Washington State Patrol

Crime Laboratory Division

Materials Analysis
Instrumentation and Techniques Training Manual

May 2023

Contents

1 INTRODUCTION

1.1 TRAINING PLANS

The supervisor in consultation with the employee will determine what subdiscipline(s) an employee will receive training in. A comprehensive training plan to accomplish training in a subdiscipline will follow the requirements in the current QOM. Training plans may include remedial readings, remedial training modules, and written tests deemed necessary. Training will be customized to meet the needs of the trainee. For training plans that include many chapters, the technical lead may be listed as the trainer and assign instructors for different chapters.

The supervisor in coordination with the technical lead(s) or their designee will review and assess a new trainee's strengths and deficiencies in the following areas:

- Knowledge of:
  - Analytical chemistry
  - Organic chemistry
  - Inorganic chemistry
  - Physical chemistry
  - Microscopy
  - Instrumentation
  - Physics
- Laboratory experience
- Aptitude for:
  - Color comparisons
  - 2D pattern recognition
  - 3D pattern recognition

Trainees who have prior related training and experience may need modifications to the chapter requirements and/or chapter testing. Preapproval of such modifications must be obtained from the technical lead. The required documentation of such related training and/or experience shall be left to the supervisor in coordination with the technical lead(s) or their designees.

1.2 PURPOSE AND SCOPE

This manual is designed to learn the necessary instruments and techniques listed as prerequisites for training in a specific subdiscipline with the Material Analysis functional area. Each chapter stands alone. The focus is on the theory of these instruments and techniques. Although practical exercises are given, the trainee is expected to gain more practical experience with these instruments and techniques during the specific subdiscipline training. Courtroom training will also be covered in specific subdiscipline training.

Pipettes and Glass Refractive Index Measurement (GRIM) are used solely by one subdiscipline. Those instruments and techniques are covered under their respective subdiscipline training manuals and are not included here.

1.3 EXPECTATIONS

Some of the chapters have a "prerequisite" instrument or technique listed. The trainee must have fulfilled all of the requirements for a prerequisite, including passing the written test, before beginning such chapters.

The instructor shall be experienced in the instrument/technique covered by that chapter. The instructor shall also discuss with the trainee the training and reference materials (if any) available on the FLSB Isilon drive. The instructor is expected to review the study questions and practical exercises with the

trainee. Reviews may be performed in written or oral form, but documentation of the contents of that review must be included maintained. The review should include discussion of the results of the practical exercises.

Although the trainee's primary interaction shall be with the assigned instructor, this program promotes and encourages discussions with other experienced examiners. When possible, the trainee should also take outside courses related to that instrument/technique.

The trainee shall record and retain notes and observations for each study segment. The trainee notes should be neatly maintained in either written or electronic form (or a combination thereof), kept up to date throughout training, and should be present during conversations with the trainer. Upon completion of training, the trainee should maintain all training documentation including notes for the duration of their career.

Instrument authorization must be received from the laboratory manager that has the instrument prior to the trainee working on the instrument. In addition, the trainee must obtain the approval of the trainer before working on, and especially manipulating any parameters, on the instrument.

The instructor has the option to recommend modifications or additions to a specific chapter. The recommended modifications or additions must be pre-approved by the technical lead prior to implementing those changes. Documentation of that approval should be included with the trainee's records (i.e. copy of an email).

Instrumental data should be saved in an electronic format in addition to printouts so that the instructor and trainee can review and compare the original data together if needed.

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 before training continues. It is important that these deficiencies be openly and promptly discussed among the trainee, instructor(s), technical lead(s), and/or supervisor, as appropriate. Repeating training steps and testing may be necessary to satisfactorily complete this training program.

Each chapter will have a set of written exam questions that must be passed by 80%. Exams may cover multiple chapters, but each chapter will be graded separately. Exams will be closed book.

The trainer is responsible for writing an interoffice communication (IOC) to the trainee's supervisor when the trainee has successfully completed a chapter. IOCs for completion of a specific instrument or technique chapter may be delayed in order to group multiple chapters together. The trainee's supervisor shall maintain copies of training IOCs and authorizations.

1.4 ORGANIZATION OF THE TRAINING MANUAL

Each chapter is designed to stand-alone and is organized into the following eight parts:

Reminders

- A recap of expectations relevant to a specific chapter.

Prerequisites

- A list of other chapters that must be completed prior to starting on that chapter.

Objectives

- A list of the goals for each chapter

Topic Areas

- A list of subjects and vocabulary that will be covered during training.
- Prior to taking an exam, the trainee should review the topic areas list to make sure none of the topics were missed or confusing.

Safety

- A list of precautions to be taken during the practical exercises of that chapter

Readings

- The list of minimum required readings to complete the training.
- A subset of readings are listed under Resources. These readings are typically books or other readings that are not required to be read in full, but should be skimmed as they may aid in the completion of study questions and/or practical exercises.
- The readings are designed to not be overly duplicative or extensive.
- Additional readings are available within the system.
- The technical leads continue to build comprehensive bibliographies of relevant readings available within Materials Analysis. Please check the Isilon drive and/or contact a technical lead if additional readings are needed for the different instruments, techniques, and subdisciplines.

Study Questions

- A series of questions that are expected to have written answers.
- The questions are designed to ensure reading comprehension & encourage discussions.
- Written answers will be maintained in the training documentation.

Practical Exercises

- These are hands on activities that are to develop understanding of the theory as well as the practical application.
- Notes, observations, and printouts on the practical exercise will be maintained in the training documentation.

2 BALANCES (SEIZED DRUGS)

2.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument(s) during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will be authorized to use the instrument in casework upon successful completion.

2.2 PREREQUISITES

- None

2.3 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.

2.4 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 balances
    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
    d. Top-loading 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
9. ***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
10. WSP CLD MA quality control requirements

2.5 SAFETY

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

2.6 READINGS

1. Anonymous **(2015)** *Weighing the Right Way*. Mettler-Toledo AG, pamphlet 720906A.
2. Anonymous **(unknown)** *Correct Use and Handling of Analytical and Microbalances*. Sartorius Weighing Technology GmbH, publication No. W—0135-a12065.
3. Berg C **(1996)** *The Fundamentals of Weighing Technology: Terms, Methods of Measurement, Errors in Weighing*. 1st edition, English translation by Sartorius AG Translation Services Department, Sartorius AG.
4. Hill K **(2019)** "Analytical balances and proper weighing practices" in *Lab Manager* e-publication dated May 22, 2019.
5. Current MATP chapter.

2.7 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 drug cases? What can be done to minimize the physical influences that impact weighing seized drug items?
   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?

2.8 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 sugar and artificial sweetener packages.
   d. Penny, nickel, dime, & quarter

3 BALANCES (NON-SEIZED DRUGS)

3.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument(s) during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will be authorized to use the instrument in casework upon successful completion.

3.2 PREREQUISITES

- None

3.3 OBJECTIVES

- To familiarize the scientist or technician (other than seized drug scientists) with the appropriate use of electronic balances, good weighing practices and to prevent deleterious adjustments of seized drug balances.
- To familiarize the scientist or technician (other than seized drug scientists) with the common terms associated with weighing and electronic balances.

3.4 TOPIC AREAS

1. Mass versus weight
2. Electronic scales
    a. Load cell transducers measure force that occurs when gravity acts on a mass.
    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.
    c. As gravity changes, it is necessary to calibrate the scale to accommodate for the change in gravity.
3. Calibration versus adjustment
    a. Calibration - Determining the deviation between the measurement value and the true value of the measurement variable under specified measuring conditions.
    b. Adjustment – Correcting the deviation between the measurement value and the true value
    c. DO NOT perform any calibration functions including the use of internal or auto calibration.
4. Terms used in weighing
    a. Accuracy – a qualitative name for the degree to which test results match the reference value, which can be the correct or expected value, depending on the definition or agreement.
        i. How close the balance display comes to the actual weight of the sample.
    b. Corner Load – deviation of the measurement value through off-center (eccentric) loading. The corner load increases with the weight of the load and its removal from the center of the pan support.
        i. If the display remains consistent even when the same load is placed on different parts of the weighing pan, the balance does not have corner-load deviation. For this reason, with high precision balances, it is important to make sure the weighing sample is always placed exactly in the middle.

   c. Linearity – refers to the quality of delivering identical sensitivity throughout the weighing capacity of a balance or scale.
   d. Precision – the closeness of agreement between independent measurement values obtained under stipulated conditions. Precision depends only on the distribution of random errors and does not relate to the true value of the measurement variable (accuracy).
      i. Precision can only be evaluated when there are several measurement values.
   e. Readability – the smallest difference between two measured values that can be read on the display. With a digital display, this is the smallest numerical increment, also called the scale interval.
   f. Repeatability – a measure of the ability of a balance to show the same result in repetitive weighings with one and the same load under the same measurement conditions.
      i. Same person, same time, same conditions
   g. Reproducibility – similarity between the measurement values of the same measured variable, even though the individual measurements are carried out under different conditions.
      i. Different person, different time, different conditions
5. Types of balances
   a. Precision balance/top-loading – 1 g to 0.001 g readability.
   b. Semi-micro balance – 0.00001 g
   c. Analytical balance – 0.0001 g
6. Good weighing practices
   a. Balance location
      i. On a weighing bench which is stable, antimagnetic, protected from electrostatic charges, wall or floor installation
   b. Vibration free
   c. Free from drafts
   d. Under constant temperature
   e. Stable humidity ideally between 45 -60% relative humidity
   f. Never in direct sunlight (radiant heat)
   g. Level surface
      i. Only a level balance can deliver reliable results
7. Power
   a. Balances should be left on at all times.
      i. Balances need to “warm up” if they have been turned off.
      ii. Semi-micro and analytical balances – 120 minutes
      iii. Precision/top-loading balances – at least 30 minutes
8. Weighing vessels
   a. Use the smallest possible vessel.
   b. Plastic weighing vessels are not recommended as they can cause static electricity.
   c. The sample, weighing vessel and balance should all be at the same temperature
      i. If the objects weighed (vessel) are too warm, the value displayed will be too low.
      ii. If the objects weighed (vessel) are too cold, then the value will be too high.
   d. Always place the vessel in the middle of the weighing pan (to prevent corner load errors).
   e. Best practice is to not touch weighing vessels with your bare hands. Wear gloves when weighing.
9. Weighing procedure
   a. A smooth, steady pace improves accuracy
   b. Do not lean on the table or bench while weighing
   c. If the balance has a draft shield, use it.

d. Position the weighing vessel in the center of the pan.
e. Zero the balance.
    i. Zero "0" – Should be used in most applications.
        1. When the Zero button is pressed, the user manually sets a new zero point on the balance. This allows the balances to "ignore" the weight of the weighing vessel.
    ii. Tare "T" – generally will not be used
        1. The tare button is used to manually add a tare point to the weighing process. This is useful when weighing a sample into a beaker, where the weight of the beaker is also important, or when you need to add more than one sample to one single container. The balances records gross, tare, and net weights.
f. Place sample in the weighing vessel. Alternatively, the weighing vessel may be removed from the balance and the sample placed into the weighing vessel. The weighing vessel is then returned to the balance pan.
g. Allow the balance to stabilize and record the weight.
    i. All electronic balances give a visual indication of weight stability.

10. Cleanliness
    a. Cleanliness reduces disturbances. Keep the pan and weighing chamber clean at all times.
    b. Use a fine brush to remove residues.
    c. Use an absorbent, lint-free cloth to remove liquids.
    d. Note – most balance pans can be removed for cleaning so as not to damage the balance itself.
11. ***<u>When in doubt, ask a seized drug scientist to help you with the balance.</u>***

3.5 SAFETY

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

3.6 READINGS

1. Anonymous **(2015)** *Weighing the Right Way*. Mettler-Toledo AG, pamphlet 720906A.
2. Anonymous **(unknown)** *Correct Use and Handling of Analytical and Microbalances*. Sartorius Weighing Technology GmbH, publication No. W—0135-a12065.
3. Hill K **(2019)** "Analytical balances and proper weighing practices" in *Lab Manager* e-publication dated May 22, 2019.
4. Current MATP chapter.

3.7 STUDY QUESTIONS

1. Why should the balance be left on at all times? How long do you need to let a balance "warm up" before use?
2. What calibration or adjustment functions should be performed on a balance before use?
3. Describe an appropriate weighing vessel.
4. Which balance should you use to weigh the following samples:
    a. 1.8 kilograms
    b. 325 grams
    c. 10 grams
    d. 0.3 gram

3.8 PRACTICAL EXERCISE

1. Demonstrate proper use of a precision balance for weighing a sample.

4 CAPILLARY ELECTROPHORESIS

4.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

4.2 PREREQUISITES

- None

4.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

4.4 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 dependent
ii. Temperature dependent

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. Electrophoretic 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. Calculated by:

$$N = \frac{\mu El}{2D}$$

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 increases

$$R = \frac{1}{4}\sqrt{N}\,\frac{\Delta\mu}{\bar{\mu}}$$

where $\Delta\mu = \mu_2 - \mu_1$

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

c. Quantitation
    i. Migration dependent
    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 (buffer pH 8.2).
      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. Adapted from Knops LA, Northrop DM, and Person EC (2006) “Capillary electrophoretic analysis of phosphorus species in clandestine methamphetamine laboratory samples” in J. For Sci. Journal of Forensic Sciences 3551(1):82-86.
    ii. Cation analysis
      1. Uses a commercially available buffer kit (buffer pH 3.2).
      2. Detection is achieved by indirect UV detection.

3. Adapted from Riviello JM and Harrold MP (1993) “Capillary electrophoresis of inorganic cations and low-molecular-mass amines using a copper-based electrolyte with indirect UV detection” in *Journal of Chromatography A* 652(2): 385-392.

iii. Organic species analysis
1. Micellar electrokinetic separation method that allows for the separation of neutral species based on partitioning of analytes with a micellar pseudophase.
2. Uses a SDS/borate buffer (pH 8.4).
3. This method uses direct UV detection.
4. Adapted from Northrop DM, Martire DE, and MacCrehan WA (1991) “Separation and identification of organic gunshot and explosive constituents by micellar electrokinetic capillary electrophoresis” in *Analytical Chemistry* 63(10): 1038-1042.

iv. General drug screen
1. This method is similar to the organic species method.
2. This is a modified micellar electrokinetic method that includes a small fraction of an organic solvent in the buffer (15% ACN) to broaden the dynamic elution range for organic species
3. Adapted from Weinberger R and Lurie IS (1991) “Micellar electrokinetic capillary chromatography of illicit drug substances” in *Analytical Chemistry* 63(8): 823-827.

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).

4.5 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.

4.6 READINGS

1. Hägele JS, Hubner EM, and Schmid MG **(2019)** “Chiral separation of cathinone derivatives using β-cyclodextrin-assisted capillary electrophoresis – Comparison of four different β-cyclodextrin derivatives used as chiral selectors” in *Electrophoresis* 40(14):1787-1794.
2. Hauser FM, Hulshof JW, Rößler T, Zimmermann R, and Putz M **(2018)** “Characterisation of aqueous waste produced during the clandestine production of amphetamine following the Leuckart route utilizing solid-phase extraction gas chromatography-mass spectrometry and capillary electrophoresis with contactless conductivity detection” in *Drug Testing and Analysis* 10(9):1368-1382.
3. Jorgenson JW and Lukacs KD **(1981)** “High-resolution separations based on electrophoresis and electroosmosis” in *Journal of Chromatography A* 218:209-216.
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12. Current MATP chapter.

4.7 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?

4.8 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 a set of unknowns from the trainer. The same unknown sample may be run on multiple methods.
    a. Obtain at least one cation unknown and use the cation method to identify the unknowns.
    b. Obtain at least one anion unknown and use the anion method to identify the unknowns.
    c. Obtain at least one organic unknown and use the micellar method to identify the unknowns.
    d. Obtain at least one drug unknown and use the drug screen method to identify the unknowns.
    e. Collect and compare UV spectra for one of the above unknown samples.

5 CHEMICAL AND PHYSICAL CHARACTERIZATION

5.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee will be authorized to use these techniques in casework upon successful completion.

5.2 PREREQUISITES

- Microscopy - Basic

5.3 OBJECTIVES

- To familiarize the trainee with the theory and application of microchemical methods.
- To provide practical experience in using microchemical methods to the identification of unknown materials.

5.4 TOPIC AREAS

1. Introduction to microchemical methods of Chamot and Mason
2. Solubility, miscibility, extraction, and recrystallization
3. Use of chemical test strips (liquid sampling)
4. Identification of magnetic properties
5. Use of Dräger sampling tubes (vapor sampling)
6. Use of flame tests (wires, pipettes)
7. Immunoassay test strips (aka lateral flow immunoassay systems)
8. Effervescence type tests
9. Microchemical Methods
    a. Spot or Color Tests
    b. Microchemical Crystal Tests
        i. Tests for Inorganics
            1. Cation Tests
            2. Anion Tests
        ii. Tests for Organics
            1. Functional Group Tests
            2. Organic Compound Tests
10. Microchemical reactions for common cations and anions

5.5 SAFETY

- Tools for the manipulation of small particles are sharp and pose cutting and puncture-wound hazards.
- Care must be exercised in the use of fine tweezers, scalpels, tungsten needles, and other sharp tools.
- Strong acids are corrosive on contact with skin.
- Caution must be exercised when performing flame tests to prevent a fire.

5.6 READINGS

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2. Chamot EM and Mason CW **(1958)** *Handbook of Chemical Microscopy Volume I* New York (NY): John Wiley & Sons [Read chapter 13 "Chemical Crystallography; Preparation of Crystals for Study", pp 384-418].
3. De Forest PR **(2002)** "Foundations of Forensic Microscopy" in: Saferstein R. *Forensic Science Handbook*. Vol. 1, 2nd Ed. Upper Saddle River (NJ): Prentice Hall. [Read "Microchemical Methods" on pp 311-313].
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11. Current MATP chapter.

RESOURCES

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3. Feigl F **(1966)** *Spot Tests in Organic Analysis*. 7th Ed. New York (NY): Elsevier Publishing.
4. McCrone WC, Draftz RG, and Delly JG **(1967)** *The Particle Atlas*. Ann Arbor (MI): Ann Arbor Science Publishers.
5. McCrone WC, McCrone LB, and Delly JG **(1984)** *Polarized Light Microscopy*. Chicago (IL): McCrone Research Institute.

5.7 STUDY QUESTIONS

1. What is the difference between solubility and miscibility? How could these techniques be used to characterize unknown materials?
2. What is magnetism and how could this be useful in characterizing materials?
3. Explain the difference between chemical spot tests and microchemical tests.
4. Explain Chamot and Mason methods I, II, III, and IX for microchemical testing.
5. What conditions must exist for microchemical tests to work properly?
6. Describe a method to differentiate ammonium ion from potassium ion.
7. Describe an analytical procedure to identify an unknown crystalline material using microchemical methods.
8. How could effervescence assist with particle characterization?
9. Describe the theory behind a flame test.
10. Describe three different chemical test strips or indicator papers which could be useful for characterizing material received in case samples.
11. What are Dräger sampling tubes and in what kind of case samples would they be used?
12. Compare and contrast the mechanism of immunoassay test strips versus chemical test strips.

5.8 PRACTICAL EXERCISES

1. Determine solubility of ten unknown samples. Test each unknown solubility in at least: water, dilute acid, dilute base, methylene chloride/chloroform, and methanol/ethanol. Use (attempted) water solutions for the next exercise.
2. Use pH paper to estimate the pH of each water solution/attempted water solution from exercise 1. Be sure to also test the water you used, for comparison.
3. Recrystallize ten known water soluble compounds and obtain photomicrographs of the resulting crystals. Use the method described in Chamot and Mason (Volume 1, 1958).
4. Recrystallize sulfur from chloroform or other appropriate solvent and take photomicrographs.
5. Practice performing microchemical methods for common cations and anions suggested by your trainer based on the methods of Chamot & Mason, Hopen & Kilbourn, Hollifield, and others.
6. Conduct a flame test on at least four different metal salts and describe their color.

6 EVIDENCE RECOVERY

6.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee will be authorized to use these techniques in casework upon successful completion.

6.2 PREREQUISITES

- None

6.3 OBJECTIVES

- To familiarize the trainee to recognize multiple types of evidence that may present on items submitted for materials analysis.
- To familiarize the trainee of the advantages and disadvantages of different collection methods.
- To familiarize the trainee of the advantages and disadvantages of different packaging materials.
- To familiarize the trainee of the advantages and disadvantages of different storage temperatures.
- To familiarize the trainee with the safety recommendations for specific types of evidence.
- To familiarize the trainee to document, collection, and package various types of evidence.
- To familiarize the trainee with background levels of airborne trace materials in different environments.
- To familiarize the trainee with substrates which may contain latent prints and/or biological evidence and the proper preservation of this evidence.

6.4 TOPIC AREAS

1. Locards Principle
    a. Transference
        i. Types
            1. Primary Transfers
            2. Secondary Transfers
            3. Probative Value transfers (Association)
            4. Non-probative value transfers (Background)
        ii. Methods
            1. Airborne
            2. Breakage/Fractures
    b. Persistence
2. Collection Methods
    a. Entire Object
    b. Dismantled Object
    c. Picking
        i. Clean Gloved Hand
        ii. Hand Tools (e.g. forceps, picks, spatulas, pipettes, swabs)
    d. Lifting
        i. Sticky Notes
        ii. Tape Lifts

e. Cutting
    i. Manual
    ii. Power
f. Scrapings
    i. Brush down with spatula
    ii. Gently shaking textiles
g. Vacuumings

3. Packaging
    a. Properties
        i. Inert to the evidence
        ii. Prevents loss
        iii. Sturdy
        iv. Safety
    b. Types of Materials
        i. Paper
        ii. Plastic
        iii. Metal
        iv. Glass
    c. Size
        i. Outer package no smaller than large envelope (8.5 x 11 inches)
        ii. Contents should fill no more than 2/3 of package
        iii. Allow space for other analyst's work products
    d. Labels/Seal Locations
        i. Allow another analyst to open evidence without breaking a seal
        ii. Allow another analyst to reseal the evidence without covering labels
    e. Sub-packaging
        i. To prevent loss
        ii. To provide convenience for another discipline
4. Creating Child Items
    a. LIMS
    b. Bench Notes
    c. RFLE
    d. Reports

6.5 SAFETY

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

6.6 READINGS

1. Gardner RM **(2005)** *Practical Crime Scene Processing and Investigation*, CRC Press LLC, Boca Raton, FL [Read chapter 2 "Understanding the Nature of Physical Evidence" – pages 23-58].
2. WSP FLSB Forensic Services Guide.
3. Current MATP chapter.

6.7 STUDY QUESTIONS

Review the FLSB Forensic Services Guide. Clarify with your trainer any portions that are unclear.

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. clothing that reportedly has pepper spray on it
b. glass (one 2 x 2 inch square, one ½ cm fragment and several round spheres)
c. metal pieces (from lathe) for a metal examination
d. mylar film with impression
e. paint chip (~ ½ cm)
f. paper clip
g. putty
h. round piece of pumice (piece originated from a pocket of "stone washed jeans")
i. shoe
j. short human hairs with roots attached
k. slightly folded over piece of duct tape
l. small, thin, yellow yarns
m. smokeless powder
n. soil (dried)
o. tablets that contain a fentanyl analogue
p. fresh marijuana
q. wet cigarette (with water)
r. white powder that contains fentanyl
s. Molotov cocktail with liquid and a wick

2. Describe the different types of collection methods, including their advantages and disadvantages (entire object, sample removal, particle picking, tapings, scrapings, vacuumings). What methods are used by the different disciplines?
3. Describe the pros and cons of different packaging materials.
4. What factors should be taken into account when choosing packaging?
5. Most evidence should be stored dry and at room temperature. What materials should be stored in the freezer? In the refrigerator? Should any materials be stored "wet" (not dried out first)?
6. What precautions should you take when working with evidence that will be examined by:
    a. DNA after your analysis (e.g. clothing)?
    b. Latent Prints after your analysis (e.g. a baseball bat)?
    c. Firearms after your analysis (e.g. a gun)?
7. What is the difference between transference and persistence?
8. Describe the types of transference and their casework significance.

6.8 PRACTICAL EXERCISES

1. Discuss with your instructor repackaging of evidence for the four different Material Analysis disciplines. Include in your discussion the following points:
    a. Under what circumstances should you repackage evidence.
    b. Who do you talk to about repackaging evidence?
    c. How do you document repackaging of evidence?
    d. What to do if the evidence will be examined by two different disciplines and may need different packaging for the different disciplines (e.g. DNA/Ignitable Liquids, LP/Tape).
2. Discuss with your instructor when and how a child item should be created and documented.
3. Create two paper packets – one from a square piece of paper and one from a rectangular piece of paper.
4. Observe a demonstration of all six types of collection methods.
5. Practice the six different collection methods with samples provided by your trainer while your trainer observes you.
6. Observe a demonstration of transference. Discuss primary and secondary transfers with your trainer. Discuss persistence with your trainer.
7. Background Airborne Trace Materials 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 notes the next day and document what changes/materials are present.

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. Look 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 collection of background level materials.

7 GAS CHROMATOGRAPHY AND DETECTORS

7.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

7.2 PREREQUISITES

- None

7.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

7.4 TOPIC AREAS

GAS CHROMATOGRAPHY (GC)

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) – 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% Trifluoropropylmethylpolysiloxane
    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)

MASS SPECTROMETRY (MS) & PYROLYSIS (PY)

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

7.5 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.
- 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.
- 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.
- Caution should be exhibited when working with vacuum pumps. Waste oil should be treated as hazardous and should be handled and disposed of appropriately.

7.6 READINGS

GAS CHROMATOGRAPHY

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2. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
3. Rood D. 1999. A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatographic Systems, Systems. 3rd Ed. New York:Wiley-VCH.
4. 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.
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7. Instrument manufacturer manuals.
8. Current MATP chapter.

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.

MASS SPECTROMETRY & PYROLYSIS

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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.
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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.
23. Current MATP chapter.

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.

7.7 STUDY QUESTIONS

GAS CHROMATOGRAPHY

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)
15. What is a good value for the HETP? And why? How is the # of N related to column efficiency?
16. 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?
17. Diagram and explain the van Deemter plot. Why does the lab use helium or hydrogen as a carrier gas for its instruments?
18. What is the Kovats retention index (I)? What does it mean if I = 650?
19. 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
20. Discuss the sample introduction of gases and vapors, volatile liquids and solids into a GC.
21. What factors govern the amount of sample to be injected? How much sample/component can the average capillary column hold? What factors influence this?
22. What temperature should the injection port be under normal circumstances and why?
23. What type of septa are recommended for GC work and why?
24. What are the differences and purposes of split, splitless, pulsed split, pulsed splitless, on-column, and direct on-column injection?
25. What is an injection port liner? What is it made of? Why is it used? Describe the packing process including the materials used.
26. 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)?
27. 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?
28. 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?

29. 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
30. Explain how derivatization is performed, including why it is used sometimes for analysis.
31. If two drug compounds were to co-elute on the GC, what could be done to resolve the peaks?
32. Explain as to a jury how a GC operates.

MASS SPECTROMETRY & PYROLYSIS

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.

7.8 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. 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?
6. 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?
7. 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.
8. Obtain an unknown spectrum from your instructor. Using interpretive methods, give as much information about the unknown compound as possible.
9. Obtain mass spectra for groups of similar compounds (e.g., phenethylamines, homologous series of alcohols, etc.) and compare data from each compound.
10. Obtain pyrograms for several polymers, including polyethylene, polypropylene, polystyrene, polymethyl methacrylate, and nylon. Determine degradation mechanisms for each, and identify major degradation products.

11. Test reproducibility of pyrolysis 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?

8 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

8.1 REMINDERS

- This chapter may be covered by a basic HPLC course sponsored by a vendor.
- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

8.2 PREREQUISITES

- None

8.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

8.4 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 quantitative 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

8.5 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.
- 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 current CLD Safety Manual.

8.6 READINGS

1. Anonymous **(2023)** Beginner's Guide to Liquid Chromatography published by Waters Corporation online and electronically downloaded from https://www.waters.com/nextgen/us/en/education/primers/beginner-s-guide-to-liquid-chromatography.html on March 21, 2023 [Read bookmarked sections What is HPLC? and How does HPLC work?].
2. Kupiec T, Slowason M, Pragst F, and Herzler M **(2004)** "High Performance Liquid Chromatography" chapter 29 in Moffat AC, Osselton MD, Widdop B, and Galichet LY [Eds]

Clarke's Analysis of Drugs and Poisons – Volume 1, 3rd Edition, London: Pharmaceutical Press, pp 500-534.
3. Northrop DM **(2020)** "Forensic Applications of High Performance Liquid Chromatography and Capillary Electrophoresis" chapter 9 in Saferstein R [Ed.] *Forensic Science Handbook*. *Vol. 1*, 3rd Ed. Upper Saddle River (NJ): Prentice Hall, pp 495-562.
4. van Deemter JJ, Zuiderweg FJ, and Klinkenberg A **(1956)** "Longitudinal diffusion and resistance to mass transfer as causes of non-ideality in chromatography" in *Chemical Engineering Science* 5(6): 271-289.
5. Instrument manufacturer's manuals.
6. Current MATP chapter.

RESOURCES

1. Bayne S and Carlin M **(2010)** *Forensic Applications of High Performance Liquid Chromatography*, Boca Raton (FL): Taylor and Francis Group (CRC Press).
2. Willard HH, Merritt LL Jr, Dean JA, Settle FA Jr **(1988)** *Instrumental Methods of Analysis*. 7th Edition. New York: Van Nostrand.
3. Other general text on chromatography.

8.7 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. Theoretical plate (n)
    h. Effective theoretical plate (N)
    i. Theoretical plate height /height equivalent to a theoretical plate (H or HETP)
    j. 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.

8.8 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 a 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.

9 IMAGING AND VISUALIZATION

9.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainer may authorize a qualified analyst as an instructor for remote training. Analysts from CSRT may be an instructor for the photography exercises. Analysts from DNA may be an instructor for ALS exercises.
- The trainee will be authorized to use these techniques in casework upon successful completion.
- The trainee is encouraged to take continuing education courses (e.g. photography, use of Photoshop, etc.)

9.2 PREREQUISITES

- None

9.3 OBJECTIVES

- To familiarize the trainee with the advantages and disadvantages of the different imaging and visualization equipment and techniques available in the CLD.
- To have the trainee demonstrate how to use a point and shoot camera to produce a high quality documentation image.
- To have the trainee demonstrate how to use a digital SLR camera to produce a high quality exemplar image.
- To have the trainee demonstrate how to use the available alternate light sources.
- To have the trainee demonstrate how to use equipment and software for image enhancement.

9.4 TOPIC AREAS

1. Alternate light sources
    a. OSAC lexicon definition
    b. Equipment examples
    c. Light interactions used for detection
        i. Absorption
        ii. Reflection
        iii. Transmission
        iv. Fluorescence
        v. Scattering
    d. Excitation wavelengths
        i. UV (blacklight, shortwave, longwave)
        ii. Visible
        iii. IR
    e. Barrier filters (colored glasses/goggles)
    f. UV protection options (for eyes)
    g. IR protection options (for camera)
2. Directional light sources
    a. Equipment examples
    b. Oblique lighting
3. Digital image capture devices
    a. Types of devices
        i. SLR cameras
        ii. Compact cameras (aka “Point and Shoot”)

iii. Dedicated microscope cameras
iv. Scanners
v. IR/UV cameras

b. Camera Settings
i. F stop/Aperture
ii. Shutter Speed
iii. ISO
iv. White Balance
v. Bracketing
vi. Bulb Setting
vii. Metering

c. Image files
i. Metadata
ii. Lossless file types
iii. Data compression (lossy file types)

d. Camera Distortions
i. Barrel Distortion
ii. Pin cushion
iii. Vignetting

e. Quality images
i. Fill the screen
ii. White balance
iii. Depth of field
iv. Focus
v. Lighting direction
vi. Scales

f. Types of images
i. Documentary images
ii. Exemplar images

4. Image Enhancement
a. Equipment
i. Camera filters (e.g. polarizers)
ii. Light diffusers (e.g. The Cloud Dome)

b. Application Software Examples
i. Adobe Photoshop
ii. Adobe Lightroom
iii. Leica Application Suite – X (LAS-X)
iv. Printer Settings

c. Factors that Effect Resolution on a Final Product
i. Image Capture Source
ii. Original File Type resolution
iii. Final Product Type
1. Image file
2. Printout
a. Paper Type
b. Printer settings
3. Scanned document

iv. Software Algorithms
1. related to Image Insertion into an electronic document
2. related to conversion of file type
3. different types of software (e.g. Word, PowerPoint, Photoshop, Acrobat)

v. Software Settings
1. Compression

2. Output Settings

vi. Printer Settings
1. Paper Type
2. Resolution Settings

d. Introduction to Adobe Photoshop & Related Software
i. Save Original Image
ii. Work Only with Copy of Image
iii. Image Modes
1. Grayscale
2. RGB
3. CMYK
4. Lab Color
iv. Layers
v. Channels
vi. Image Size
vii. Simple image enhancements for documentary images
1. Brightness
2. Contrast
3. Tone
4. Color
viii. Image enhancements for exemplar images
ix. Presentation/Reports
1. Contact Sheets
2. Templates

9.5 SAFETY

- Ultra-violet wavelengths of light are not safe to observe directly. UV protective glasses/goggles must be worn if working with UV emitting sources.

9.6 READINGS

1. Breeding K **(2008)** “Basic theory behind alternate light sources” in *Evidence Technology Magazine* 6(1): 30-33.
2. Causin V and Guzzine G **(2018)** “Light for Crime Scene Examination” chapter 1 in Miolo G, Stair JL, and Zloh M [Eds.] *Light in Forensic – Science Issues and Applications,* Croydon, UK; European Society for Photobiology, Volume 17 in the Comprehensive Series in Photochemistry and Photobiology, pp 3-25.
3. Davenport J **(2017)** “The dPS Ultimate Guide to Photography for Beginners” pamphlet published by Darren Rowse online and electronically downloaded from https://digital-photography-school.com/ultimate-guide-photography-beginners/ on February 3, 2023.
4. Ferrucci M, Doiron TD, Thompson RM, Jones JP, Freeman AJ, and Neiman JA **(2016)** “Dimensional review of Scales for forensic photography” in *Journal of Forensic Sciences* 61(2): 509-519.
5. Forensic Technology Center of Excellence **(2018)** “Landscape study of alternative light Sources” U.S. Department of Justice, National Institute of Justice, Office of Investigative and Forensic Sciences [read Glossary pp 7-8, Introduction pp 9-12, ALS Photography pp 34-35].
6. Gonen N, Gilad Y, Ziv D, Rajs N, and Finkelstein NS **(2022)** “Using an alternate light source to recover sticker marks from a vehicle” in Journal of Forensic Identification 72(3): 313-319
7. Graphic Mills **(2023)** “Color Spaces” electronic download from https://www.graphicsmill.com/docs/gm/color-spaces.htm on February 3, 2023.
8. Pierce G **(2009)** “Resizing digital images to actual size (1:1) using Adobe® Photoshop®” in *Journal of the Association for Crime Scene Reconstruction* 15(1):13-16.
9. Reis G **(2007)** *Photoshop CS3 for Forensics Professionals: A Complete Digital Imaging Course for Investigators*. Indianapolis: Wiley Publishing [read chapters 1-8 and 15].

10. Ringsmuth S **(2017)** "The dPS Ultimate Guide to Photography Terms – A Glossary of Common Words and Phrases" pamphlet published by Darren Rowse online and electronically downloaded from https://digital-photography-school.com/ultimate-guide-photography-terms-glossary-words/ on February 3, 2023.
11. Robinson EM **(2016)** "Basic Exposure (Nonflash) Concepts" chapter 4 in Robinson EM [Ed.] *Crime Scene Photography, 3rd Edition*, San Diego: Elsevier, pp 125-199.
12. Robinson EM **(2016)** "Electronic Flash" chapter 6 in Robinson EM [Ed.] *Crime Scene Photography, 3rd Edition*, San Diego: Elsevier, pp 267-364.
13. Robinson EM **(2016)** "Ultraviolet, Infrared, and Fluorescence" chapter 7 in Robinson EM [Ed.] *Crime Scene Photography, 3rd Edition*, San Diego: Elsevier, pp 365-409.
14. Russ JC **(2016)** *Forensic Uses of Digital Imaging*, 2nd Edition, CRC Press, Taylor & Francis Group, LLC, Boca Raton, FL [read chapters 1 and 2].
15. Schotman TG, Westen AA, van der Weerd J, and De Bruin KG **(2015)** "Understanding the visibility of blood on dark surfaces: A practical evaluation of visible light, NIR, and SWIR imaging" in *Forensic Science International* 257:214-219.
16. Scientific Working Group on Digital Evidence (SWGDE) "Digital image compression and file format guidelines" Version 1.0 **(2016-06-23)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 3Feb2023.
17. Scientific Working Group on Digital Evidence (SWGDE) "General photography guidelines for the documentation of evidence items in the laboratory" Version 1.0 **(2019-07-16)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 24Jan2023.
18. Scientific Working Group on Digital Evidence (SWGDE) "Guideline for low light crime scene photography" Version 1.0 **(2022-01-13)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 24Jan2023.
19. Scientific Working Group on Digital Evidence (SWGDE) "Guideline for the use of infrared radiation (IR) in forensic photography" Version 1.0 **(2020-09-17)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 24Jan2023.
20. Scientific Working Group on Digital Evidence (SWGDE) "Guideline for the use of reflected ultraviolet radiation (UV) in forensic photography" Version 1.0 **(2020-09-17)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 24Jan2023.
21. Scientific Working Group on Digital Evidence (SWGDE) "Image Processing Guidelines" Version 1.0 **(2016-02-08)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 3Feb2023.
22. Scientific Working Group on Digital Evidence (SWGDE) "Lighting techniques in forensic photography" Version 1.0 **(2022-06-09)**, electronic download from https://www.swgde.org/documents/published-complete-listing on 24Jan2023.
23. Staggs S **(2014)** "Lighting methods for copy and evidence close-up photography" electronically downloaded from https://www.crime-scene-investigator.net/closeup.html on February 3, 2023.
24. Witzke D **(2016)** "Digital Imaging Technologies" chapter 10 in Robinson EM [Ed.] *Crime Scene Photography, 3rd Edition*, San Diego: Elsevier, pp 587-625.
25. Current MATP Imaging and Visualization chapter
26. Manual(s) for camera(s).
27. Manual(s) for alternate light source(s).

9.7 STUDY QUESTIONS

1. Using the OSAC Lexicon (https://www.nist.gov/organization-scientific-area-committees-forensic-science/osac-lexicon), provide definitions for the following terms:
    a. Alternate light source
    b. Image
    c. Image enhancement
    d. Image processing
    e. Imaging technology
    f. Oblique lighting

2. Describe how an alternate light source works.
3. Is a handheld UV light an alternate light source? Why or why not?
4. Why do you take pictures of evidence?
5. When and why should you use a scale during documentation of case work?
6. plane of the object in the photograph impact the resultant image?
7. Define “digital image capture device” and give examples.
8. What is the difference between a lossy and lossless file type? Include examples and when they might be used.
9. What is image compression and how does it affect an image.
10. Explain the following types of camera distortion:
    a. Barrel distortion
    b. Pin cushion
    c. Vignetting
11. What is depth of field?
12. How do you increase the depth of field?
13. What does aperture refer to?
14. How can you adjust the aperture on the camera?
15. What does shutter speed mean?
16. How do you adjust the shutter speed on the camera?
17. What is the bulb setting? When would you use this setting?
18. What is ISO?
19. How do you adjust ISO on the camera?
20. What is the image histogram?
21. 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?
22. What are the components to a quality image?
23. Explain bracketing as it applies to photography.
24. What is the difference between a documentary image and an exemplar image?
25. When should images be stored in ADAMS?
26. When should images be stored in LIMS?
27. Explain the factors that affect the final resolution of a photo.
28. Explain what a color space and use examples.
29. When processing your image why is it important to do the editing on the copy of the image rather than the original?

9.8 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. All of these exercises require discussion with your trainer.

1. 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:
    i. ~ ½ teaspoon of table salt
    ii. image(s) of your shoes including outsoles
    iii. a pair of adult jeans
    iv. a particle (e.g. fingerprint, smudge) on the reflective face of a mirror

2. Get comfortable in the operation of your lab's digital SLR camera by practicing taking images of various items.
    a. *Aperture, Varied f/stop:* Use the appropriate aperture settings to take a series of images of 2 objects (such as books) at different distances (one at 3 feet and one at 6 feet) from the camera.
        i. Capture an image of the closest book to the camera being the only object in focus. Record the settings of your camera for review.
        ii. Capture an image of the farthest book to the camera being the only object in focus. Record the settings of your camera for review.
        iii. Capture an image of both books being in focus. Record the settings of your camera for review.
    b. *Shutter Speed:* Using the low and high shutter speed settings of your digital SLR camera, take photographs of a moving vehicle.
        i. Capture an image so that the car in your image appears not to be moving. Record the settings of your camera for review.
        ii. Capture an image so that the car in your image appears to be a blur. Record the settings of your camera for review.
    c. *ISO Settings*: Take a series of images at different ISO setting. Keep all other settings the same. Note the effect of the ISO setting on image noise, brightness, and contrast.
        i. Capture the image series of an object in a well lit room.
        ii. Capture the image series of the same object with the room lights off or low.
3. Take an exemplar quality photo of an impression on a shirt.
4. Working with your instructor you will become familiar with the ALSs in your facility (e.g. handheld "blacklights" and UV lamps, Foster + Freeman Crime-Lite instruments, LEEDS LSV2 instruments). 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:
        i. Seminal fluid
        ii. Saliva
        iii. Vomit
        iv. Blood on a dark cloth
        v. Gun shot through a dark cloth
5. 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 and capture images of your observations.
6. 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 and capture images of your observations.
7. Get several pieces of duct tape and look at the adhesive face with a handheld "black light" or UV lamp. Document and capture images of your observations.
8. Watch the Forensic Technology Center of Excellence (FTCoE) presentation "Best Practices for Digital Image Processing" by David "Ski" Witzke. (https://forensiccoe.org/best-practices-image-processing/)

9. Work with your trainer (trainee should operate mouse) on multiple ways to enhance an image for the same desired goal using Adobe Photoshop. Use best procedures: work on image copies and document the steps used to obtain the final image. Print out original and final images. Use multiple ways to:
    a. Optimize brightness
    b. Optimize contrast
    c. Optimize tone
    d. Optimize color
10. Work with your trainer (trainee should operate mouse) on multiple ways to enhance an image for the same desired goal using Adobe Lightroom. Use best procedures: work on image copies and document the steps used to obtain the final image. Print out original and final images. Use multiple ways to:
    a. Optimize brightness
    b. Optimize contrast
    c. Optimize tone
    d. Optimize color
11. Print the same image with different adjustments to the printer settings. Write on each printout what the printers setting were (e.g. type of paper in settings versus type of paper actually used, brightness and/or contrast settings, DPI setting).
12. Create montages of 2, 4, and 6 images using Adobe Bridge. Include the file names underneath the images, and a header with the “Practical Exercise 9.8.21”, and the date you took the image.
13. Create montages of 2, 4, and 6 images using Adobe Lightroom. Include the file names underneath the images, and a header with the “Practical Exercise 9.8.22”, and the date you took the image.
14. Use Adobe Acrobat DC to add comments to one of the montages created in the previous exercise. Print your montage.

10 INFRARED SPECTROSCOPY

10.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive, especially the Ed Suzuki lecture series.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

10.2 PREREQUISITES

- Microscopy (Basic)

10.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required performance verifications and maintenance of the instrument.

10.4 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. CO_2
        ii. H_2O
    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

10.5 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.

10.6 READINGS

1. Cooper JW **(1980)** *Spectroscopic Techniques for Organic Chemists*. New York: John Wiley & Sons. [read pp 22-52].
2. Suzuki EM **(2020)** "Infrared Spectroscopy in Forensic Sciences: A Comprehensive Discussion" chapter 6 in Saferstein R [Ed.] *Forensic Science Handbook. Volume 1*, 3rd Edition. Upper Saddle River (NJ): Prentice Hall, pp 301-423.
3. Current MATP chapter.

RESOURCES

1. Willard HH, Merritt LL Jr, Dean JA, Settle FA Jr **(1988)** *Instrumental Methods of Analysis*. 7th Edition. New York: Van Nostrand.
2. Other general texts on infrared spectroscopy.

10.7 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?

10.8 PRACTICAL EXERCISES

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

1. Run a performance verification on 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. Review the example spectra collected by various sample accessories. What differences are observed in the spectra?
8. You will be given an unknown spectrum by your instructor, attempt to identify the substance.
9. Analyze a series of unknowns given to you by your instructor.

11 MICROSCOPY (BASIC)

11.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- Trainee has received authorization from laboratory management to use the instruments.
- The trainee will be authorized to use the instrument in casework upon successful completion.

11.2 PREREQUISITES

- None

11.3 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.
- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

11.4 TOPIC AREAS

1. Properties of Light
    a. Wave Properties
        i. Wavelength
        ii. Amplitude
        iii. Oscillation within a Plane
        iv. Phase / Interference
    b. Ray Properties
        i. Reflection
        ii. Law of Reflection
        iii. Refraction
        iv. Snell's Law
        v. Critical angle
        vi. Dispersion
        vii. Diffraction
2. Image Formation
    a. Refraction Lenses
        i. Convex
        ii. Concave
    b. Reflection Lenses/Mirrors
        i. Convex

        ii. Concave
    c. Ray Diagrams
    d. Optical Trains
        i. Transmitted light
        ii. Reflected light
3. Stereomicroscopes
    a. Designs
        i. Greenough
        ii. CMO (Common Main Objective)
        iii. Zoom Configuration
    b. Illumination
        i. Reflected
            1. Ring
            2. Oblique
        ii. Transmitted
4. Compound Microscopes
    a. Lenses
    b. Magnification
    c. Numerical Aperture
    d. Aberrations/Corrections
        i. Chromatic
        ii. Spherical
        iii. Curvature of Field
    e. Coverslip Thickness
    f. Mounting Medium
    g. Basic Components
        i. Oculars
        ii. Objectives
        iii. Condensers
        iv. Illumination Sources
    h. Conjugate Planes
        i. Illumination
        ii. Image
    i. Kohler Illumination
    j. Contrast Enhancement
    k. Optical Trains in WSP
        i. Brightfield
        ii. Reflection (epi)
    l. Resolution
        i. Diffraction
        ii. Airy Disc
        iii. Rayleigh approach
        iv. Abbe approach
5. Polarization Microscopy
    a. Polarized Light
        i. Calcite
        ii. Polaroid
    b. The Optical Train
        i. Polarized Light
        ii. Cross Polarized Light
        iii. Wave Filters
    c. Particle Interactions with Polarized Light
        i. Pleochroism
        ii. Birefringence

1. Isotropic
2. Anisotropic
    a. Uniaxial
    b. Biaxial

iii. Retardation
iv. Michel-Levy Chart
v. Sign of Elongation
vi. Extinction

6. Working With Microscopes
    a. Proper Handling
        i. Moving
        ii. Rotating Objective Lenses
    b. Ergonomics
    c. Particle Picking
        i. Forceps
        ii. Tungsten Needles
        iii. Magnetism
    d. Particle Characterization
        i. Shape (e.g. polygonal, irregular, angular, slivers, flakes, clumps, aggregates, etc.)
        ii. Color (visual, stereomicroscopy with reflected light, stereomicroscope with transmitted light, light microscope no polarizers, pleochroism, etc.)
        iii. Luster/Reflectivity (e.g. shiny, translucent, mirrored, highly reflective, etc.)
        iv. Edges (e.g. sharp, blunt, abraded, manufactured, etc.)
        v. Surfaces (e.g. bumpy, pebbled, scratches, abraded, wavy, crinkly, etc.)
    e. Contrast versus Resolution
    f. Determination of the Ocular Units Conversion Factor for the Eyepiece Micrometer
    g. Basic Maintenance
7. Awareness
    a. Topics to Be Covered in Refractive Index Determination
    b. Topics to be Covered in Special Applications in Microscopy

11.5 SAFETY

- Glass microscope slides and cover slips should be considered a sharp and should be handled with care.
- Never look directly into a light source.
- 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.

11.6 READINGS

1. Abramowitz M **(2003)** *Microscope Basics and Beyond*, Olympus America, New York.
2. Bloss FD **(1999)** *Optical Crystallography*, Mineralogical Society of America, Washington DC [Read pp 1-37].
3. DeForest PR **(2002)** “Foundations of Forensic Microscopy” in *Forensic Science Handbook Volume 1*, 2nd Edition, Ed. Richard Saferstein. Upper Saddle River (NJ): Prentice Hall, pp 215-319 [read topics covered by pages 216-232 and 234-257].
4. Goldberg O **(1980)** “Köhler illumination” in *The Microscope* 28:15-21.
5. McCrone WC, McCrone LB, and Delly JG **(1984)** *Polarized Light Microscopy*, McCrone Research Institute, Chicago. [Read chapters I-IV, and pp 4953 of chapter V – based upon 11th printing].

6. Petraco N and Kubic T **(2004)** *Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators*, CRC Press, New York [Read chapters 1, 2 and "Micrometry" of chapter 3].
7. Stoiber RE and Morse SA **(1994)** *Crystal Identification with the Polarizing Microscope*, Chapman and Hall, New York. [Read chapters 1, 2, 5, 6 and pp 31-38 of chapter 2]
8. Microscope manuals supplied with the microscopes in your laboratory.
9. Current MATP chapter.

11.7 STUDY QUESTIONS

1. What property of light determines the color of a light wave?
2. What property of light determines the brightness (intensity) of a light wave?
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. Name and describe the components of a stereomicroscope and explain how they work.
7. What is the difference between a Greenough and Common Main Objective stereomicroscope?
8. What is oblique lighting on a stereomicroscope?
9. What is an optical train?
10. What is chromatic aberration in lenses?
11. What property of light and what property of a lens are used to determine resolution?
12. What is the maximum theoretical resolution possible with a 40X/0.65 NA objective?
13. What is the highest theoretical Numerical Aperture for a dry objective?
14. Name and describe the components of a polarized light microscope and explain how they work and what they do.
15. How does a brightfield optical path differ from a polarization optical path?
16. What is extinction in polarized light microscopy? Name and describe at least 5 different types of extinction.
17. What is pleochroism?
18. Is pleochroism any different than dichroism?
19. What's the difference between a uniaxial and a biaxial crystal?

11.8 PRACTICAL EXERCISES

1. Adjust the ocular lenses on you microscopes (stereo and PLM) for your eyes.
    a. Adjust the inter-ocular distance such that a single field of view is observed.
    b. The oculars should initially be rotated to the "0" point.
    c. With your dominant eye, focus on an object rotating the focus control knob. (The non-dominant eye should be closed or covered.)
    d. With your non-dominant eye, focus on the object by rotating the ocular lens for that eye. (The dominant eye should be closed or covered.)
2. Determine if your stereomicroscope is a Greenough or CMO design.
3. Determine the ocular unit conversation factor for an eyepiece reticule of a PLM.
4. Observe a set of samples (provided by your trainer) with a ring light and again with an oblique light on a stereomicroscope. For each sample, note the texture(s), surface feature(s), adherent materials, particles shapes, and luster (e.g. shiny, glossy, reflective, matte, dull, etc.).
5. Observe a demonstration of transmitted light stereomicroscopy with the previous sample set.
6. Observe demonstrations of sharpening tungsten needles and sharpening of forceps.

7. Particle pick and separate small particles supplied by the trainer using forceps, tungsten needles, and a magnet. Describe the characteristics of these particles (color, shape, luster, magnetic, etc.)
8. Work with your trainer to clean your microscopes.
9. Observe a demonstration of preparing wet mount slides.
10. Set up two different models/brands of polarized light microscopes for Köhler illumination.
11. Observe a sample of diatomaceous earth (mounted in water) at 400X with brightfield. Note any differences observed when the condenser aperture is closed down versus when it is open.
12. Characterize a set of samples with PLM based on morphology, color, pleochroism, isotropic/anisotropic, and if anisotropic describe the extinction.

12 MICROSCOPY (SPECIAL APPLICATIONS)

12.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- Observation of additional samples is encouraged (no approval needed).
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- Trainee has received authorization from laboratory management to use the instruments.
- The trainee will be authorized to use these techniques in casework upon successful completion.

12.2 PREREQUISITES

- Microscopy – Basic

12.3 OBJECTIVES

- To familiarize the trainee with the theory behind the different microscopies available in the CLD.
- To have the trainee demonstrate the setup and use for each of the different optics.

12.4 TOPIC AREAS

1. Review the Topic Areas from Microscopy (Basic)
    a. Properties of Light
    b. Image Formation
    c. Compound Microscopes
2. Compound Microscope Contrast Methods
    a. Brightfield
    b. Phase Contrast
    c. Darkfield
    d. Fluorescence
3. Specialized Optical Trains
    a. Reflected
    b. Oblique
    c. Comparison Microscopes
4. Color Balance
    a. Lightness Constancy
    b. Weber's Law
    c. Simultaneous Color Contrast

12.5 SAFETY

- Tools for the manipulation of small particles are sharp and pose cutting and puncture-wound hazards.
- Care must be exercised in the use of fine tweezers, scalpels, tungsten needles, and other sharp tools.
- Do not open the housing or handle a mercury-vapor lamp housing while it is illuminated or hot; in addition to the possibility of burns there is always the danger of explosion.

12.6 READINGS

1. Abramowitz M. 1985. Microscope Basics and Beyond. Vol. 1. Lake Success (NY): The Olympus Corporation.

2. Abramowitz M. 1987. Contrast Methods in Microscopy-Transmitted Light. Vol. 2. Lake Success (NY): The Olympus Corporation.
3. Abramowitz M. 1990. Reflected Light Microscopy-An Overview. Vol. 3. Lake Success (NY): The Olympus Corporation.
4. Abramowitz M. 1993. Fluorescence Microscopy-The Essentials. Vol. 4. Lake Success (NY): The Olympus Corporation.
5. Bradbury S, Evennet P. 1996. Contrast Techniques in Light Microscopy. Bios Scientific Publishers.
6. DeForest PR **(2002)** "Foundations of Forensic Microscopy" in Forensic Science Handbook Volume 1, 2nd Edition, Ed. Richard Saferstein, Upper Saddle River (NJ): Prentice Hall, pp 215-319 (read comparison microscopes 232-234, visibility and image contrast 258-269, oblique illumination method 302, darkfield illumination 303, dispersion staining 303-305, phase contrast microscopy 305).
7. Delly JG. Essentials of Polarized Light Microscopy. Most current edition available. Various chapters as recommended by the trainer.
8. Gilchrist AL **(2007)** "Lightness and brightness" Current Biology 17(8) 267-269.
9. McCrone WC, McCrone LB, Delly JG. 1984. Polarized Light Microscopy. Chicago (IL): McCrone Research Institute.
10. McCrone W, Kilbourn JH. 1985. Fusion Methods: Identification of Inorganic Explosive. The Microscope.33 (2):73-90.
11. Nassau K (2001) The Physics and Chemistry of Color: The Fifteen Causes of Color. John Wiley & Sons, Inc.; New York. (Read Color Perception, pp 20-23).
12. Robertson J, Grieve M. 2003. Forensic Examination of Fibres. 2nd Ed. Philadelphia (PA): Taylor & Francis. p. 171-172.
13. Wood EA. 1977. Crystals and Light: An Introduction to Optical Crystallography. 2nd Ed. New York (NY): Dover Publications.
14. Current MATP chapter.

12.7 STUDY QUESTIONS

1. Phase Contrast
    a. Sketch the light path for transmitted phase contrast.
    b. What property of light is the basis for phase contrast?
    c. Explain how contrast is generated in phase contrast.
    d. What is the difference between a phase ring (phase plate) and a phase annulus?
    e. What is the difference between positive and negative phase contrast.
    f. What types of samples are good for phase contrast (list sample properties and examples)?
    g. If no sample is present on the slide, what would you see with phase contrast?
    h. What will you see if slides are not thoroughly cleaned for phase contrast?
    i. What causes the "halo" effect in phase contrast?
    j. How can the "halo" effect be minimized?
    k. What symbols would be inscribed on a phase objective to distinguish it from other objectives?
2. Darkfield
    a. Sketch the light path for transmitted darkfield.
    b. What property of light is the basis for darkfield?
    c. Explain how contrast is generated in darkfield.
    d. What types of samples are good for darkfield (list sample properties and examples)?
    e. With no specimen present on a slide, what would you see using transmitted darkfield illumination?
    f. What will you see if slides are not thoroughly cleaned for darkfield?

3. Reflection
    a. Sketch the light path for reflected brightfield.
    b. What property of light is the basis for brightfield?
    c. Explain how contrast is generated for brightfield.
    d. What types of samples are good for reflected brightfield (list sample properties and examples)?
    e. Can polarization, phase contrast, and/or darkfield be adapted to the reflected light path? If so, give examples of good samples (list properties and examples).
4. Oblique
    a. How would you obtain oblique illumination using the PLM in transmitted brightfield mode?
    b. How does oblique illumination work?
    c. Sketch a ray path diagram for oblique illumination.
    d. What is the difference between reflection, darkfield, and oblique light?
5. Fluorescence
    a. Describe the difference between photoluminescence and fluorescence.
    b. What property of light is the basis for fluorescence?
    c. Explain how contrast is generated in fluorescence.
    d. Sketch the light path for reflected fluorescence.
    e. What is the difference between reflected fluorescence and epifluorescence?
    f. What types of samples are good for fluorescence (list sample properties and examples)?
    g. If no sample is present on the slide, what would you see with fluorescence?
    h. What will you see if slides are not thoroughly cleaned for fluorescence?
    i. Why are quartz slides recommended for fluorescence microscopy?
    j. What is the edge effect?
    k. What is photobleaching?
    l. List and explain the purpose of the three different filters in a fluorescence cube.
    m. Explain the differences between a mercury arc lamp, and xenon arc lamp, and an LED lamp for fluorescence microscopy
    n. Can fluorescence be adapted to the transmitted light path? If so, give examples of good samples (list properties and examples).
6. Comparison Microscopy
    a. Sketch the light path for transmitted comparison brighfield.
    b. What other lights paths may be used with comparison microscopy?
    c. What features of particles may be compared with comparison microscopy?
    d. What is the difference between brightness and relative lightness?
    e. How does relative lightness pertain to comparison microscopy?
    f. Explain Weber's Law.
    g. How does simultaneous color contrast pertain to comparison microscopy?
    h. When is comparison microscopy required versus comparison of photomicrographs?
    i. Discuss with your trainer how comparison microscopes with two different lights sources may be color balanced.

12.8 PRACTICAL EXERCISES

1. Phase Contrast
    a. Observe a demonstration of how to setup up and align a microscope for phase contrast.
    b. Set up and align your microscope for phase contrast. Have your trainer check it.
    c. Examine the following unstained specimens mounted on slides using both transmitted polarized light and phase contrast. Describe your observations, including the differences seen between PLM and phase contrast. Take photographs of each specimen using the two methods.
        i. Semen
        ii. Buccal cells (and/or skin cells)
        iii. Head hair
        iv. Glass particles having the same RI as mounting media
        v. Hornblende
        vi. Diatomaceous earth
2. Darkfield
    a. Observe a demonstration of how to setup up and align a microscope for darkfield using a phase contrast condenser.
    b. Observe a demonstration of how to create and use a home-made darkfield stop out of black paper for darkfield.
    c. Set up and align your microscope for darkfield. Have your trainer check it.
    d. Examine the following unstained specimens mounted on slides using both transmitted polarized light and darkfield. Describe your observations, including the differences seen between PLM and darkfield. Take photographs of each specimen using the two methods.
        i. Semen
        ii. Buccal cells (and/or skin cells)
        iii. Head hair
        iv. Glass particles having the same RI as mounting media
        v. Hornblende
        vi. Diatomaceous earth
3. Reflection
    a. Observe a demonstration of how to setup up and align a microscope for reflection for the different relevant contrast methods.
    b. Set up and align your microscope for reflection (choose at least 2 contrast methods). Have your trainer check it.
    c. Examine an assortment of different specimens such as a penny, dust, polished rock or concrete directly placed on a slide with reflected brightfield. Take photographs.
    d. Examine the following unstained specimens mounted on slides using reflection (with the 2 contrast methods you selected). Describe your observations, including the differences seen between reflected and transmitted contrast methods. Take photographs of each specimen using the different methods.
        i. Buccal cells (and/or skin cells)
        ii. Head hair
        iii. Aluminum flakes (e.g. from flash powder)

4. Oblique
    a. Observe a demonstration of how to setup a microscope for oblique light.
    b. Examine an assortment of different specimens such as a penny, dust, polished rock or concrete directly placed on a slide. Take photographs.
    c. Examine the following unstained specimens mounted on slides using reflection (with the 2 contrast methods you selected). Describe your observations, including the differences seen between reflected and transmitted contrast methods. Take photographs of each specimen using the different methods.
        i. Buccal cells (and/or skin cells)
        ii. Head hair
        iii. Aluminum flakes (e.g. from flash powder)
5. Fluorescence
    a. Obtain a set of images from your trainer
        i. For each image, identify if fluorescence is present or absent.
        ii. If fluorescence is present, state the color.
        iii. Label each image as to what particles are fluorescing and artifacts present.
    b. Observe a demonstration of how to setup up and align a microscope for fluorescence.
    c. Set up and align your microscope for fluorescence. Have your trainer check it.
    d. Examine the provided slides with fluorescence microscopy using all available cubes. Describe your observations, including the differences seen between the different cubes. Take photographs of each specimen.
    e. Mount the provided particles on glass and quartz slides in the following mounting media. Observe the differences in fluorescence intensity and color using the difference cubes and record your observations. Take photographs.
        i. Glycerol (glycerin/methanol)
        ii. Permount
        iii. Water
        iv. Neoclear
6. Comparison Microscopy
    a. Obtain a set of images from your trainer
        i. Each image should be the view through the oculars of two particles in a comparison microscope. Each image will have a label of the type of illumination, what the particles are, and what feature is compared.
        ii. Checkmark the images that have the particles and dividing line (between fields of view) oriented appropriately for the feature being compared.
        iii. For images where the orientation of the particle(s) or the dividing line is incorrectly setup, write below the images what correction(s) is needed.
    b. Observe a demonstration of how to setup up and align a comparison microscope for the different relevant contrast methods.
    c. Set up and align your comparison microscope for at least two contrast methods. Have your trainer check it.
    d. Examine the following unstained specimens mounted on slides (set of 2 slides for each specimen) using comparison microscopy (with the two contrast methods you selected). Describe your observations, including what is similar and different between the slides. Take photographs of each comparison.
        i. Buccal cells (and/or skin cells)
        ii. Glass particles having the same RI as mounting media
        iii. Hornblende
        iv. Diatomaceous earth

13 MICROSPECTROPHOTOMETRY

13.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

13.2 PREREQUISITES

- Microscopy – Basic

13.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

13.4 TOPIC AREAS

1. Color Physics
    a. Hue (i.e. Color)
    b. Saturation (i.e. Chroma, Purity)
    c. Brightness (i.e. Luminous Intensity)
    d. Electromagnetic Spectrum
    e. Mixing
        i. Additive
        ii. Subtractive
        iii. Metamerism
    f. Color Order Systems
        i. CIE chromaticity
        ii. CIE Lab
        iii. Munsell
2. Color Chemistry
3. Instrument
    a. Components
    b. Operation
    c. Analysis Modes
        i. transmission
        ii. reflectance
        iii. fluorescence

4. Advantages & Limitations
5. Quality Assurance

13.5 SAFETY

- Care should be taken to avoid looking directly at light coming from the lamps as eye damage can occur. The lamps also use high voltage power supplies that could result in serious electrical shock if contacted while on. They also generate significant heat so care should be taken not to make contact with them while in operation.
- Replacing the dust cover over the instrument should not be done until the lamps have cooled down.
- Do not open the housing or handle a mercury-vapor lamp housing while it is illuminated or hot; in addition to the possibility of burns there is always the danger of explosion.

13.6 READINGS

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2. Elert G **(2018)** "3.3.7. Color: Discussion and Summary". In *The Physics Hypertextbook*. Electronic download 2/13/18.
3. Eyring MB **(2002)** "Chapter 6: Visible Microscopical Spectrophotometry in the Forensic Sciences". In Richard Saferstein (Ed.) *Forensic Science Handbook, Volume I, Second Edition* (pp 321-387). Upper Saddle River, NJ: Prentice Hall.
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5. Martin P **(2003)** "Technical Bulletin: Changing Xenon and Mercury Arc Lamps in a Microspectrophotometer". A CRAIC Technologies Paper.
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9. Palenik CS, Beckert JC, and Palenik S **(2016)** "Microspectrophotometry of Fibers: Advances in Analysis and Interpretation". National Criminal Justice Reference Service Document 250437 (Award Number 2012-DN-BX-K040). U.S. Department of Justice.
10. Walbridge-Jones S **(2009)** "Microspectrophotometry for textile fiber color measurement". In Max M. Houck (Ed.) *Identification of Textile Fibers* (pp 165-180). New York, NY: CRC Press.
11. User's manual for the specific instrument being used.
12. Microspectrophotometry chapter in the Materials Analysis Technical Manual

13.7 STUDY QUESTIONS

1. How does a microspectrophotometer work?
2. What range in the electromagnetic spectrum is used in microspectrophotometry?
3. List ways in which samples are prepared for microspectrophotometry?
4. What are some advantages of microspectrophotometry?
5. What are the limitations of microspectrophotometry?
6. How can microspectrophotometry be used to identify metameric colors?
7. How does sample thickness and sample surface morphology affect microspectrophotometry?
8. How does sample heterogeneity affect microspectrophotometry?

9. What is pleochroism?
10. How does pleochroism affect MSP data?
11. Can the microspectrophotometer be used to identify a chemical compound? Explain your answer.
12. What are dyestuffs, chromophores, and auxochromes?
13. Define colorimetry.
14. What are color order systems?
15. What factors affect the microscopic color of a fiber? Of a paint smear?
16. Explain the difference between calibration and instrument verification for the microspectrophotometer?
17. What calibrations are performed?
18. What instrument verifications are performed?
19. What is the purpose of the dark scan? How often should they be taken?
20. What is the purpose of the reference scan? How often should they be taken?
21. Is there a reason why transmittance or absorbance measurements are preferable in the photometry of dye solutions? In the microspectrophotometry of dyed fibers?
22. What parameters should be taken into account when making a comparison of a set of questioned and known samples? Specify whether the parameters are independent or dependent on the type of sample?
23. What are the two different methods for comparing a questioned to a known sample?

13.8 PRACTICAL EXERCISES

1. Instrument Quality Assurance
    a. Run and log an instrument verification.
    b. Observe the difference between the current calibration certificate and the previous calibration certificate. Review with your trainer the computer file that holds the certificate information.
    c. Review with your trainer the wavelength graphs associated with each filter cube.
2. Comparison Methods
    a. Prepare a glass slide of fibers from a colored cotton sample mounted in Cargille 1.520E.
    b. Collect all data with the same collection time using transmittance optics (absorbance mode) and the same reference spectrum.
    c. Collect spectrum from 1 point on each of 10 different fibers (K set).
    d. Collect spectrum from 3-10 different points on a single fiber (Q set).
    e. Follow the technical manual for a comparison by range overlap.
    f. Follow the technical manual for a comparison by averages.
3. Reflectance Optics
    a. Obtain the following paint chips (surface is color coat, no clear coats)
        i. Colored, high gloss, non-metallic
        ii. White, high gloss, non-metallic
        iii. White, semi-gloss, non-metallic
        iv. White, matte, non-metallic
    b. Collect five spectra of the colored paint sample by reflectance using the white high gloss paint for the reference spectrum.
    c. Collect five spectra of the colored paint sample by reflectance using the white semi-gloss paint for the reference spectrum.
    d. Collect five spectra of the colored paint sample by reflectance using the white matte paint for the reference spectrum.
    e. Compare the data with the different reference sources. Is there a difference? Which reference gives the best result? Why?
4. Fluorescence Optics
    a. Mark a glass slide with neon-colored dry erase inks.
    b. Collect three spectra for each ink per filter cube. Use all the filter cubes.

c. Examine the pattern changes for the same ink with the different cubes.

5. Preparation Variation
    a. Prepare the following slides with the same sample of red man-made fibers (uniform thickness).
        i. One long fiber in Cargille 1.520E oil on a glass slide.
        ii. One long fiber in xylenes (or xylene substitute) on a glass slide
        iii. One long fiber in Cargille 1.520E oil on a quartz slide (quartz coverslip)
        iv. One long fiber in glycerin on a quartz slide (quartz coverslip)
    b. Collect 10 data points from each sample prep (4 slides, each different sample prep).
        i. Use same collection time with transmittance optics
        ii. Use same reference spectrum for each sample point on the same slide.
        iii. Collect new reference spectrum for each sample prep (different slide).
    c. Compare data from different sample preps by average and/or range overlap
        i. Cargille 1.520E glass slide versus quartz slide
        ii. Cargille 1.520E versus xylenes on glass slides
        iii. Cargille 1.520E versus glycerin on quartz slides
        iv. Cargille 1.520E versus diamond anvil cell
        v. Cargille 1.520E versus adhesive film lift
6. Sample Thickness Inter-Variation
    a. Prepare a glass slide of hand sections of an automotive paint mounted in a Cargille oil.
    b. Collect one spectrum from the colored layer from each of five different sections.
    c. Collect all data with the same collection time using transmittance optics and the same reference spectrum.
    d. Compare the spectra to each other. Do the locations of the peaks and valleys vary? Do the slopes between the peaks and valleys vary? Do the intensities vary?
    e. If you have access to a rotary microtome, repeat this exercise with microtome sections from an embedded sample. Do you think there are thickness differences with the microtome sections?
7. Sample Thickness Intra-Variation
    a. Prepare a glass slide of a smear from the colored layer of an automotive paint mounted in a Cargille oil.
    b. Collect five spectra from different regions of the smear.
    c. Collect all data with the same collection time using transmittance optics and the same reference spectrum.
    d. Compare the spectra to each other. Do the locations of the peaks and valleys vary? Do the slopes between the peaks and valleys vary? Do the intensities vary?
8. Mounting Medium Variation over Time
    a. Mount a long, blue man-made fiber (uniform thickness) in Permount on a glass slide.
    b. Collect a set of data from the fiber for each time point as follows:
        i. Time Points (relative to slide preparation) – same day, 1 day, 3 days, 5 days.
        ii. Use the same collection time for all data points and transmittance optics.
        iii. Collect 10 data points using the same reference spectrum.
        iv. Collect 10 data points with a new reference spectrum taken immediately prior to and adjacent to each data point.
    c. Compare data from different data sets by average and/or range overlap
        i. Difference over time points
        ii. Difference within same time point using same reference versus new reference for each data point

9. Effect of Lamp Warm-Up (setup is overnight, so plan at end of another exercise)
    a. Focus on a uniform thickness man-made green fiber with transmittance optics.
    b. Leave slide on instrument and turn everything off.
    c. Next day, startup computer but leave transmittance lamp off.
    d. Turn lamp on and quickly collect data point immediately after turning lamp on.
    e. Continue to collect spectra every 1-2 minutes until 20 minutes has passed.
10. Compare data points to each other and see if there is a pattern.

14 RAMAN SPECTROSCOPY

14.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

14.2 PREREQUISITES

- None

14.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

14.4 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 path length 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

14.5 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. Direct exposure of laser light can cause severe damage to the retina as the power levels of lasers used for Raman spectroscopy are much greater than those used in a FT-IR instrument (light from a Nd:YAG laser, which is typically used in a FT-Raman instrument, can be 107 times as intense as the helium neon laser used for a FT-IR instrument). When using a laser that is visible to the eye (which would include most of those used with dispersive CCD instruments), viewing the sample with the laser light on is not normally a problem since only diffusely reflected light reaches the eye, and highly reflective samples (such as a mirror) are not usually subject to analysis. The laser light of an FT-Raman instrument, however, cannot be observed so its path and location on the sample are not discernable, and its beam is

particularly damaging because of the high power levels involved. Because of this, the laser light of a FT-Raman instrument is not allowed into the sampling chamber when the chamber is open, so there is less of a potential hazard involved with this type of instrument. For either type of system, strict adherence to the safety guidelines outlined in the manufacturer's instruction manual should be maintained.

- A few samples, including paint pigments and explosives, have ignited from the heat of the laser. Although this is not a problem with the vast majority of samples, it is still a good idea to analyze only a minimal amount of material, particularly if a colored sample is involved.

14.6 READINGS

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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.
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10. Current MATP chapter.

14.7 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

14.8 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 ratio 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.

15 REFRACTIVE INDEX (RI) DETERMINATION

15.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- Observation of additional samples is encouraged (no approval needed).
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainer may authorize a qualified analyst as an instructor.
- The trainee will be authorized to use these techniques in casework upon successful completion.
- Training on the GRIM instrument is part of the Glass Training Manual and not covered here.

15.2 PREREQUISITES

- Microscopy - Basic

15.3 OBJECTIVES

- To familiarize the trainee with the theory behind RI determination, including the basics of optical crystallography, and its application to forensics.
- To familiarize the trainee of the advantages and disadvantages of RI determination methods.
- To have the trainee demonstrate how to perform and interpret RI determination methods.

15.4 TOPIC AREAS

1. Review the Topic Areas from Microscopy (Basic)
    a. Ray Properties of Light
    b. Compound Microscopes
    c. Polarization Microscopy
    d. Determination of the Ocular Unit Conversation Factor for an Eyepiece Micrometer
2. Optical Crystallography
    a. Crystal Forms
    b. Crystal Habits
    c. Miller Indices
3. Optical Screening of Crystals
    a. Immersion Method of RI Determination
        i. Becke Lines
        ii. Contrast Relief
        iii. Bracketing with Cargille Oils
    b. Dispersion Staining to Determine RI
    c. Conoscopy
        i. Determination of Optic Sign
        ii. Measurement of the Optical Axial Angle (2V)

15.5 SAFETY

- Tools for the manipulation of small particles are sharp and pose cutting and puncture-wound hazards.
- Care must be exercised in the use of fine tweezers, scalpels, tungsten needles, and other sharp tools.
- Refractive index immersion liquids may pose health hazards if there is skin/eye contact or ingestion.

15.6 READINGS

1. Bloss FD **(2000)** *Crystallography and Crystal Chemistry* Washington (DC): Mineralogical Society of America. (Read chapters 1, 2, 5, and 8).
2. Chamot EM and Mason CW **(1958)** *Handbook of Chemical Microscopy Volume I* New York (NY): John Wiley & Sons [Read the following chapters:
   "10 Study of Doubly Refractive Materials by Means of the Polarizing Microscope" pp 270-310
   "11 Determination of Refractive Indices of Liquids and Salts" pp 311-334
   "12 Relation of Optical Properties to Structure of Aggregates and Crystals" pp 335-383
   "13 Chemical Crystallography; Preparation of Crystals for Study", pp 384-418].
3. DeForest PR **(2002)** "Foundations of Forensic Microscopy" in *Forensic Science Handbook Volume 1*, 2nd Edition, Ed. Richard Saferstein, Upper Saddle River (NJ): Prentice Hall, pp 215-319 (read Brief Introduction to PLM 269-293, Calibrated Liquids 299, Becke Line Method 301, Determination of Optic Sign 309, and Measurement of Optic Angle 310-311).
4. Phillips WR **(1971)** *Mineral Optics Principles and Techniques*, WH Freeman and Company, San Francisco (read chapters 3, 6, and 8).
5. Petraco N and Kubic T **(2004)** Color Atlas and Manual of Microscopy for Criminalists, Chemists, and Conservators, CRC Press, New York (read chapter 3).
6. Stoiber RE and Morse SA **(1994)** *Crystal Identification with the Polarizing Microscope*, Chapman and Hall, New York. (Read pp 39-48 from chapter 2 and chapters 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 16)
7. Wood EA **(1977)** *Crystals and Light: An Introduction to Optical Crystallography*. 2nd Ed. New York (NY): Dover Publications.
8. Current MATP chapter.

RESOURCES

1. McCrone WC, Draftz RG, and Delly JG **(1967)** *The Particle Atlas*. Ann Arbor (MI): Ann Arbor Science Publishers.
2. McCrone WC, McCrone LB, and Delly JG **(1984)** *Polarized Light Microscopy*. Chicago (IL): McCrone Research Institute.
3. Winchell AN and Winchell H **(1989)** *The Microscopical Characters of Artificial Inorganic Solids Substances: Optical Properties of Artificial Minerals*. Chicago (IL): McCrone Research Institute.
4. Winchell AN **(1987)** The Optical Properties of Organic Compounds. Republication of Second Edition. Chicago (IL): McCrone Research Institute.

15.7 STUDY QUESTIONS

1. Review the following topics from the Microscopy (Basic) chapter with your trainer
   a. Ray Properties of Light
   b. Compound Microscopes
   c. Polarization Microscopy
   d. Determination of the Ocular Unit Conversation Factor for an Eyepiece Micrometer
2. What is a "Becke" line?
3. Explain "contrast relief".
4. Define the word "crystal".
5. What is a "unit cell"?
6. Explain Miller indices.
7. Explain the difference between a crystal system, crystal habit, and crystal form.
8. What is twinning?
9. Define the crystal systems.
10. Describe common crystal forms observed in the different crystal classes.
11. Define retardation in terms of the behavior of polarized light in an anisotropic crystal.
12. What is an isogyre?

13. What is an interference figure?
14. What is dispersion?
15. What is a 'stop' in dispersion staining and where is it positioned?
16. What is the difference between central stop and annular stop dispersion staining?
17. Describe how you would set-up a microscope for dispersion staining.
18. What are the key differences between the series A and series E Cargille Liquids and how are they used?
19. Can you use dispersion staining successfully on isotropic colored specimens such as garnet?
20. What information can be gathered from using dispersion staining?
21. Explain how dispersion staining can be used to confirm a refractive index?
22. Define the optic sign in a uniaxial crystal and in a biaxial crystal.
23. What is the difference between an optic sign and a sign of elongation?
24. What is the acute bisectrix in a biaxial positive crystal?
25. Define optic axial angle.
26. Explain how to measure the principle refractive indices of a uniaxial crystal.
27. Explain how to determine optic sign of uniaxial and biaxial crystals using conoscopic observations.
28. Explain microscope set-up for conoscopic observations.
29. Define dispersion as it relates to refractive index of a material.

15.8 PRACTICAL EXERCISES

1. Immersion Method – Becke Lines and Bracketing with Cargille Liquids
    a. Observe a demonstration of this method to determine the RI of an isotropic crystal.
    b. Discuss with your instructor how you would determine the RI of an anisotropic crystal using this method.
    c. Prepare and observe a set of at least three knowns (at least one of the knowns should be anisotropic). Each set should include the known in at least three refractive index liquids (slightly above, at or near, and slightly below the RI of the known). Describe the contrast relief and colors of the Becke line of the known crystal in each refractive liquid.
    d. Determine the RI of two unknown crystals using this method.
2. Dispersion Staining (Central Stop)
    a. Observe a demonstration of this method to determine/verify the RI of an isotropic crystal.
    b. Discuss with your instructor how you would determine the RI of an anisotropic crystal using this method.
    c. Prepare a set of samples of sodium chloride (RI nD = 1.544) in series E Cargille liquids (e.g. 1.540, 1.545, 1.550). What colors do you see in liquid is higher? Lower?
    d. Prepare a second set of samples of sodium chloride (RI nD = 1.544) in series A Cargille liquids (e.g. 1.540, 1.542, 1.544, 1.546, and 1.548). What additional colors do you see? What differences do you see between the series A and series E for 1.540 liquid?
3. Conoscopy
    a. Observe a demonstration of this method to observe the interference figures of biaxial and uniaxial crystals. Discuss how to determine the optic sign of uniaxial and biaxial crystals. Discuss how to determine 2V of biaxial crystals.
    b. Photograph the expected interference figure for at least one known uniaxial crystal.
    c. Photograph the expected interference figure for at least two known biaxial crystals.
    d. Determine whether the crystal is uniaxial or biaxial, the optic sign, and 2V (if uniaxial) for at least two unknown anisotropic crystals using this method. You instructor will recommend what refractive index liquids the unknowns should be mounted in.

4. Determination of Unknowns (Optical Screening)
    a. Review the Optical Screening of Crystalline Materials chart with your trainer.
    b. Observe a demonstration of how to identify an isotropic crystal using the RI determined from immersion methods and Winchell's Inorganics.
    c. Identify the isotropic unknowns from exercise 1d above.
    d. Observe a demonstration of how to identify uniaxial and biaxial crystals using the RI determined from immersion methods, the optical data from conoscopy, and Winchell's Inorganics.
    e. Identify the anisotropic unknowns from exercise 3d above.

16 SCANNING ELECTRON MICROSCOPY/ENERGY DISPERSIVE X-RAY SPECTROSCOPY

16.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.
- The trainee will NOT place the XRF Bruker blocks into an SEM-EDX.

16.2 PREREQUISITES

- Microscopy (Basic)

16.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

16.4 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.

16.5 SAFETY

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

16.6 READINGS

1. ASTM E766 (current version) – Standard Practice for Calibrating the Magnification of a Scanning Electron microscope.
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7. Johnson R. Environmental Scanning Electron Microscopy: An Introduction to ESEM. http://www.cb.uu.se/~ewert/SEM.pdf
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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.
16. Current MATP chapter.

16.7 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?

16.8 PRACTICAL EXERCISES

1. Demonstrations and Quality Assurance Exercises

This set of exercises are to be performed with the assistance of the trainer or a designated instructor. This set of exercises must be performed prior to the self-paced practical exercises. At minimum, documentation of the demonstrations will be the trainee's written notes (electronic or paper) and/or a set of trainee notations on a copy of a quick review document (also electronic or paper). The trainee should practice operating the SEM-EDX instrument immediately after the demonstrations under the supervision of the trainer/instructor.

a. Logbooks and Other Materials – Observe the storage location of all logbooks, user manuals, standard/reference materials, sample preparation and storage supplies, and any other materials associated with the instrument. This will include electronic and physical locations.
b. Sample Handling – Observe a demonstration and take notes on different methods to prepare, handle, and store samples. This demonstration will include a discussion of aluminum stubs, carbon tape, Spectro Tabs, liquid graphite, and carbon evaporation.
c. Instrument Components – Observe a demonstration and take notes on the components of the instrument. This demonstration will include a discussion of any safety precautions and damage prevention for this instrument.
d. Start Up – Observe a demonstration and take notes on the start-up procedures of the instrument. This demonstration will include loading and removing samples from the sample compartment, evacuation of the sample chamber, beam start up, raising the sample into the incident beam.
e. Data Collection & Analysis – Observe a demonstration and take notes on how to use the instrument. This demonstration should include changing magnifications, focusing at low and high magnification, imaging using the SE and BS detectors, checking/adjusting the astigmatism (wobble), EDX data collection methods (Bulk, Spot, and Mapping), saving data, the reporting module, and printouts. It will also include use of the element identification software and review of the user manual's recommended settings.
f. QA Exercises – Perform and document the procedures according to the SEM-EDX Quality Assurance section of the MATP under the supervision of the trainer or instructor.
g. Shut Down – Observe a demonstration and take notes on how to shut down of the instrument.

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. Place the sample stubs with the penny halves in the SEM chamber.
   c. Evacuate the chamber using the high vacuum mode.
   d. Check the tuning of the instrument.

e. 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.
f. Set the X-ray parameters to obtain approximately 1000 counts per second and 30 to 40 percent dead time.
g. Collect an X-ray spectrum scanning across the entire cross section for 300 live seconds.
h. 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.

17 SCREENING AND EVALUATION OF TRACE EVIDENCE

17.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee will be authorized to use these techniques in casework upon successful completion.

17.2 PREREQUISITES

- Evidence Recovery
- Microscopy (Basic)

17.3 OBJECTIVES

- To instruct the trainee on how to recognize and classify types of trace evidence.
- To instruct the trainee on how to separate and screen trace evidence.
- To instruct the trainee on how to determine if a particle may be important for a case.
- To have the trainee demonstrate competence in performance of bloodstain presumptive testing.

17.4 TOPIC AREAS

1. Particle Characterization
    a. Shape (e.g. polygonal, irregular, angular, slivers, flakes, clumps, aggregates, etc.)
    b. Color
        i. visual (macro)
        ii. stereomicroscope with reflected light
        iii. stereomicroscope with transmitted light
        iv. light microscope
            1. white light (no pols)
            2. polarized light (single pol)
            3. pleochroic
    c. Luster/Reflectivity (e.g. shiny, translucent, mirrored, highly reflective, etc.)
    d. Edges (e.g. sharp, blunt, abraded, manufactured, etc.)
    e. Surfaces (e.g. bumpy, pebbled, scratches, abraded, wavy, crinkly, etc.)
2. Classification (Categorization) of Trace Evidence
    a. Botanicals
    b. Hairs
    c. Fibers
    d. Glass
    e. Tape
    f. Paints
    g. Miscellaneous
3. Methods of Separation of Collection of Loose Particles
    a. Particle Picking
    b. Tuning Fork
    c. Magnet
    d. Filtration/Sieving
    e. Sonication
    f. Density in Water

4. Screening Considerations
    a. Targeted versus General Examinations
    b. Physical Properties
    c. Chemical Properties
    d. Mounting Media
        i. Refractive Indices
        ii. Solubility
    e. Adhesives
        i. Interference in observations
        ii. Separation of a particle from adhesive
    f. Type of Microscope
    g. Type of Illumination
    h. Alternate Light Sources
5. Evaluating the Significance of Trace Evidence
    a. Case Scenario Information
    b. Foreign versus Background Particles
    c. Quantity of Specific Particles

17.5 SAFETY

- Use precautions when searching articles of clothing, sharp objects such as syringes, glass and knives may be concealed in fabric and pockets. Wear gloves and a lab coat when handling blood stained clothing.
- Exposure to blood and other body fluids carries the risk of exposure to blood-borne pathogens. Universal precautions should be exercised when examining items with potential blood-borne pathogens.

17.6 READINGS

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8. Current MATP chapter.

17.7 STUDY QUESTIONS

1. Define the following terms and describe their forensic significance:
    a. Botanicals (e.g. leaf fragments, seeds, grass, rootlets, needles)
    b. Car seat foam
    c. Cordage
    d. Dust
    e. Fabric
    f. Feather

g. Fiber
h. Food particles
i. Glass
j. Glitter
k. Hair – differences between animal and human
l. Insect parts
m. Insulation
n. Lamp filament
o. Makeup (e.g. lip stick, foundation, eyeliner)
p. Metal turnings
q. Paint chip
r. Pressure sensitive tape
s. Road beads
t. Rope/twine/thread
u. Sand
v. Smokeless powder
w. Soil
x. Toothpaste
y. Wood

2. What is the difference between categorizing and characterizing particles?
3. Describe the different methods for separating a collection of loose particles. Include the advantages and disadvantages of each method.
4. How does case scenario information help determine what may be potentially significant in a collection of debris?
5. How can you determine what is “background” and what is “foreign” in a debris collection?
6. What microscopical tests/methods can be used to identify blood? Describe phenolphthalein presumptive blood testing (including the steps) to a jury.

17.8 PRACTICAL EXERCISES

1. Examine a set of known samples by stereomicroscopy. Document the characteristics used and photograph examples.
2. Examine particles present on a textile with a close range bullet hole (i.e. the partially burned and unburned smokeless powder). Document the characteristics and photograph.
3. Examine tape lifts with a stereomicroscope and list broad categories of materials present.
4. Examine a set of known debris collections. Categorize the particles present. Document the characteristics used and photograph examples. Discuss the significance of the particles present in relation to the source with your trainer.
5. Observe demonstrations of the following:
    a. Separation of particles by a tuning fork.
    b. Separation of particles by a magnet.
    c. Separation of particles by sonication and filtration.
    d. Separation of particles by sieving.
    e. Removal of particles from a tape lift.
6. Categorize the separated particles (by stereomicroscopy) from the demonstrations. Document the characteristics used and photograph examples.
7. Characterize and identify to class (e.g. hair, fiber, feather) 10 unknowns.
8. Examine the debris from two clothing items and write two practice screening reports.
9. Perform phenolphthalein testing on various types of stains as specified by your trainer to determine specificity of the test and which substances may give positive results other than blood.

18 THIN LAYER CHROMATOGRAPHY

18.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee will be authorized to use these techniques in casework upon successful completion.

18.2 PREREQUISITES

- None

18.3 OBJECTIVES

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

18.4 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 2 test for Seized Drugs Analysis
        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.

18.5 SAFETY

- UV radiation can be harmful to the eyes and care should be exercised to avoid direct exposure to UV radiation.
- Visualizing reagent sprays may be hazardous.
- 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.

18.6 READINGS

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.
5. Current MATP chapter.

18.7 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.

18.8 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.

19 VOLATILES SAMPLING

19.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee will be authorized to use these techniques in casework upon successful completion.

19.2 PREREQUISITES

- Gas Chromatography & Detectors
- Infrared Spectroscopy

19.3 OBJECTIVES

- To understand existing procedures of ignitable liquid recovery methods in the analysis of other types of evidence besides fire debris. This may include general chemical analysis requests or requests where the evidence may dictate the application of these technical procedures.
- To acquire skills in the collection and analysis of volatile compounds and gases from evidence.
- To develop evidence preservation techniques for volatile compounds and gases.

19.4 TOPIC AREAS

1. Amenable compounds
    a. Some gases (e.g. difluoroethane, nitrous oxide, isobutene)
    b. Liquids (e.g. toluene, alkyl nitrites)
    c. Flavorings and fragrances (e.g. esters, terpenes)
2. Evidence handling
    a. Upon receipt, exhibits need to be examined for proper packaging and storage
    b. Vapor tight packaging
    c. Cold storage (freezer or refrigerator)
3. Outline analytical approach
    a. Ensure integrity of the evidence
    b. Prevent the escape of volatile compounds of interest
    c. Least destructive to more destructive sampling
4. Sample preparation
    a. Ambient headspace (AHS)
    b. Heated headspace (HHS)
    c. Passive adsorption/elution (PAE)
    d. Isolating volatile compounds from a complex matrix
5. Sample analysis
    a. GC/MS
        i. Adjust instrument parameters if looking for a target compound
        ii. Manual injection of a headspace sample
        iii. Auto-injection of a pseudo headspace
    b. IR (vapor)
        i. Gas cell
        ii. Adjust instrument parameters for obtaining a vapor spectra
        iii. Collecting a background
        iv. Collecting a sample
6. Sample preservation

a. Spoliation
b. Long term sample storage
c. LIMS requirements

19.5 SAFETY

- Puncturing metal cans with awls or other tools should be conducted with caution.
- Carbon disulfide and other extraction solvents should be handled in a fume hood.

19.6 READINGS

1. ASTM E1386, Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction. Current edition.
2. ASTM E1388, Standard Practice for Sampling of Headspace Vapors from Fire Debris Samples, Current edition.
3. ASTM E1412, Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration With Activated Charcoal, Current edition.
4. Dietz WR. Improved Charcoal Packaging for Ignitable Liquid Recovery by Passive Diffusion. J Forensic Sci. 1991; 36:111-121.
5. Juhala J. A Method for Adsorption of Flammable Vapors by Direct Insertion of Activated Charcoal into the Debris Samples. Arson Analysis Newsletter, 1982; 6:3240.
6. Lentini JJ, Armstrong AT. Comparison of the Eluting Efficiency of Carbon Disulfide with Diethyl Ether: The Case for Laboratory Safety. J Forensic Sci. 1997; 42:307-311.
7. Newman RT, Dietz WR, Lothridge MSM. The Use of Activated Charcoal Strips for Fire Debris Extractions by Passive Diffusion. Part 1: The Effects of Time, Temperature, Strip Size, and Sample Concentration. J Forensic Sci. 1996; 41:361-370.
8. Phelps JL, Chasteen CE, Render MM. Extraction and Analysis of Low Molecular Weight Alcohols and Acetone from Fire Debris Using Passive Headspace Concentration. J Forensic Sci.1994; 39:194-206.
9. Williams M R, Fernandes D, Bridge C, Dorrien D, Elliott S, Sigman M. Adsorption Saturation and Chromatographic Distortion Effects on Passive Headspace Sampling with Activated Charcoal in Fire Debris Analysis. J Forensic Sci. 2005; 50:316-325.
10. Current MATP chapter.

19.7 STUDY QUESTIONS

1. Define the following terms:
    a. Elution
    b. Volatile Organic Compounds (VOC)
    c. Purge and Trap
    d. Headspace sampling
2. Compare and contrast adsorption and absorption.
3. Describe the nature and use of some adsorbent materials in the laboratory, including activated carbon.
4. What can cause distortion in activated charcoal sampling? What are two considerations to overcome the impact of this distortion?
5. What is the difference between phosgene and phosphine? Under what circumstances could these compounds be found in case work?
6. What instrument modifications are necessary when analyzing gases and VC for GC-MS? For IR?
7. Describe how you would preserve a carbon strip used in analysis. What must be done in LIMS and on the RFLE for created samples?
8. Describe quality control requirements for the following:
    a. A new lot of C-strips
    b. A new bottle of carbon disulfide
    c. Oven thermometer

19.8 PRACTICAL EXERCISES

EXERCISE 1: HEADSPACE (HS) VERSUS PASSIVE ADSORPTION-ELUTION (PAE)

Goals of the Exercise:

1. To become familiar with ambient headspace (AHS), heated headspace (HHS), and passive adsorption/elution (PAE) sampling techniques for isolating volatile compounds from sample matrices.
2. To become familiar with challenges in the detection of volatile compounds using AHS, HHS, and PAE.

Procedure:

1. Prepare three sets of lined quart cans for three different flavors of lip balm from the same brand. One set should be labeled AHS, one should be labeled HHS, and the final set labeled PAE. Place a Kim wipe or other absorbent toweling in each can. Unroll each tube, weigh out at least 1 gram of lip balm, and place into the appropriate can. Secure a lid on each can for AHS and HHS.
    i. Example:
    ii. AHS: Cherry – Mint – Strawberry
    iii. HHS: Cherry – Mint – Strawberry
    iv. PAE: Cherry – Mint – Strawberry
2. PAE Samples
    a. Place an ACS in an alligator clip and magnetically secure the clip to the lid's interior while having a magnet on the exterior of the lid.
    b. Prepare a blank can to be used as a preparatory blank to demonstrate the can, ACS, clip, solvents, and general process are free from interfering compounds.
    c. Heat the cans in a 60-80°C oven for a minimum of two hours. Record the temperature and length of heating time.
    d. While the cans are heating label GC vials with sample information.
    e. Remove the cans and allow them to cool to room temperature.
    f. Remove the C-strips from each can and place in GC vial and elute with carbon disulfide containing internal standard. Prepare a corresponding blank for each sample. Be sure to change gloves and lab bench covering between each sample.
        i. C-strips may need to be folded or rolled to fit into the GC vial.
    g. Analyze each blank and sample via GC/MS.
3. AHS Samples
    a. "Clean" the syringe by heating in a 60-90° oven for a few minutes. After heating allow the syringe to cool to room temperature. Pump the syringe several times with air.
    b. Withdraw 0.5 to 2.0 mL of air and inject into the GC for a blank. Analyze using a manual injection method.
    c. Create a hole in the can lid such that the syringe needle can be introduced. Seal the hole with aluminum foil tape.
    d. Puncture the syringe through the tape and withdraw 0.5 to 2.0 mL of headpace from the can.
    e. Immediately inject the headspace sample in the GC and analyze using a manual injection method.
    f. Reseal the hole in the can with aluminum foil tape to prevent volatile loss in case an additional sampling is required.
    g. While the sample is running on the GC, clean the syringe as described above in preparation for the next sample.
    h. Repeat until all AHS samples have been analyzed. Running a blank between each sample is not required but it is recommended that several blanks be run throughout this exercise to demonstrate whether or not the syringe cleaning procedure is effective.

4. HHS Samples
    a. Place the first HHS labeled can in a 60-90° oven for ~30 minutes.
    b. "Clean" the syringe by heating in a 60-90° oven for a few minutes. After heating allow the syringe to cool to room temperature. Pump the syringe several times with air.
    c. Withdraw 0.5 to 2.0 mL of air and inject into the GC for a blank. Analyze using a manual injection method.
    d. Remove the sample can from the oven and do not wait for the can to cool. Create a hole in the can lid and cover with tape.
    e. Puncture the syringe through the tape and withdraw 0.5 to 2.0 mL of headpace from the can.
    f. Immediately inject the headspace sample in the GC and analyze using a manual injection method.
    g. Reseal the hole in the can with tape to prevent volatile loss in case an additional sampling is required.
    h. While the sample is running on the GC, clean the syringe as described above in preparation for the next sample.
    i. Repeat until all HHS samples have been analyzed. Running a blank between each sample is not required but it is recommended that several blanks be run throughout this exercise to demonstrate whether or not the syringe cleaning procedure is effective.

Data Evaluation:

For each PAE, AHS, and HHS sample, evaluate the chromatograms and mass spectral data produced by each lip balm. Using the data answer the following questions:

1. Compare TIC of the three lip balms and the sampling technique. Does one of the methods produce better results?
2. Do any of the TICs show the petroleum/wax matrix?
3. Based on the data, which method would you choose to use and when? What are the advantages and disadvantages of each way of proceeding?

EXERCISE 2: CARTRIDGE SAMPLING AND VOLATILE PRESERVATION

Goals of the Exercise:

1. To become familiar with sampling strategies for CO2 and N2O gas cylinders.
2. To become familiar with sample preservation strategies for CO2 and N2O evidence.

Procedure

1. Obtain CO2 and/or N2O cylinders and associated crackers or dispensers. Practice dispensing gas using the various sampling methods. If these are not available to you, research various methods online.
2. Package the remaining gas and/or cylinders for long term storage. Check back on the packaging after two weeks to see if the packaging was effective for preserving the gas.

Data Evaluation:

1. Which sampling method was easiest to use? Which method best controls the cylinder for additional sampling?
2. How effective was the long term storage technique?

20 X-RAY FLUORESCENCE

20.1 REMINDERS

- Modifications to this chapter may be made as needed with the approval of the technical lead.
- The trainer should review and discuss with the trainee any related training materials on the Isilon drive.
- The trainee received authorization from the lab manager to use the instrument during training.
- The trainer has set guidelines on what parameters and settings the trainee may alter.
- The trainee will save all electronic data for the trainer to review.
- The trainee will be authorized to use the instrument in casework upon successful completion.

20.2 PREREQUISITES

- Microscopy (Basic)

20.3 OBJECTIVES

- To familiarize the trainee with the theory behind the instrument and its application to forensics.
- To familiarize the trainee with the advantages and disadvantages of this instrument.
- To familiarize the trainee with the instrument components and software used in the CLD.
- To familiarize the trainee with the safety recommendations for this instrument.
- To have the trainee demonstrate the operation of the instrument, including optimizing parameters.
- To have the trainee demonstrate how to prepare samples for the instrument.
- To have the trainee demonstrate how to interpret data from the instrument.
- To have the trainee demonstrate how to perform and document required calibrations, verifications, and maintenance of the instrument.

20.4 TOPIC AREAS

1. Elemental Analysis Theory
    a. Electromagnetic Spectrum
        i. Photons
            1. X-ray photons
            2. Visible light photons
        ii. Electrons
        iii. X-rays
    b. Electron Interactions with Matter – Type of Events
        i. Elastic Scattering (Backscattered Electrons)
            1. between electron beam and specimen nuclei
            2. between electron beam and specimen electrons
        ii. Inelastic Scattering
            1. Secondary Electrons
                a. between electron beam and specimen electrons
                b. between backscattered electrons and specimen electrons
            2. Continuum X-rays (Bremsstrählung)
                a. between electron beam and specimen nuclei
                b. between backscattered electrons and specimen nuclei
        iii. Recombination (Shell Filling)
            1. Cathodoluminescence
            2. Auger Electrons
            3. Characteristic X-rays

iv. Transmission (Transmitted Electrons)

c. X-ray Beam Interactions with Matter
   i. Absorption
   ii. Diffraction
   iii. Emission (Fluorescence)
   iv. Scattering
   v. Rayleigh Scattering (elastic scattering)
   vi. Compton Scattering (inelastic scattering)
   vii. Transmitted X-rays
d. Critical Energy
e. Spatial Resolution of Emissions
f. Characteristic X-ray Nomenclature
   i. Bohr model
   ii. K series
   iii. L series
   iv. M series

2. Instrumentation in our Laboratory System
   a. microXRF
      i. Beam Source - X-Ray Tube
      ii. Beam Focus – Capillary Optics
      iii. Detector
   b. Comparison of Elemental Analysis for Both Instruments (XRF & SEM-EDX)
      i. Detection Limits
      ii. Sensitivity (Curve)
      iii. Spectral Resolution
      iv. Penetration Depth
      v. Sample Preparation
      vi. Damage/Degradation
      vii. Instrument Start Up
   c. Collection Modes
      i. Single Spot
      ii. Multi Spot
      iii. Autofocus
      iv. Area
      v. Mapping
3. Evaluation of X-ray Spectra
   a. Voltage
   b. Current
   c. Matrix Effects
   d. Topology Effects
   e. Sum peaks
   f. Escape peaks
   g. Diffraction peaks
   h. Compton and Rayleigh scattering (XRF)
   i. Argon Peaks (XRF)
   j. Dead time

20.5 SAFETY

- A safety interlock is in place to shield the user from the x-ray beam. This safety mechanism should not be tampered with.
- The detector is located close above the measurement position. It has a beryllium window, which should never be touched. The exposure to beryllium is poisonous. This part is protected by a sheet. Never remove this sheet. It is also not permitted to introduce tools or

other objects into the measuring part, because this can lead to the destruction of the detector or the primary beam optics.
- The trainee will read the safety chapter of the user manual for their instrument. For the Bruker M4 Tornado, that means Chapter 2 (Safety Instructions), Appendix C (General Safety Precautions of the M4 Tornado), and Appendix D (Biological Effects of X-ray Radiation).

20.6 READINGS

1. Bruker **(2009)** Physical Principles of Micro-X-ray Fluorescence. Berlin: Bruker AXS Microanalysis GmbH.
2. Bruker **(2013)** M4 Tornado XRF User Manual (read chapters 3 & 4)
3. Haschke M **(2014)** *Laboratory Micro-X-Ray Fluorescence Microscopy: Instrumentation and Applications.* New York: Springer. [read Chapter 1 – all sections (pp 1-15); Chapter 2 – sections 2.1 (pp 19-33), 2.2.5.4 – 2.2.5.5 (pp 55-66), and 2.3 – 2.5 (pp 69-115); Chapter 3 – section 3.4 up to 3.4.5.1 (pp 133-136); Chapter 5 – all (pp. 201.209); Chapter 7 – section 7.4.6 (pp 308 – 315)]
4. LearnXRF **(2015)** Retrieved July 20, 2015 from http://learnxrf.com. (read pages 29-31)
5. Newbury D **(2009)** "Mistakes encountered during automatic peak identification of minor and trace constituents in electron-excited energy dispersive x-ray microanalysis" *Scanning* 31:91-101.
6. 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" *Microscopy and Microanalysis* 11:545-561.
7. Vaughan D **(2008)** *Energy Dispersive X-Ray Microanalysis: An Introduction.* Thermo Fisher Scientific (TM50627_E 01/08M).
8. Current MATP chapter.

20.7 STUDY QUESTIONS

BACKGROUND THEORY

1. List and briefly define the different types of interactions of matter with an electron beam.
2. List and briefly define the different types of interactions of matter with an x-ray beam.
3. What is critical excitation energy?
4. What is Bremsstrählung radiation?
5. What is spatial resolution?
6. Define characteristic x-ray nomenclature using the Böhr model.
7. What is a K series? L series? M series?
8. 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?
9. What are Kα, Kβ and Kab?

MICROXRF INSTRUMENT

10. Describe the components of a microXRF instrument, what each does, and how an X-ray spectrum is produced.
11. What is the spot size and how do you adjust it?
12. What is a secondary target?
13. What is a filter?
14. What is the difference between mono-capillary and poly-capillary optics?
15. What effect does changing the voltage and current have on the X-rays produced?
16. 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.

17. What factor (or factors) determines the analysis depth of XRF spectrometry?
18. Mylar, Prolene, and Kapton are trade names for what polymers?
19. What can be done to improve the signal/noise ratio in an x-ray spectrum?

INSTRUMENTAL COMPARISONS

20. Briefly define other instruments besides microXRF that are used in forensics for elemental analysis? Include a comparison of the advantages and disadvantages of those instruments to microXRF, including element detection by atomic number and concentration.
21. How is beryllium used in an SEM-EDX versus a microXRF?
22. What is fluorescence? Why is this term applied to characteristic x-rays generated by microXRF and not to characteristic x-rays generated by a SEM–EDX?
23. How is Bremsstrahlung radiation produced in an SEM-EDX versus a microXRF?
24. Is there any difference of why and when a vacuum is used on an SEM-EDX versus a microXRF?

ANALYSIS

25. What is a sum peak? How is it recognized?
26. What are escape peaks?
27. What are system peaks?
28. What are diffraction peaks?
29. What is shot noise?
30. Define spectral resolution.
31. Define dead time and list the factors that affect it.
32. What is the optimal range for dead time?
33. What happens if the dead time is too high?
34. Why do peak overlaps occur in x-ray spectra?
35. What methods are available in an SEM-EDX versus a microXRF to distinguish between overlapping peaks in an x-ray spectrum?
36. For which element or elements are all three series observed between 1 and 40 KeV?
37. Where in the x-ray spectrum of helium would you expect the L series to occur?
38. An argon peak is present in an x-ray spectrum generated from a sample of steel in an XRF. Where is the peak coming from?
39. You are analyzing a laminate that is situated in the instrument with a flat orientation in an XRF. 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.

20.8 PRACTICAL EXERCISES

1. Training Samples

The following samples will be used for the practical exercises. The materials should be gathered prior to beginning the demonstrations. Many of the samples are provided as part of the “Training Kit”. This kit should be available near the instrument in each laboratory.

Sample Name	Practical Exercises	Sample Description	Source
Bruker Reference Blocks	Absorption Edge, Element Sensitivity,	This sample is a set of 3 blocks of samples (each sample in a separate “well”) provided by Bruker with the instrument.	Stored with Instrument

Pennies	Collection Methods	The sample is an intact 1980s or later USA penny ("Newer Penny") and an intact 1979 or earlier USA penny ("Older Penny").	Training Kit
Sodium Carbonate	Backgrounds	This sample contains materials for creating sodium carbonate preps on 4 different support substrates. It includes: • Kapton film • Prolene film • Mylar film • XRF cups • Tombow film • XRF cup accessory • a clear styrene box • vial of mineral oil • vial of sodium carbonate	Training Kit
Black Glass	Sample Thickness	This sample consists of 2 fragments of a black art glass. One fragment is "thick" and one is "thin".	Training Kit
Sodium Chloride	Diffraction Peaks	This sample contains materials for creating a sodium chloride XRF sample cup. It includes: • Kapton and/or Prolene films • XRF cups • Tombow film • vial of sodium chloride	Training Kit
Chrome	Sum Peaks, Tube Settings	A piece of chrome on an XRF cup.	Training Kit
Bornite	Surface Topology	An irregularly shaped chunk of bornite – a copper iron sulfide mineral (aka peacock ore)	Training Kit
Green Glass	Run Time	A "thick" piece of green glass.	Training Kit
Random Objects	Spectrum Interpretation	This sample is a set of objects to be collected by the trainee. It may include objects that other trainees have collected. Some ideas include rocks, minerals, coins, ceramics, glass, jewelry, plastics, coated objects, inks, and a GC/MS filament.	Collected by Trainee

2. Demonstrations and Quality Assurance Exercises

This set of exercises are to be performed with the assistance of the trainer or a designated assistant. This set of exercises must be performed prior to the self-paced practical exercises. At minimum, documentation of the demonstrations will be the trainee's written notes (electronic or paper) and/or a set of trainee notations on a copy of a quick review document (also electronic or paper). The trainee should practice operating the XRF instrument immediately after the demonstrations under the supervision of the trainer/assistant.

a. Sample Handling – Observe a demonstration and take notes on different methods to prepare, handle, and store samples. This demonstration will include a discussion of different films for XRF cups, double films, use of Tombow tape, clear plastic boxes with mineral oil coating, and pasteboard boxes for storage. The "Sodium Carbonate" and "Sodium Chloride" sample materials may be used to practice preparation and storage.

b. Instrument Components – Observe a demonstration and take notes on the components of the instrument. This demonstration will include a discussion of any safety precautions and damage prevention for this instrument.

c. Start Up – Observe a demonstration and take notes on the start-up procedures of the instrument. This demonstration will include loading and removing samples from the sample compartment, evacuation of the sample chamber, beam start up, raising the sample into the incident beam, and resetting the stage z-axis. *Care in loading multiple height samples and a warning about the detector window will be emphasized.*

d. Safety Interlock Verification – Perform and document a safety interlock verification according to the MATP under the supervision of the trainer or assistant. What is the purpose of this verification? Why is this verification performed prior to data collection and analysis?

e. Data Collection & Analysis – Observe a demonstration and take notes on how to use the instrument. This demonstration should include the different cameras, the mosaic function, the detector settings, the data collection methods (Point, Multipoint, Line, Area/Mapping), saving data, the Bruker reporting module, using Word to prepare reports, and printouts. It will also include use of the deconvolution software and review of the user manual's recommended settings.

f. Shut Down – Observe a demonstration and take notes on how to shut down of the instrument.

g. Logbooks and Other Materials – Observe the storage location of all logbooks, user manuals, standard/reference materials, sample preparation and storage supplies, and any other materials associated with the instrument. This will include electronic and physical locations.

h. Calibration – Perform and document a spectral calibration according to the MATP under the supervision of the trainer or assistant. Be sure to verify with any Glass analysts that they do not have any open casework PRIOR to performing the calibration. What value(s) change when a calibration is performed?

i. In Service XRF Verification – Perform and document an in service XRF verification according to the MATP under the supervision of the trainer or assistant.

j. XRF Spot Size Verification – Perform and document an XRF spot size verification according to the MATP under the supervision of the trainer or assistant. What is the actual size of the spot?

3. Self-Paced Exercises

This set of exercises are to be performed by the trainee. The trainee may consult with the trainer and/or assistant at any time during these exercises, but it is expected that the trainee will be working much if not all of these exercises independently. The order of the exercises is designed to build comprehension. All spectra are to be saved for review by the trainer. Unless otherwise stated, the trainee should collect each spectrum for 15 live seconds at 200uA/50 kV under atmosphere (no vacuum). If "under vacuum" is required, set to at least 20 mBar. At minimum, documentation of these exercises will be a report printout (electronic or paper) of the spectra with the collection conditions (included sample prep) as required by the MATP.

The trainee will take care not to have ANY samples penetrate the detector window area.

a. Collection Methods – Collect multiple spectra using Point and Multi Point methods using the "Pennies" sample set. Identify the main and the trace element peaks. Compare the

spectra from the two different pennies. What are the advantages and disadvantages of the Point and Multiple Point tabs?

b. Backgrounds – Prepare 4 different sodium carbonate samples from the "Sodium Carbonate" set of materials. Three of the samples will be particles mounted onto Tombow film on XRF cups with a different film (Kapton, Prolene, Mylar). The fourth sample will be particles mounted in mineral oil on the styrene box. Collect spectra from the backgrounds alone (films, films + Tombow, box, box + oil) and with the particles (films + Tombow + particles, box + oil + particles). Collect each spectrum for 120 live seconds at 200 µA, 50 kV, under vacuum. Determine what trace elements are present in the different materials. Note what level these elements are present. Prepare reports for each sample of the bicarbonate particles and the background. Prepare another report with one sodium carbonate spectrum from each sample. What trace elements are present in the different films, in the Tombow tape, in the styrene box, and in the mineral oil? What are the common components to the background? How does the background differ between the films and the styrene box? Why?

c. Sample Thickness – Collect spectra from the large and small fragments of the "Black Glass" sample. Collect spectra for at least 60 live seconds under vacuum. How do the spectra vary with sample thickness? Identify the elements present. Use the deconvolution software to confirm your identifications. Run the quantitation (use "A," "oxides," and the same element list for both spectra). Print the quant results as Net Intensity and, for each spectrum, calculate the following net intensity ratios: Sr/K, Sr/Zn, and Ca/K. How do these ratios vary with sample thickness? Identify the Compton and Rayleigh scattering (from rhodium K peaks).

d. Diffraction Peaks – Prepare a sample of sodium chloride using the "Sodium Chloride" materials of the training kit. Collect all spectra under vacuum. Use the "Focus Point" function to move the sample while the "Preview" function is active to locate different diffraction patterns. Collect at least 3 spectra with different diffraction patterns (you may have to use different particles). Can the same particle have multiple diffraction patterns? Why does sodium chloride have diffraction peaks but not sodium carbonate (spectra collected from "Backgrounds" exercise)?

e. Sum Peaks – Analyze the "Chrome" sample at 50 kV, 600 µA, vacuum, for 100 live seconds or more. Look up the energies for Cr Ka, Cr Kb, Fe Ka, Fe Kb, Ni Ka and Ni Kb, and identify as many sum peaks (pile up peaks) as you can. There may also be trace elements and diffraction peaks.

f. Absorption Edge – Using the Mo reference on the Bruker sample block, collect XRF spectra at 18kV, 20kV, and 22 kV. Look up the Kab for Mo and compare to what you have observed.

g. Tube Settings – Collect XRF spectra using the Point mode from the "Chrome" sample. Collect spectra from the same location (don't move the sample) under the following conditions: 100 µA at 20 kV, 100 µA at 40 kV, 200 µA at 40 kV, and 600 µA at 40 kV. Each spectrum should be collected for 30 live seconds at atmosphere (no vacuum). Compare the different spectra and notate the pattern in dead time, background, sum peaks, and scattering. Explain these patterns.

h. Surface Topography – Analyze the "Bornite" sample (under vacuum). REMEMBER, the rock must not be allowed to touch the detector window! Collect at least three spectra, each from a face of the specimen with a distinctly different orientation (you may have to reposition the specimen). Compare the spectra. How does the shape of the object affect the XRF results? What would be the ideal orientation for elemental analysis? How can this orientation effect be compensated for in casework?

i. Element Sensitivity – Using the Bruker sample blocks, collect XRF spectra for Al, Si, Ti,, Fe, Zn, Zr, Mo, and Sn. Collect all spectra at 50kV, 600 uA, under vacuum for 30 live seconds. Be sure to focus carefully on each sample surface at 100X, to insure comparable spectra. Overlay the resulting spectra.

How does the sensitivity of the instrument vary:

As atomic number changes?
Between elements with similar atomic numbers?
Between elements with significantly different atomic numbers?

For those elements that show both K and L lines, describe the relative intensities of the K and L series for the same element. How do the K and L intensities vary with atomic number?

How does this Mo spectrum compare with the three Mo spectra collected in the "Absorption Edge" exercise?

j. Run Time – Analyze the "Green Glass" sample at 50 kV, 600 µA, under vacuum at 15 live seconds, then 60 live seconds, then 300 live seconds. What are the benefits of collection for a longer time? Identify as many of the peaks in the final (300 live seconds) spectrum as you can, then use the deconvolution software to verify your results, looking closely for any possible overlapping peaks.

k. Spectrum Interpretation – Analyze several objects from the "Random Objects" set. REMEMBER, the objects must not be allowed to touch the detector window! Identify elements, sum peaks, and diffraction peaks. Be sure to include at least one small particle in your set. If possible, analyze the actual (coiled) filament from a GC/MS filament.