or trying to document these types of evidence when they are on a dark substrate. However, this is a trial by error method, if the substrate also absorbs IR, then there is no

contrast between the two substances and thus they cannot be readily photo-documented.

3. Imaging Technology
    a. Digital SLR Cameras
        i. These cameras vary from film SLR cameras in many ways. The biggest difference is the way in which the image is recorded. The D-SLR captures and image and stores the digital information to some sort of media device so that it can later be read by a computer of directly by a printer. A comprehensive discussion on the difference between film based and digital SLR cameras is well beyond the scope of this document. It is important to understand the different files and types of photographs that can be saved using a digital SLR.
    b. Compact Digital Cameras - Often referred to as “point and shoot”.
        i. Compact digital cameras represent the newest generation of the point and shoot camera. They differ from SLR cameras in that they do not possess the ability to changes lenses and sometimes do not possess other functions, such as manual control of both the shutter and aperture at the same time.
        ii. These cameras are extremely versatile and often provide many useful features that are not found on SLR cameras. These features may include a rotating viewing screen, the ability to easily mount to a microscope, and macro capabilities without having to switch lenses. These cameras often provide high quality photographs without any setup. For our purposes, they are best utilized for taking general photographs of evidence items with the lab.
    c. Digital microscope cameras
        i. These devices are connected directly to microscopes and can be used in photomicrography, live video and video-recording.
    d. IR/UV digital cameras
        i. Certain substances will either absorb or reflect different wavelengths that are outside the visible region. For example, blood and gunshot residue will both absorb infrared and x-rays. This can be useful when searching for or trying to document these types of evidence when they are on a dark substrate. However, this is a trial by error method, if the substrate also absorbs IR, then there is no contrast between the two substances and thus they cannot be readily photo-documented.
    e. Since each laboratory in the Division:
        i. Has different cameras and imaging systems, the trainer for this module in each lab will describe the main features of each camera that is used, the reason for using one camera over another in a particular situation, and how the cameras are attached to the microscope.
        ii. Has different imaging processing software the trainer for this module in each lab will describe the imaging processing software used in that laboratory and go over the basic points of image duplication, adjusting size, adjusting brightness, contrast, color, hue and saturation, affixing scales or magnification descriptors to images.
4. Digital Imaging System
    a. Knowledge of a digital imaging system is imperative to producing high quality photographs that may be used for comparisons or analysis.
    b. A digital imaging system may consist of a computer, an imaging capture source, printer and scanner. Any of the devices may be the limiting factor in the resolution of the final product.
    c. The computer must be of sufficient quality to allow for digital enhancements of digital images.
    d. The scanner must be of sufficient quality to properly represent the item being scanned.
    e. The final output, the printer must be of sufficient quality to display the image to the end user.

5. Photography Techniques
    a. To be able to use the lighting techniques discussed above with the camera(s) available and be able to capture the observations of interest.
6. Adobe® Photoshop®
    a. To be able to have an understanding of how Adobe® Photoshop® may be used as an evidence screening tool for contact transfer examinations.
    b. George Reis' book (See Readings and References) contains a small sampling of the capability of Photoshop. The trainee should be familiar with a series of these skills including:
        i. Levels adjustment
        ii. Color balance
        iii. Color isolation
        iv. Measuring objects

21.3 SAFETY

Some wavelengths of light may not be safe to observe directly (especially if the ALS can operate in the UV range).

21.4 SUGGESTED READING

1. DeForest PR, Gaensslen RE, Lee HC. 1983. Appendix 3: Fundamentals of Photography. In: Forensic Science: An Introduction to Criminalistics. New York: McGraw-Hill. p. 426-350.
2. Delly J G. 1988. Photography Through the Microscope. Eastman Kodak Company.
3. McCrone WC, McCrone LB, Delly JG. 1995. Photomicrography. In: Polarized Light Microscopy. Chicago: McCrone Research Institute, Inc. p. 69-94.
4. Reis G. 2007. Photoshop CS3 for Forensics Professionals. Indianapolis:Wiley Publishing.
5. Scientific Working Group Imaging Technology (These documents can be accessed through the International Association for Identification website, www.theiai.org.)
    a. Section 1: Overview of SWGIT and the Use of Imaging Technology in the Criminal Justice System, Current Version.
    b. Section 3: Guidelines for Field Applications of Imaging Technologies in the Criminal Justice System, Current Version.
    c. Section 5: Recommendations and Guidelines for the Use of Digital Image Processing in The Criminal Justice System, Current Version.
    d. Section 6: Guidelines and Recommendations for Training in Imaging Technologies in the Criminal Justice System, Current Version.
    e. Section 11: Best Practices for Documenting Image Enhancement, Current Version.
6. www.forensictv.net/Downloads/digital_imaging_and_photography/forensic_scientists_guide_to_photography_by_brian_gestring.pdf
7. User manual from your camera.
8. Other manuals suggested by your trainer.

21.5 STUDY QUESTIONS

1. Why do you take pictures of evidence?
2. When should you use a scale during documentation of case work?
3. Why should a scale be used with photography? How does the plane of the scale versus the plane of the object in the photograph impact the resultant image?
4. Why is it important to know the workings and controls of your camera(s)?
5. Why is it important to have your camera mounted to a copy stand, tripod, or other type of stationary device while taking close-up images?

6. When processing your image why is it important to do the editing on the copy of the image rather than the original?

21.6 PRACTICAL EXERCISES

Record everything you do to obtain the images in the following exercises in your notes. Make sure all images have an indication of the scale and/or magnification.

1. Your instructor will provide you with an item of clothing that had been "seeded" with a variety of trace evidence (paint, fibers, other) for you to search using oblique lighting. Document your observations and discuss with your instructor.

2. Create dusty shoe impressions on a non-carpeted floor and flat substrates such as cardboard, wood, plastic and glass. Using a flash light and, if available, a high intensity light source, search using different angles of lighting for the impressions which were created. Note if there are any impressions that become more apparent or if the angle of light makes any difference. Document your observations and discuss with your instructor.

3. Get several pieces of duct tape and look at the fibers with a "black light". Document your observations and discuss with your instructor.

4. Working with your instructor you will become familiar with the ALS in your facility. Your instructor will demonstrate safety, wavelengths and filters, and common forensic materials/fluids that fluoresce. The trainee will search known samples on textiles and document their observations and ALS settings. The known samples must include:

   Seminal fluid
   Saliva
   Vomit
   Blood on a dark cloth
   Gun shot through a dark cloth

   Discuss your results with your instructor.

5. Get comfortable in the operation of your lab's point and shoot digital camera by practicing taking images of various items. Be familiar with using different colored backgrounds (white, gray and black) and various rulers. Take images with and without flash. Take images with various settings of the cameras white balance. Items to be photographed for review must include:

   ~ ½ teaspoon of table salt
   image(s) of your shoes including outsoles
   a pair of adult jeans
   a mirror

   Discuss your results with your instructor.

6. Get comfortable in the operation of your lab's digital SLR camera by practicing taking images of various items. Take a series of images while adjusting the f/stop (keeping the aperture fixed) of 2 objects (such as books) at different distances (one at 3 feet and one at 6 feet) from the camera.
   a. Capture an image of the closet book to the camera being the only object in focus. Record the settings of your camera for review.
   b. Capture an image of the farthest book to the camera being the only object in focus. Record the settings of your camera for review.

   c. Capture an image of both books being in focus. Record the settings of your camera for review.

7. Using the low and high shutter speed settings of your digital SLR camera, take photographs of a moving vehicle.
   a. Capture an image so that the car in your image appears not to be moving. Record the settings of your camera for review.
   b. Capture an image so that the car in your image appears to be a blur. Record the settings of your camera for review.

8. If your laboratory has an image capture system your instructor will give the trainee a basic overview and demonstrate how to utilize.

9. Your instructor will give the trainee a basic overview of the Photoshop software and its uses in the field of forensics.

10. Additional exercises may be added at the discretion of the trainer.

22 EVIDENCE RECOVERY

22.1 OBJECTIVES

- The trainee will be able to recognize multiple types of evidence on submitted items.
- The trainee will be able to document, collect, and preserve trace evidence from various items of evidence.
- To become familiar with the proper packaging of debris and trace material.
- The trainee will become aware of the relationship of the physical characteristics of trace evidence and substrates and the persistence of transferred evidence.
- To familiarize the trainee with substrates which may contain latent prints and/or biological evidence and the proper preservation of this evidence.

22.2 TOPIC AREAS

1. Locard's Exchange Principle states in principle, whenever objects come into contact, a transfer of material will occur between the objects. Trace evidence that is transferred can be used to demonstrate a connection between objects, individuals, vehicles, and locations. An understanding of the transfer and persistence of various types of materials on different substrates will aid the examiner in interpreting the significance of the trace evidence.
2. The importance of trace material(s) as associative evidence relies on the detection of the material, the documentation of the location of the material on the substrate, a description of the material, a description of the substrate, and the appropriate collection of the material to preserve the integrity of the evidence. Patterns or markings found on trace evidence may require additional written and photographic documentation. Since a physical match between a questioned item of evidence and its source is possible, care should be taken to protect the edges and markings that may be on both items.
3. It is important to recognize those materials that are commonly found in a specific type of evidence, such as reflective beads in road debris, as they may be of little evidential value. Alternatively, the case scenario may dictate that some more common types of trace materials are critical evidence.
4. The item may also bear evidence for examination in other areas of expertise, such as biological stains and fingerprints. Coordination of the order of multiple examinations is critical to maintain the integrity of all evidence types. Preservation of all types of evidence is critical during collection and documentation of trace materials.

22.3 SAFETY

1. Hands must not be blindly placed into packaging or into evidence clothing pockets.
2. A particulate mask should be worn when scraping down evidence as this process can generate airborne dust and blood particles.

22.4 SUGGESTED READING

1. Locard E.1930. The Analysis of Dust Traces. Part I. American Journal of Police Science. 1:276-298.
2. Pounds CA, Smalldon KW. 1975. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part I. J Forensic Sci Soc.15:17-27.
3. Pounds C A, Smalldon KW. 1975. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part II. J Forensic Sci Soc.15:29-7.
4. Pounds C A, Smalldon KW. 1975. The transfer of fibres between clothing materials during simulated contacts and their persistence during wear, part III. J Forensic Sci Soc.15:197-207.

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

22.5 STUDY QUESTIONS

1. Using your knowledge/experience and the FLSB Forensic Services Guide describe how each of the following should be packaged and what types of examinations may be conducted on these types of evidence:
    a. tobacco leaves
    b. wet cigarette (with water)
    c. short human hairs with roots attached
    d. small, thin, yellow yarns
    e. putty
    f. paper clip
    g. soil (dried)
    h. paint chip (~ ½ cm)
    i. smokeless powder
    j. slightly folded over piece of duct tape
    k. metal pieces (from lathe) for a metal examination
    l. glass (1 2 x 2 inch square, 1 ½ cm fragment and several round spheres)
    m. shoe
    n. mylar film with impression
    o. round piece of pumice (piece originated from a pocket of "stone washed jeans")

22.6 PRACTICAL EXERCISES

1. Debris collection exercise
    a. Collect debris from various indoor and outdoor sources such as:
        i. Floors and carpeting from a residence and vehicles
        ii. Airborne debris fallen onto tape left in indoor and outdoor settings
        iii. Road and gutter debris
        iv. Air dried debris from the banks of fresh and saltwater sources
        v. Tape lifts of debris from clothing, furniture, vehicles, etc.
    b. Examine the debris visually with overhead and oblique lighting, with UV light, and with stereomicroscopy at low to high magnification. Examine tape lifts visually and with stereomicroscopy.
    c. Separate like materials into groups such as hairs, fibers, vegetative materials, glass, plastic, etc.
    d. Mark items on the tape lifts, excising selected items for further examination (see your trainer for which items).
    e. Describe the materials in your notes and sketch items.
    f. Determine the dimensions of representative samples of each group with a ruler or a microscale.
    g. Transfer representative samples of each group to the appropriate container; paper packets, Post-its, cushioned boxes, etc.
    h. Photograph the debris and selected samples.
2. Prepare "evidence items" so that there will be duplicates to examine in exercise "c" - Persistence of Trace Evidence. Consult the trainer for specifics of preparation of these items.
    a. Examine items loaded with known trace evidence such as:
        i. Materials such as glass, wood, wallboard, duct tape, electrical tape, architectural and automotive paint, forcefully transferred to clothing and weapons such as wrenches, baseball bats, axe handles, etc.

ii. Clothing items, other items that may be submitted as items of evidence loaded with debris, food and drink stains, biological stains, weapon damage including gunshot holes, building materials, footwear/tire tread impressions, etc.

b. Examine each item visually with intense and oblique lighting, alternate light sources, and with stereomicroscopy.
c. Document and collect obvious trace evidence found on the items.
d. Document the position and physical properties.
e. Brush down the item to collect debris (taking care to preserve stains and damage), separating the debris into groups.
f. Separate, examine, document and preserve debris materials as described in Exercise 1.

3. Persistence of Trace Evidence exercise
    a. Prepare (load) a variety of clothing types to include nylon windbreaker, wool sweater, and denim jeans with known brightly colored fibers. Wear each garment for the same period of time (4-8 hours), and tape lift each and compare the amount of fibers transferred to the tape.
    b. Examine or re-examine items; documenting and collecting trace evidence as described in previous lessons.
    c. Compare numbers and types of trace materials collected from “new” vs. “old” evidence on the different substrates.
4. Contamination Exercise
    a. Observe the adhesive of several new sticky notes under a stereomicroscope using at least 250X magnification. Place these notes in multiple exam areas that you may use. Observe the changes in materials present the next day.
    b. Put on a pair of exam gloves and observe them under a stereomicroscope using at least 250X magnification. Type on a keyboard, write a few notes, take a photo with the camera, and/or touch some clothing. What do the gloves look like after 20 minutes? An hour?
    c. Survey the laboratory for in-use two-inch tape roll dispensers with attached tape. Using a stereomicroscope, examine the tape edges for contamination, specifically fibers. Record what you find.
    d. Obtain a two-inch tape roller dispenser. Attach a roll of new clear tape used for tape lifting to the roller. Examine the edges for any contamination using a stereomicroscope. Record what you find. Find several willing participants and gently rub the tape roll over their clothing. Drop the tape on the floor several times. Examine the edges for fibers and trace evidence and record what you find. Always remember to store the tape lifting tape rolls in a zip lock bag when not in use to prevent contamination.

PRIMARY FOUNDATION MODULE 1 CHECKLIST

Trainee:	Trainer:		
	Trainee Initials/Date	Trainer Initials/Date	Time for Completion
General Forensic Procedures			
Intro/orientation/HR paperwork			
TAS/TEMS/SharePoint			
QM/OM/FSG			
LIMS			
Evidence handling			
Lab Safety			
Safety tour/orientation			
Reading			
Study Questions			
Practical Exercises			
Criminalistics 101			
Ethics			
Reading			
Lecture/Discussion			
Cognitive Bias			
Reading			
Study Questions/Practical Exercises			
Law Basics & Court Testimony			
Reading			
Lecture/Discussion			
Observation of testimony			
Scientist Observed	Date	Type of case	

PRIMARY FOUNDATION MODULE 2 CHECKLIST - PAGE 1

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Balances and Weighing				
	Reading			
	Study questions			
	Practical exercises			
Thin Layer Chromatography				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Gas Chromatography/Flame Ionization Detector				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Mass Spectrometry & Pyrolysis				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Capillary Electrophoresis				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Infrared Spectroscopy				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			

PRIMARY FOUNDATION MODULE 2 CHECKLIST - PAGE 2

		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Gas Chromatography/FTIR				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Raman Spectroscopy				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
High Performance Liquid Chromatography				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Scanning Electron Microscopy/Energy Dispersive X-Ray				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Spectroscope				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
X-Ray Fluorescence Spectrometry				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Comprehension examination				

PRIMARY FOUNDATION MODULE 3 CHECKLIST

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Basic Practical Microscopy				
	Reading			
	Review PowerPoint presentations			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Comprehension examination				
Competency testing				

PRIMARY FOUNDATION MODULE 4 CHECKLIST

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Imaging & Visualization				
	Reading			
	Review PowerPoint presentations			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Evidence Recovery				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Comprehension examination				
Competency testing				