COURSE NAME: Forensic Chemistry
COURSE CODE: 4024586-2

Learning outcomes

- By the end of this course student should have known the following:

a. Know the meaning of forensic chemistry and quality in chemical analysis.

b. Be able to describe techniques to obtain representative sampling and problems associated during sample preparation.

c. Provide a background in statistical analysis of data.

d. Be able to describe basic instrumentation used in forensics analysis and the principles behind their function.

e. Know the methodologies involved in analysing forensic samples including: fingerprints, hair, Forgery of Banknotes , documents …. Etc.

Distribution the marks of the module

Course Grades		
	First periodic exam	20 %
	Second periodic exam	20 %
	Participation and projects	10 %
	Final	50 %
Guidelines:	➢No make ups for missing quizzes ➢Only urgent excuses could be revised and may accepted ➢Any caught action of cheating will result in a zero on exam or special project. ➢Any misconduct in the classroom will result in the loss of the 10 %. ➢Final exam will be comprehensive.	

Lecture title: **Introduction to forensic chemistry**

The objectives

- This lecture aims at giving students a general view about forensic chemistry, this is to prepare them for the rest of the coming lectures.

Forensic Chemistry:
Drug Detection
Great Value
250
Round Toothpicks

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- What is forensic chemistry?
- Its history.
- Why do we need to study it?
- Essential materials in crime laboratory.
- The role of chemistry in solving crimes.

What is forensic chemistry?

- The word **forensic** is derived from the Latin word ***forensis*** which means public, to the forum of public or public discussion.
- Any science used for the purpose of establishing a law is called forensic science.
- Forensic is the application of science to civil laws, especially during criminal investigation.
- Forensic scientists collect evidences from crime scenes and analyze them in special laboratories.
- Evidences could be blood stains, sperm specimen, hair, fibers, fingerprints, toxins ……. etc.

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- Forensic science is the use of scientific methods, chemical experiments, for solving crimes.
- Crime investigations depend on forensic science. Forensic science relays on chemistry and biochemistry to uncover the facts especially in crime scenes.
- Forensic chemistry can be seen as practical analytical chemistry, this as it deals with collecting samples and doing qualitative and quantitative analysis on them.
- Also, forensic chemistry deals with matching the results of the analyzed evidences with the suspected people in order to solve crimes and help the laws.

- The goal of forensic chemistry is to determine the provenance of a sample, and link it to a person or a thing.
- Forensics use science, psychology, accounting, computer science, engineering science …..etc to solve crimes.

History of forensic chemistry

- It was stated that early civilizations like Greeks, Romans and Egyptians used poisons for murder and as a mean for execution.
- Democritus was said to be the first chemist to study poisons.
- A law against poisoning was stated by ancient Romans in 82 BC.
- Arsenic (As), in the form of arsenic trioxide As_2O_3, was a popular poison at that times due to its features of odourlessness and can be easily introduced into food and drinks.

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- *Atropa belladonna* (nightshade), which was used as anesthetic for surgery as well as in making poison-tipped arrows. Digesting it could cause hallucination and death.
- *Conium maculatum* (hemlock), its consumption would cause disruption of the central nervous system, respiratory collapse and death. The Greek philosopher Socrates was given a potent infusion of hemlock plant after being accused of corrupting young men in Athens.
- Other poisons were Strychnine and Curare.

- The 18th century witnessed what could be the first forensic test for poisoning during Mary Blandy trial in 1752 AD. Mary spiked the food of her father with arsenic which caused his death. Dr. Addington did a test based on the residue for traces of arsenic and prove it to be the source of death. Mary was charged with the crime and was hanged accordingly.
- In 1892, Sir Francis Galton classified fingerprints into 8 types.
- In 1910, the first police crime laboratory was established.

- In 1937, the development of luminol, a chemical that shines blue when get in contact with blood, was witnessed.
- Americans scientists develop methods to inspect the gunshot residues with scanning electron microscope in 1974.
- Major breakthrough was in 1984 when Sir Alec Jeffreys developed a method for DNA fingerprinting and profiling.

Importance of studying forensic chemistry

- Why do we need to study it?

A chemist conduct qualitative and quantitative analysis on chemicals found on people, various objects and in solutions.

A chemist analyze drugs, food, paints, fire debris, residues of gunshots, soil samples, remnants of explosives. This analysis is done to find biological toxins (biological weapons), toxic chemicals (chemical weapons) and radioactive substances (nuclear weapons).

A chemist can also help in cases of environmental pollution by finding the pollution sources and tracing their origins.

Forensic chemistry vs. analytical chemistry

- There are similarities between the two, such as they deal with finding the composition of the samples (like the whole compound or a proportion in a mixture). Also, they use similar instruments.
- However, there are few differences between the two. The source of the samples in forensic needs to be determined whereas it is known in the case of analytical chemistry.

The components of crime laboratories

- Any forensic laboratory must have the following components, administration area (this to keep a record of the in-coming and out-coming samples with detailed procedure which they have undergone up-t-date).
- Various range of instruments that would help analyzing the samples and revealing their secrets.
- Storage system, so the samples can be preserved and kept to be used for later need.

- The investigations in any forensic laboratories can be divided into biological, chemical, DNA and toxicology investigations. This is as there are various evidences could be collected from crime scenes like blood and urine (for alcohol analysis whereas breath analyser can be used on-site), drugs and poisons, firearms, fingerprints as well as traces evidences (from explosives, paints, glass, soils and fires) …etc.

- The samples under investigations could be classified to organic (based on carbon as the structural backbone) or inorganic compounds.
- Another classification of the evidences could be:

1. Biological samples, like blood, urine, saliva, tissues, hair and nails.
2. Non-biological samples, like powders, liquids and gases.

Role of chemistry in solving crimes

- Chemical science plays vital role in revealing crimes ambiguities, this is by performing qualitative and quantitative analysis on specimen obtained from crime scenes. Also, forensic chemistry encompasses arson investigation, toxicology and serology.
- Evidences collected from the crime scenes are analyzed for finding the source who/which caused the problem.
- Forensic chemist usually attend court sessions during trials to give their testimony as expert witnesses, this is to help the law establishing justice.

- A wide variety of instruments have been employed in the field of forensic chemistry, ranging from simple devices like density gradient column for the comparison of soil samples to sophisticated ones like mass spectroscopy and neutron activation analysis for qualitative and quantitative analysis of unknown samples. Other instruments are UV-Vis spectrophotometers, gas chromatography, atomic absorption spectroscopy and infrared spectroscopy.

- The physical evidences collected from the crime sites are examined by chemists who have been trained on using various techniques. At the crime laboratories, forensic serologists examined body fluids whereas human remains are analyzed by pathologists, firearms and explosives are examined by firearms technicians whereas forensic chemists determine the composition and identity of the materials.

References

Forensic chemistry: fundamentals and applications. Jay A. Siegel. 2016. John Wiley & Sons Ltd.

Forensic Chemistry. Max M. Houck. 2015. Advanced Forensic Science Series. Elsevier Inc.

Basic principles of forensic chemistry. JaVed I. Khan, Thomas J. Kennedy and Donnell R. Christian, Jr. 2012. Human Press (Springer Science + Business Media).

Lecture title: **the quality in chemical analysis - quality assurance - quality control - quality management - Internal quality control - External quality control**

The objectives

- This lecture aims at giving students a flavor of different qualities employed in chemical laboratories; mainly analytical ones.

The quality in chemical analysis

- Before going into details about quality in chemical analysis, we need to fully understand the meaning of measurements. Measurements can be defined as a set of operations having the object of determining a value of quantity.
- Quantity itself can be defined as an attribute of a phenomenon, body or a substance that may be distinguished qualitatively and determined quantitatively.
- Quantities can be mass, volume, length, amount of a substance, current and voltage …etc.

- Mole is the only unit used for the amount of a substance.
- Measurements are done on samples (analyte and matrix).
- In analytical chemistry, there are many steps involved in any analysis (procedure). If any step performed in an inaccurate way, then the final result will be wrong.
- We live in the era of quality. Quality is measured, analysed and discussed. Quality is applied everywhere; product quality, measurement quality, teaching quality and research quality ….etc.

- "If you think quality systems are expensive, look at the cost of not having them". Yes, by not establishing quality measurements, there would be many catastrophic, such as loss of life, loss of money, loss of business and loss of customers.

- In all aspect of lives, a role of a chemist is present; from the analysis of the pollutants and contaminations in our consumed water or air or food to the analysis of trace quantities of radioactive or explosive materials.
- In any chemical analysis, the aim is not just finding answers for problems, by performing analysis, but it is very important to ensure that the data does represent the sample and the results are confident and reliable and defendable and accepted by all laboratories (if performed by others).

Quality assurance (QA)

- QA can be defined as a documented system of protocols used to assure the accuracy and reliability of analytical results. It includes all the procedures, actions and paperwork that take place to be sure the results are accurate and reliable.
- It is part of the quality management which ensure that the confidence of the quality will be fulfilled.

- QA composed of many components, like proficiency testing, employee qualifications and training standards of the chemists performing the analysis.
- The documentation of the following:
  - Evidence collection and the handling procedures.
  - Standard validated analytical protocols to make sure that the utilized analytical methods meet the acceptable scientific standard.
  - Instruments maintenance log, reagents preparation records.

Quality control (QC)

- It refers to the measures that must be included during each assay run to verify that the test is working properly.
- In QC we aim at answering questions like:

i. Did we perform the test?

ii. Are we doing things in accurate way?

iii. Did the results pass of fail the criteria? If failed, did we perform a corrective actions?

- Both QC and QA can be useful in many ways, such as to determine the precision and accuracy in the analysis, to demonstrate the absence of interferences as well as to demonstrate the absence of contamination from sampling equipment, glassware and reagents ….etc.

- Quality control detects systematic and random errors in any measurement. This is as QC will monitor both the accuracy and precision in any obtained results.
- As for accuracy, the bias of the results will be checked with certified reference samples and control samples, this is according to the QC procedure.
- The precision can be monitor by means or replicate the analysis of test samples as well as the reference samples.

Quality control (QC)

- QC concern about fulfilling the quality requirements. It is a set of planned activities designed to verify the measurements' quality, like the analysis of blanks and samples of known concentrations.
- QC is divided to two types; internal quality control and external quality control.

- Internal quality control provides confidence to the laboratory management, this is the operations that are performed by staff as part of the measurement process to provide evidence that the system is working properly and the results are well accepted.
- External quality control provides confidence to the customer, and it concerns about providing evidences that performance of a laboratory is comparable with other laboratories when doing identical measurements.

Quality management

- It is a set of procedures and responsibilities that an organization put in place to ensure that analysts have the required resources, instruments and all the facilities they need to do their work effectively and efficiently.

References

1. Quality assurance in analytical chemistry, Elizabeth Prichard and Vicki Barwick, 2007, John Wiley & Sons, Ltd.
2. Quality assurance and quality control in the analytical chemical laboratory: a practical approach, Piotr Konieczka and Jacek Namiesnik, 2009, CRC Press, Taylor & Francis Group.
3. FAO CORPORATE DOCUMENT REPOSITORY: http://www.fao.org/docrep/w7295e/w7295e09.htm

Lecture title: **Statistics used in Analyzing the results (Absolute uncertainty, relative uncertainty, mean value, random error, systematic error - absolute error).**

The objectives

- This lecture aims at increasing students' knowledge about the statistics that are applied for any chemical measurements.

Statistics used in Analysing the results (Absolute uncertainty, relative uncertainty, mean value, random error, systematic error - absolute error, standard deviation (the population, the sample), accuracy, precision, variance, %RSD, 95% confidence interval,).

The purpose of most practical work is to observe and measure a particular characteristic of a chemical system. However, it would be extremely rare if the same value was obtained every time the characteristic was measured, or with every experimental subject. More commonly, such measurements will show variability due to measurement error and sampling variation.

So, studying statistics is needed for many purposes such as:

1. It provides the necessary tools in quantitative reasoning to obtain information from our experimental data.
2. It allows better communication of research finding and provides credibility to research methodology and conclusion.
3. It guides researchers in choosing proper characterisation, summarisation, presentation and interpretation of the results.

The need of statistics for a chemist

- “Statistics is the science of learning from data, and of measuring, controlling and communicating uncertainty; and it thereby provides the navigation essential for controlling the course of scientific and social advances”.
- Statistics provides the link between our sample and the [unknowable] population.
- Statistics enables this through the principles and laws of probability. To permit this we must make assumptions about the distribution of the errors in our measurements.

Data

Visualize the data;
analyze with
statistical models

Knowledge

Interpret
practical and statistical
significance of results

Understanding

Make scientifically
sound decisions and
communicate them

Solve Problems and
Make Decisions

Design experiments
to answer
research questions

Experiments

Control
sources of variation,
detect outliers

Absolute uncertainty, relative uncertainty

- All experimental measurements contain errors.
- There are a variety of terms used to describe errors.
- Precision, uncertainty, reproducibility, variability, accuracy, inaccuracy to list just a few.
- It is absolutely and fundamentally essential that data are presented in an unambiguous manner.
- You must determine the types of errors and their size in your measurement.

- There are three types of errors:
  i. Gross
  ii. Determinate, also known as systematic.
  iii. Indeterminate, also known as random.
- Gross errors are non-recoverable, the whole measurement must be repeated
- Determinate errors, affect accuracy
- Indeterminate errors, affect precision

Absolute uncertainty, relative uncertainty

Absolute uncertainty is the actual size of the uncertainty in the units used to measure it, whereas the size of relative uncertainty is relative to the measured value and is usually expressed as a percentage.

Relative uncertainty can be calculated by dividing the absolute uncertainty by the measured value and multiplying by 100.

Absolute uncertainty can be minimized by choosing accurate equipment whereas relative uncertainty can be minimized by making bigger measurements.

Absolute uncertainty, relative uncertainty

Mean, arithmetic mean, and average (x) are synonyms for the quantity obtained by dividing the sum of replicate measurements by the number of measurements in the set.

$$\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$$

where, x_i represents the individual values of x making up a set of N replicate measurements.

The median is the middle result when replicate data are arranged in order of size. Equal number of results are larger and smaller than the median. For an odd number of data points, the median can be evaluated directly. For an even number, the mean of the middle pair is used.

Absolute uncertainty

Range is the difference between the lowest and highest values

Average deviation (d):

$$\bar{d} = \frac{\sum_{i=1}^{i=n} \left| Xi - \bar{X} \right|}{n}$$

Relative average deviation (%):

$$\% = \frac{\bar{d}}{\bar{X}} \times 100$$

Relative average deviation (ppt):

$$ppt = \frac{\bar{d}}{\bar{X}} \times 1000$$

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The standard deviation (S)

$$S = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

The Variance (S^2)

$$S^2 = \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}$$

Coefficient of variation

$$Coefficient\ of\ Variation = \frac{S}{\bar{x}}$$

Relative Standard deviation (RSD)

$$RSD = \frac{S}{\bar{x}} \times 100\%$$

Example 1

The normality of a solution is determined by four separate titrations, the results being 0.2041, 0.2049, 0.2039 and 0.2043 N. Calculate the mean, median, range, average deviation, relative average deviation, standard deviation and coefficient of variation.

Answer

The mean, $\bar{X} = \dfrac{0.2041 + 0.2049 + 0.2039 + 0.2043}{4} = 0.2043$

The median, $M = \dfrac{0.2041 + 0.2043}{2} = 0.2042$

Range R = Highest value – lowest value

Range = 0.2049 – 0.2039 = 0.001 N

Average deviation,

$$\bar{d} = \frac{(0.2041 - 0.2043) + (0.2049 - 0.2043) + (0.2039 - 0.2043) + (0.2043 - 0.2043)}{4} = 0.0003$$

Relative average deviation (ppt),

$$= \frac{0.0003}{0.2043} \times 1000 = 1.5\,ppt$$

Standard deviation,

$$S = \sqrt{\frac{(0.2041 - 0.2043)^2 + (0.2043 - 0.2043)^2 + (0.2039 - 0.2043)^2 + (0.2049 - 0.2043)^2}{4 - 1}} = 0.0004$$

Coefficient of variation $= \frac{0.0004}{0.2043} \times 100 = 0.2\%$

Example 2

Mean, standard deviation and RSD of the following data set.

12.475	12.469	12.481	12.466
12.474	12.465	12.475	12.473
12.481	12.472	12.482	12.475
12.485	12.473	12.465	12.485
12.468	12.477	12.450	12.513

This would be a good time to get absolutely familiar and comfortable with your calculator.

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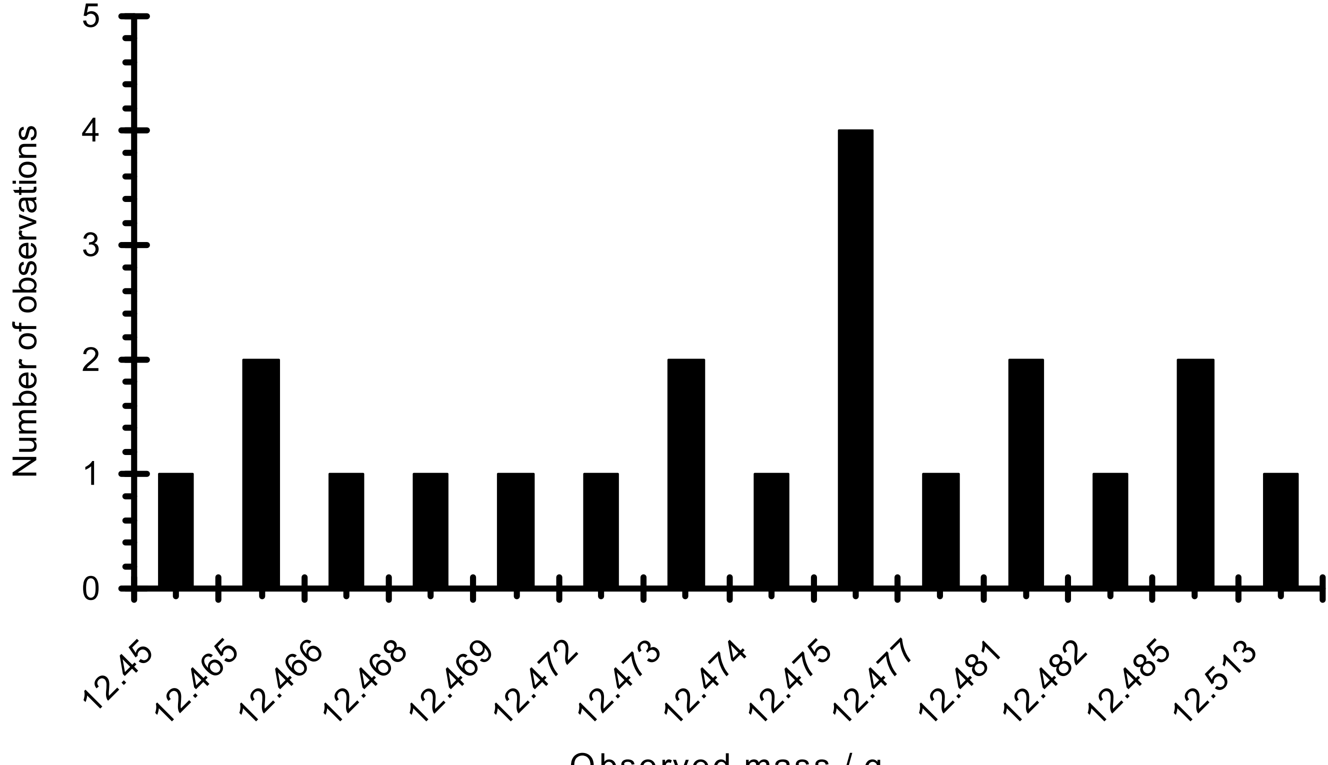
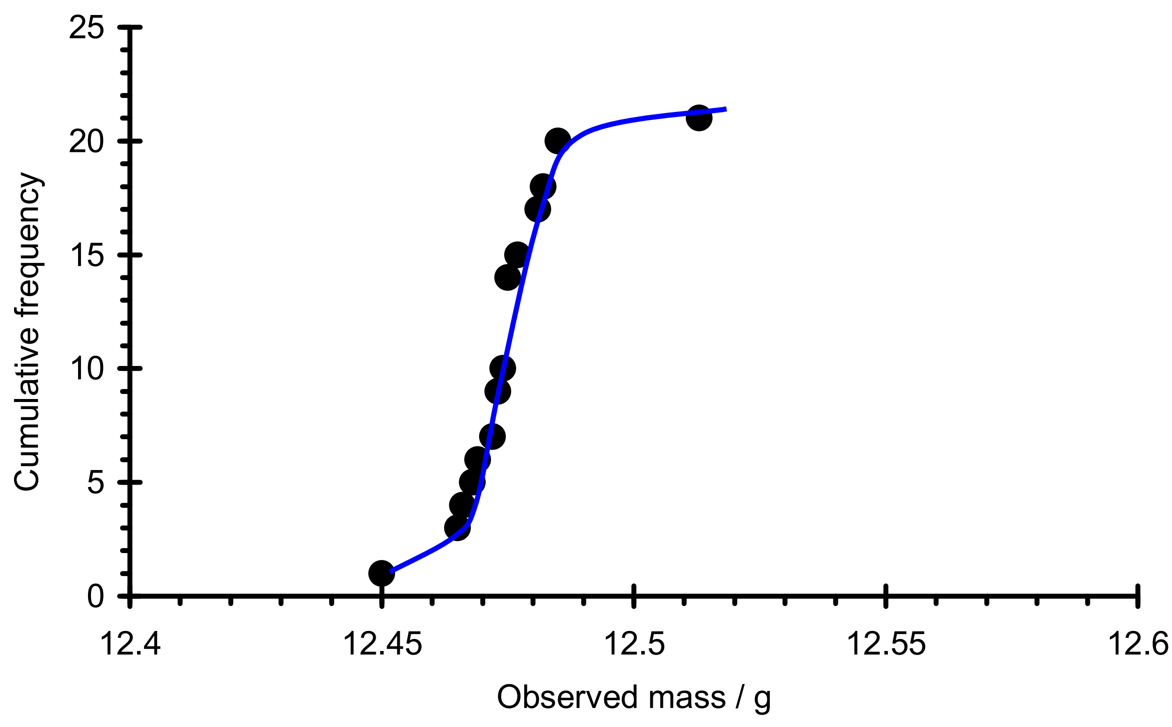
The data in example may be plotted as number of occurrences against observed value. Such a graph is known as a frequency distribution or frequency histogram



Data may be presented as summed number of occurrences against observed value. This is to produce a graph called cumulative frequency plot.



Mean value, random error, systematic error, absolute error

- The mean is the numerical average for a set of data. It can be calculated by dividing the sum of the individual data by the its size.
- The mean is the most common estimator of central tendency. It is not a robust estimator, however, if one value is far much larger or far much smaller than the rest of the data, it will influence of the mean position.

Mean value

Question: Calculate the mean of the following data 3.08, 3.094, 3.107, 3.056, 3.112, 3.174 and 3.198 g.

Answer: the values need to be added together

3.08 + 3.094 + 3.107 + 3.056 + 3.112 + 3.174 +3.198 = 21.821 g

There are 7 values, so the mean = $\frac{21.821}{7}$ = 3.117 g

The median

- If the values in a data set are arranged in sequence from the smallest to the biggest value, then another term called median can be calculated. It is the middle value (n+1)/2 in case of odd number of the set values, whereas it is (n/2)+1 for the case of even number of the set values.

Question: Find the median of the following data 3.08, 3.094, 3.107, 3.056, 3.112, 3.174 and 3.198 g.

Answer: the values need to be rearranged in a sequence

3.056, 3.08, 3.094, 3.107, 3.112, 3.174 and 3.198g

The number of the values is 7 (odd number)

The median is the middle number which is 3.107 g

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The median

- As can be noticed from the previous two examples, the mean and median provide similar estimates of central tendency when all measurements are comparable in magnitude.
- The median provides a more robust estimate of central tendency because it is less sensitive to measurements with extreme values (large difference between the biggest and smallest values).

The range

- The range is the difference between the largest value and the smallest value in a data set.

$$R = x_{\text{largest value}} - x_{\text{smallest value}}$$

- R can provide information about the total variability in a data set, but no information about the distribution of individual value.

Question: Calculate the range of the following data 3.08, 3.094, 3.107, 3.056, 3.112, 3.174 and 3.198 g.

Answer: $R = x_{\text{largest value}} - x_{\text{smallest value}}$

$R = 3.198 - 3.056 = 0.142$ g

Standard deviation 1 (the population, the sample).

- Standard deviation is a measure of variation of all values from the mean. In another word, it measures the spread about the mean, it measures the average distance from the mean value.
- It should be used only when the mean is chosen as the measure of center.
- Its value is usually positive, however it can be zero just if all the original data values are the same.
- Its unit is the same as the unit of the original data value.
- Its value can increase dramatically if the outliers (data values far away from all the others) are taken into consideration.

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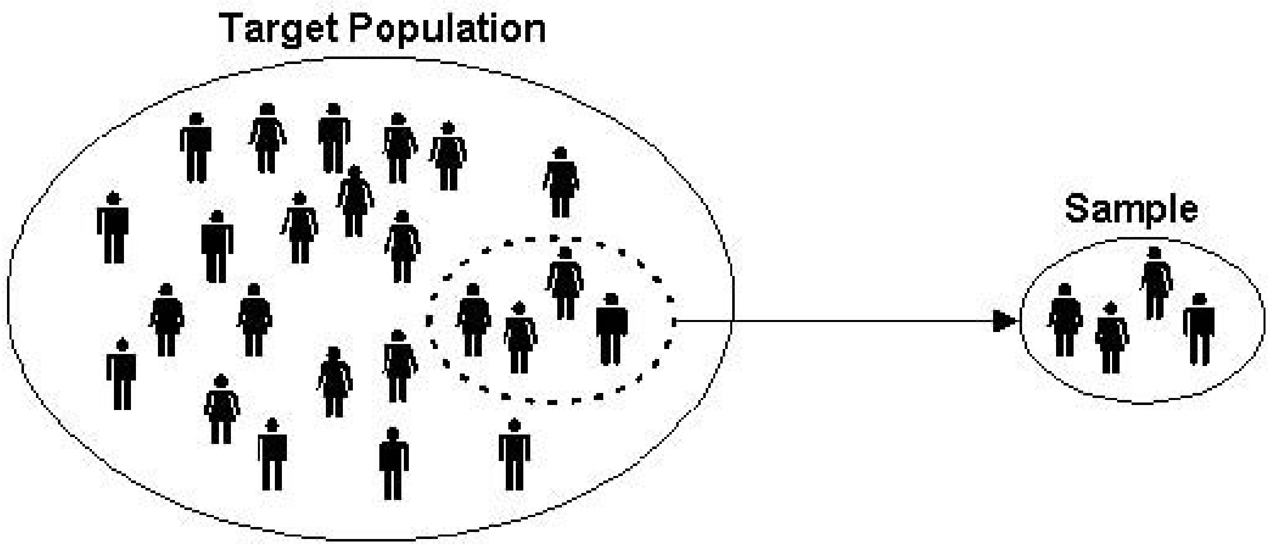
Standard deviation 2

- Population is the group of data, specimen or items from which you are to obtain your information for your statistical study. It is the entire collection to be analysed or studied.
- A sample represents a portion of the population you want to analyse or study.
- The sample should be randomly chosen so there is no bias and you can be sure that your sample covers all the characteristics of the population, otherwise the result would be invalid.

Standard deviation 3.



Lecture title: **Statistics used in Analyzing the results (accuracy, precision, variance, %RSD, 95% confidence interval).**

Accuracy and precision

- **Accuracy** measures the closeness of the obtained test results to the true value. It measures the systematic error of the analysis.

- Accuracy can be expressed as either an absolute error; *e*, or as a percentage relative error $\%e_r$
- Absolute error = mean value – the expected true value of the measurement, $e = \bar{x} - \mu$
- A percent relative error is multiplying the absolute error by 100 then dividing it by the expected true value,

$$\%e_r = \frac{\bar{x} - \mu}{\mu} \times 100$$

Example: the results of an analysis is 36.97 g compared with the accepted value of 37.06g. What is the relative error in part per thousands?

Answer:

Absolute error = 36.97 – 37.06 = - 0.09g

Relative error = $\frac{-0.09}{37.06}$ x 1000 = - 2.43

- Positive and negative signs are just to show whether the errors are high or low.

- The errors that affect the accuracy are called determinate errors, also known as systematic errors. They arise several sources, but their cumulative effect has positive or negative influence on the accuracy.
- Determinate errors may arise from sampling errors, method errors, measurement errors and personal errors.

- If the sampling strategy does not provide a representative sample, then determinate error may arise. For example, if you collect samples from one location, ignoring other locations, where a pollution occur.
- As for method error, the relation between the signal and the absolute amount of analyte, n_A, or analyte concentration, C_A, is

$$S_{total} = k_A n_A + S_{mb}$$

$$S_{total} = k_A C_A + S_{mb}$$

k_A is the method sensitivity for the analyte whereas S_{mb} is the signal form the method blank. A determinate method error exists when the values of k_A and S_{mb} are invalid.

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- The calibration of the method can minimise the determinate error in k_A.
- If the interferent in the reagents arise the determinate error, then a method blank should be carefully chosen.
- As for measurements errors, normally, statements are provided by the manufacturers of the analytical instruments and equipment showing the items' maximum measurement errors and tolerance range, like 10 mL volumetric pipet has a tolerance of ± 0.02 mL at 20 ^{o}C. There are different classes for volumetric glassware. Class A is manufactured to comply with tolerances stated by world-known institutions like NIST and ASTM. They can be normally used without calibration.

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- Class B glassware has tolerance levels twice that of class A.
- Other volumetric glassware like beakers and measuring cylinders are not suitable for measuring volumes, and they need to be calibrated.
- Exposing glassware to high temperatures, during machine washing and oven drying, would lead to changes in their actual reading.

- Determinate errors can be minimised by calibrating the equipment.

i. Balances can be calibrated by using a reference weight whose mass comply with SI standard kilogram.

ii. Digital pipets and volumetric glassware can be calibrated by determining the mass of water that it delivers and using the water density to calculate the water volume drawn by each of them.

❖ The calibration should by done on routinely basis, not just once forever.

❖ As for personal errors, they may be due to ability to notice the change in colour change during titration process, the underestimation or overestimation the values obtained by an instrument, failing to calibrate an instrument or not following the experiment procedure properly.

▪ It can be minimised by taking proper care during the course of performing an experiment.

✓ Note that the determination of the determinate error is difficult especially when the expect (true) value of an analysis is not known.

- **Precision** measures the closeness of the obtained test results to each other. It provides a measure of the random or indeterminate error of the analysis.
- There are two types of precision, repeatability and reproducibility.
- Repeatability is the precision when a single analyst completes the analysis in a single session using the same solutions, equipment and instrumentation.
- Reproducibility is the precision under any other set of conditions, including between analysts or between laboratory sessions for a single analyst.
- Reproducibility includes additional sources of variability compared to repeatability.

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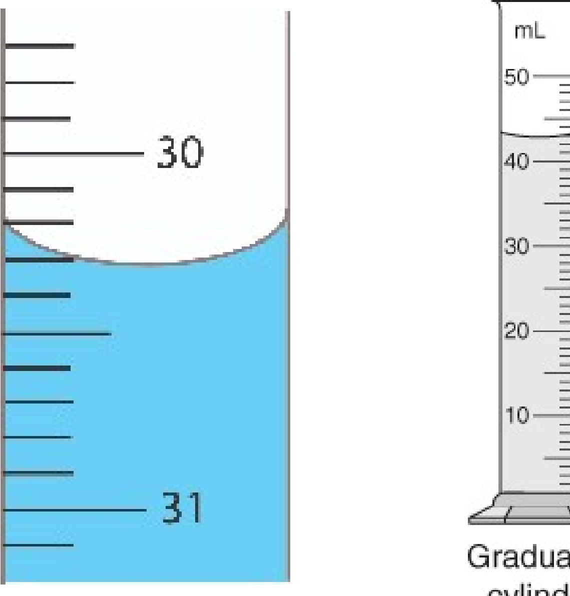
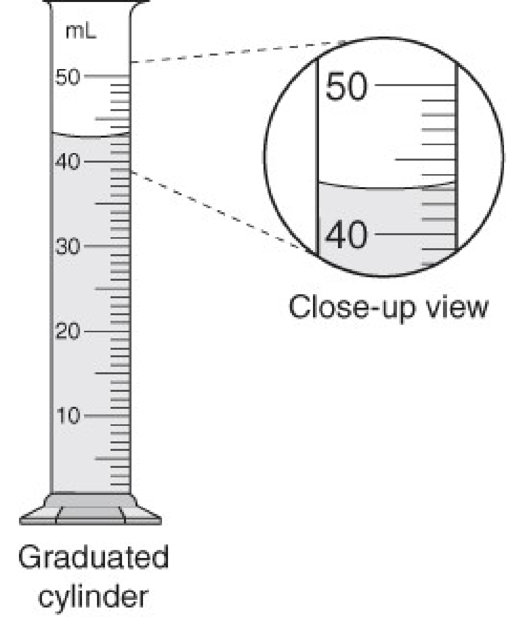
- Errors affecting the precision are indeterminate, also known as random errors, and are characterised by random variations in their magnitude and their direction.
- There are many sources of indeterminate error such as the collection of samples, manipulating samples during the analysis and performing the measurements.
- As for collecting samples, a small portion of the sample is taken which increase the chance that inhomogeneity in the sample will affect the repeatability.
- As for doing the analysis, indeterminate error may arise if all samples are not treated exactly the same way.

- As for performing the measurements, indeterminate error may arise due to limitations in reading the scale of some equipment like in measuring cylinders and burettes.
- Be aware that the change in the humidity or room temperature may have an impact on the measurement and contribute to the indeterminate error.





Graduated cylinder

- Precision can be expressed as standard deviation, deviation from the mean/median and the range of relative precision.

- An analytical chemist, a distinction between errors and uncertainty should be made.
- An error is the difference between a single measurement value and its expected (true) value. It can be divided into determinate and indeterminate error. Determinate error can be corrected whereas indeterminate one can not be corrected.
- Uncertainty accounts for all errors; determinate and indeterminate.

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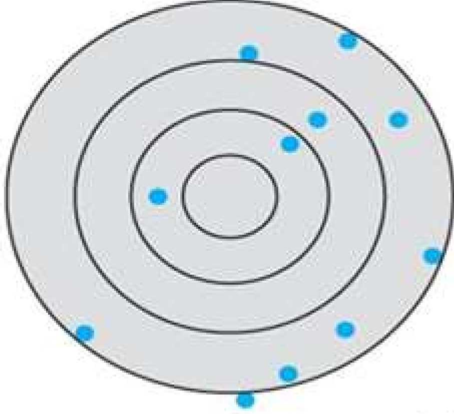
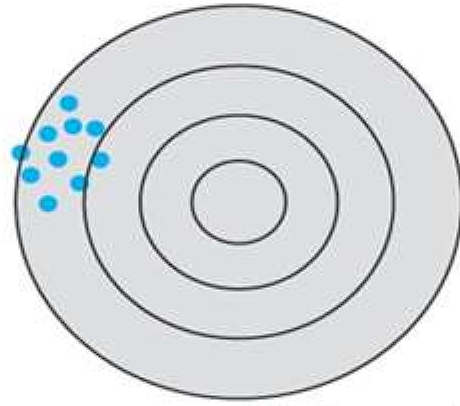
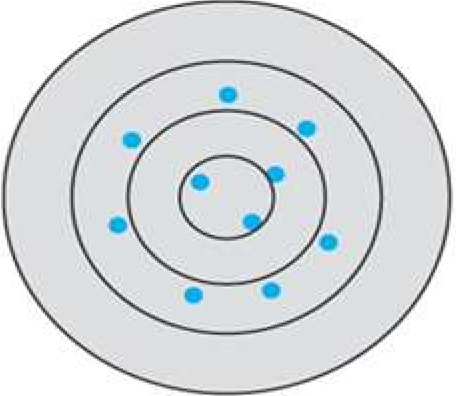
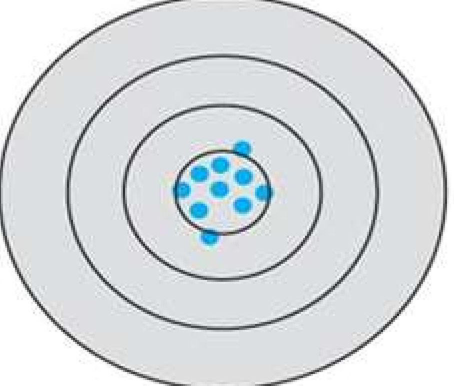
- If you purchase a 10 mL Class A pipet and use it without calibration. The tolerance of the pipet is ± 0.02 mL, also it is **its uncertainty** because your best estimate of its expected volume is 10.00 mL ± 0.02 mL. This uncertainty is determinate error. If you use the pipet to dispense several replicate portions of solution, the resulting standard deviation is the pipet's **precision**.


Low accuracy, low precision


Low accuracy, high precision


High accuracy, low precision


High accuracy, high precision

Variance, %RSD

Sample question: Find the RSD for the following set of numbers: 49, 51.3, 52.7. 55.8

Step 1: Find the standard deviation of your sample. Std dev: 2.8437065 (or 2.84 rounded to 2 decimal places).

Step 2: Multiply Step 1 by 100.   2.84 * 100 = 284

Step 3: calculate the sample mean,

$$\bar{x} = (49 + 51.3 + 52.7 + 55.8) / 4 = 208.8/4 = 52.2.$$

Step 4: Divide Step 2 by the absolute value of Step 3.
284/|52.2| = 5.44

The RSD is 52.2 ±5.4%

95% confidence interval (1)

- The purpose of taking a random sample from a lot or population and computing a statistic, such as the mean from the data, is to approximate the mean of the population. How well the sample statistic estimates the underlying population value is always an issue. A confidence interval addresses this issue because it provides a range of values which is likely to contain the population parameter of interest.

95% confidence interval (2)

- Confidence intervals are constructed at a confidence level, such as 95 %, selected by the user. What does this mean? It means that if the same population is sampled on numerous occasions and interval estimates are made on each occasion, the resulting intervals would bracket the true population parameter in approximately 95 % of the cases. A confidence stated at a $1-\alpha$ level can be thought of as the inverse of a significance level, α.

95% confidence interval (3)

- Confidence limit define a range with a probability that the true value, μ , lies within it. $\mu = \bar{x} + E$
- Confidence limit is determined by standard deviation (S) and the sample size (n).

$$E = t_{p,n-1} \frac{S}{\sqrt{n}}$$

95% confidence limit means that 1 in 20 results is likely to be incorrect.

Outliers

- An outlier is an observation that lies an abnormal distance from other values in a random sample from a population. In a sense, this definition leaves it up to the analyst (or a consensus process) to decide what will be considered abnormal. Before abnormal observations can be singled out, it is necessary to characterize normal observations.

References

Statistics and Chemometrics for Analytical Chemistry. James N. Miller and Jane C. Miller. 2010. Prentice Hall/Pearson.

Lecture title: Sample preparation , representative sampling techniques , reproducibility, replicates, duplicates, external standard, internal standard and matrix effect.
Analytical problems associated with the preparation of the sample for analysis , Selecting the suitable analytical procedure

The objectives

- This lecture aims at reading about few issues in relation to laboratory-based work, such as the following:
- Sample preparation; how a sample can be prepared.
- Representative sampling techniques.
- The need to use reproducibility, replicates, duplicates, external standard, internal standard and matrix effect on the signal obtained from a measurement.
- Addressing Analytical problems associated with the preparation of the sample for analysis, and how to select the suitable analytical procedure.

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Sample preparation , representative sampling techniques

- Samples can be gas or liquid or solid or biological material. Means of analyzing them can vary from sample to another.
- Analysis can be done to reveal chemical or physical properties, or to study its structure or its surface properties, or to reveal the sequence of proteins in genetic samples.
- However, one may think if an X sample is submitted to an instrument, all required information would be obtained straightaway. NO, this is impossible.

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- Each chemical instrument would do the analysis if samples are well prepared, if badly prepared then it may cause damage to the instrument or the obtained results would not be accurate for the original sample.

❖ There are 4 general steps for any sample to be measured instrumentally; sampling, sample preservation, sample preparation and doing the analysis.

- **Sampling**. Collect a sample representing the actual sample from the site. For instance, if you want to determine the concentration of Zn^{2+} in a lake (the site), then one sample from the lake would not represent the actual sample as the concentration of zinc varies between spots in the lake.

- **Sample preservation**. In analysis routines, there is a delay between sampling and conducting the analysis. This require a sample to be kept in certain conditions that ensure the preservation of the chemical and physical properties of the sample. Otherwise, if any changes occurs in the sample during storage, the analysis result would not be applicable to the actual sample in the first instance.

- **Sample preparation**. Most samples need special treatment before the analysis. For example, if pesticides to be determined in fish liver, then pesticides need to be extracted into a solution which can be submitted safely to an instrument. (not doing the analysis directly on the liver). The analytical technique determine the way by which a sample should be prepared. Gas chromatography can analyse the pesticides, only few microliters from the sample are needed to be injected in the GC. So, sampling and sample preservation and sample preparation all aim at producing these few microliters which represents the actual sample.

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There are many steps in the sample preparation, homogenization, size reduction, extraction, derivatization, concentration, speciation, etching, transfer to vapour phase, clean-up and analysis.

- **Analysis**. Once the sample preparation is complete, the analysis can be conducted by an instrument. Different instruments are available in the market to fit for different analysis purpose which depend on the required information to be obtained. AAS for metal analysis, capillary electrophoresis for DNA sequencing ….etc.

Reproducibility, replicates, duplicates

- Reproducibility and repeatability are ways to measure precision in any analytical analysis.
- Reproducibility is a type of precision which related to a situation in which environmental conditions and other factors will have changed and usually the results are obtained in different laboratories and at different times.

As already mentioned, any measurement need to be done multiple times, not just once, to ensure that the results are valid and reliable. Also, it is a way to detect the source of problems when the repeated measurements give different results on the same sample.

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Calibration

If a group of students attempt at measuring a line, already drawn on a paper, with their own rulers, they would not obtain similar measurement values, this is as rulers may be made from wood or plastic and they may be affected by ambient conditions. However, if there was a standard ruler and all the students' rulers were calibrated against it, there would not a difference in reading the line length. So, the term calibration was introduced to tackle the problem, and to improve the comparability of the measurements.

In chemistry, calibration can be done by using either ways; pure chemical standards or a reference material.

Reference materials can be of two types:

A reference material (RM). It is a material or a substance which has one or more properties that are sufficiently established to be effectively used for calibration.

A certified reference material (CRM). It is a material or a substance which has one or more properties that are certified by a technically valid procedure issued by a certifying body.

Chemicals can be used as standards for the calibration process, and can be applied externally or internally with respect to the sample. If they are added to a sample and measured with it, then it is called internal standard. If the standard is not added to the sample, but measured in isolation from the sample, then it is called external standard.

External standard, internal standard and matrix effect

External standards are the most common form of standardization, they are prepared with known quantities of the pure analyte.

Internal standards are added directly to a sample along way with the analyte and measured simultaneously with it.

Internal standards (IS) are added directly to a sample along way with the analyte and measured simultaneously with it. It is preferable when doing calibration for an instrument due to many reasons:

- Both IS and analyte will undergo identical processes like loss in the sample preparation step.
- IS and analyte will experience similar matrix effect during measurements.
- Simultaneous measurements will reduce any bias due to baseline drift in the measurements.

External standards (ES) are the most common form of standardization, they are prepared with known quantities of the pure analyte. They can be:

Single external standard. It can be effective, but should be tested experimentally to ensure that k_A is independent of concentration.

Multiple external standards. Standrad solution with a range of concentrations are preferred to be used for calibrations. They are used to generate a normal calibration curve.

- The benefit of external standards is that one calibration curve can be used to measure multiple sample solutions.
- The great limitation of this approach is that the standard and sample may not have the same matrix and this may alter the signal (sensitivity k_A).
- Can be mitigated by using similar matrix for both sample and standard

Matrix effect.

- A sample contains analyte and matrix, in other words matrix is other substances presented in any sample which may cause problems during the identification and quantification of the analyte.
- Matrix can cause the instrumentally measured signal to deviate from the true value; it may bind to the analyte and reduce the signal or may produce analytical signal that increase the overall response signal for the analyte. This is also called interference.
- Interference may present in the original sample or introduced to the sample during the preparation steps.

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- There are many methods to get red of the matrix effect or at least minimize its effect, such as filtering or by the addition of reagents that can equalize matrix effect in the sample.

Selecting the suitable analytical procedure

- Analytical procedures should be chosen in a way that the precision is not affected; improvement of precision.
- A combination of sample preparation and analytical instruments should be chosen to reduce the steps involved in the sample preparation and the RSD. Thus, analytical procedure where there are many handling steps, work done by hands, tend to give lower precision if compared to automated techniques.

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2. Basics of Analytical Chemistry and Chemical Wquilibria. Brian M. Tissue. 2013. John Wiley & Sons Inc.
3. Quality in the Analytical Chemistry Laboratory. Elizabeth Prichard and others. 1997. John Wiley & Sons Ltd.

Lecture title: **The most important analytical devices used in the chemical analysis process to analyze the ambiguity of the crime scene in forensic Chemistry (Gas chromatography mass spectrometry, Scanning electron microscope device, Gas chromatography**

The objectives

- This lecture aims at studying different analytical instruments that found widely usage in forensic chemistry, such as GC, SEM and GC-MS.

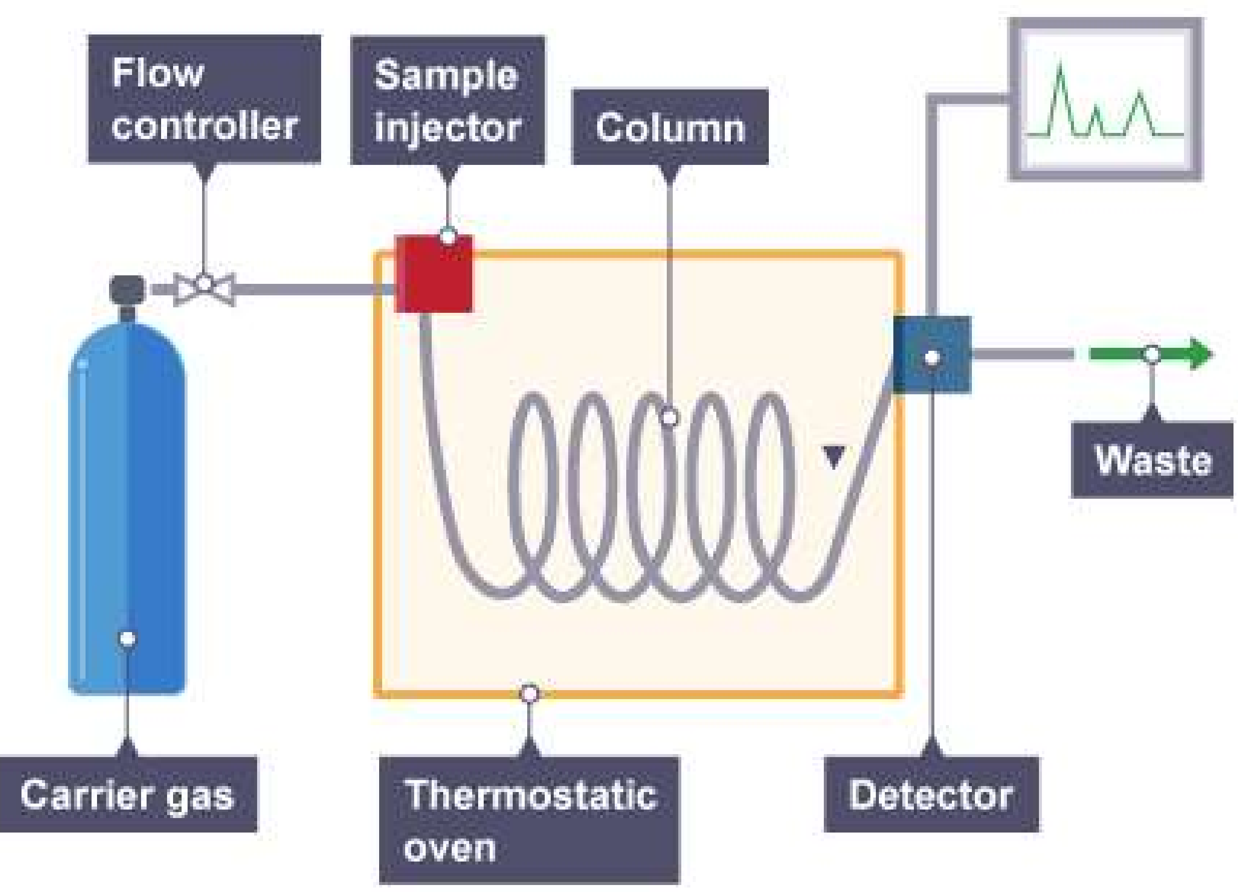
The most important analytical devices used in the chemical analysis process to analyse the ambiguity of the crime scene in forensic Chemistry (Gas chromatography- mass spectrometry, Scanning electron microscope device, Gas chromatography.

- Like any laboratory, forensic laboratory must have various instruments that have the capability of doing the qualitative and the quantitative analysis on various sample's types.
- Typical and well-known instruments are:

1. Gas chromatography, GC, which is used for the analysis of gases and volatile compounds.
2. High pressure liquid chromatography, HPLC, which is used for the analysis of non-volatile organic samples.
3. Gas chromatography-mass spectroscopy, GC-MS,
4. LC-MS
5. Atomic absorption spectroscopy-inductively coupled plasma, AAS-ICP, which is used for the analysis of toxic metals.
6. Scanning electron microscopy, SEM,

Gas chromatography (GC)

injector
autosampler
carrier gas
MS detector
capillary column
fan
oven


Flow controller
Sample injector
Column
Waste
Carrier gas
Thermostatic oven
Detector

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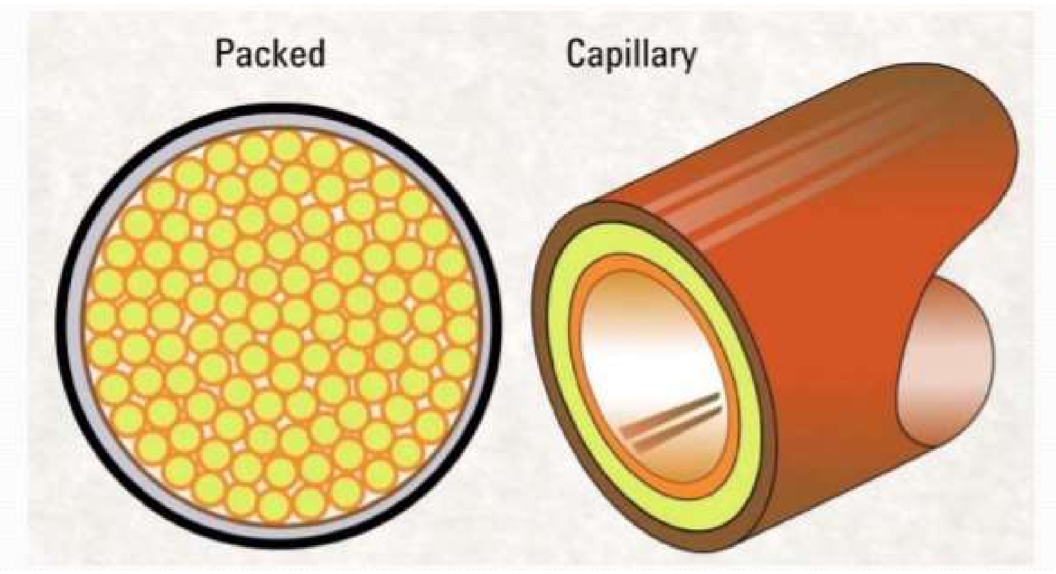
GC has the consists of various parts like:

- **Cylinders of compressed gases** to provide GC with the mobile phase. As mobile phase function is to carry the sample's components to the stationary phase, then it must be chemically inert (like He, Ar and N_2). The choice of the mobile phase is affected by the type of the used detector.
- **Heated injection port**, this is where the sample is introduced to GC. Liquid samples should be injected when the port is hot enough to volatilize their components.

- **A column**, it contains the stationary phase and is suited inside a controlled-temperature oven. It is the place where the retention of the analytes is the consequence of physical adsorption. There are two general types of columns; packed and capillary (open tubular) columns. Chromatographic columns are usually made from glass, stainless steel, Teflon, Cu or Al. Their length is 2-50 m and are shaped as coils (with diameters 10-30 cm) to fit inside the oven.



The choice of column's temperature (oven too) is based on the boiling point of the sample as well as the required degree of separation. To avoid problems of uncontrolled temperature, temperature programming is advisable; this is to increase the column temperature gradually as the separation proceeds.

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- **A detector** to monitor the eluent as they leave the column. Ideal detectors should have desirable features like non-destructive of the sample, low detection limit (adequate sensitivity), sensitive for all solutes and selective toward one class of solutes, insensitive to the change in the flow rate or temperature, have good stability (over a range of temperatures to at least 400 ^{o}C) and reproducibility as well as give a linear response over a wide range of solute concentration (to draw the calibration curve for quantitative analysis).

Examples are thermal conductivity detector (TCD), flame ionization detector (FID), electron capture detector (ECD) and mass spectrometer (MS).

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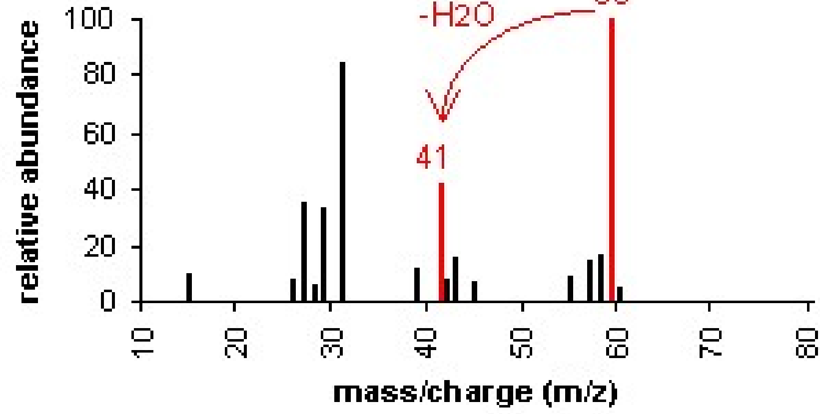
- **Data display system** to show the chemist the results of current chemical analysis.

In GC, Samples (gases or liquids) are injected through the hot injection port, by special injection syringe. Then carried by the mobile phase (An inert gas like He or N) through the capillary or packed column (stationary phase) where the sample's components are separated by their ability to partition between the two phases.

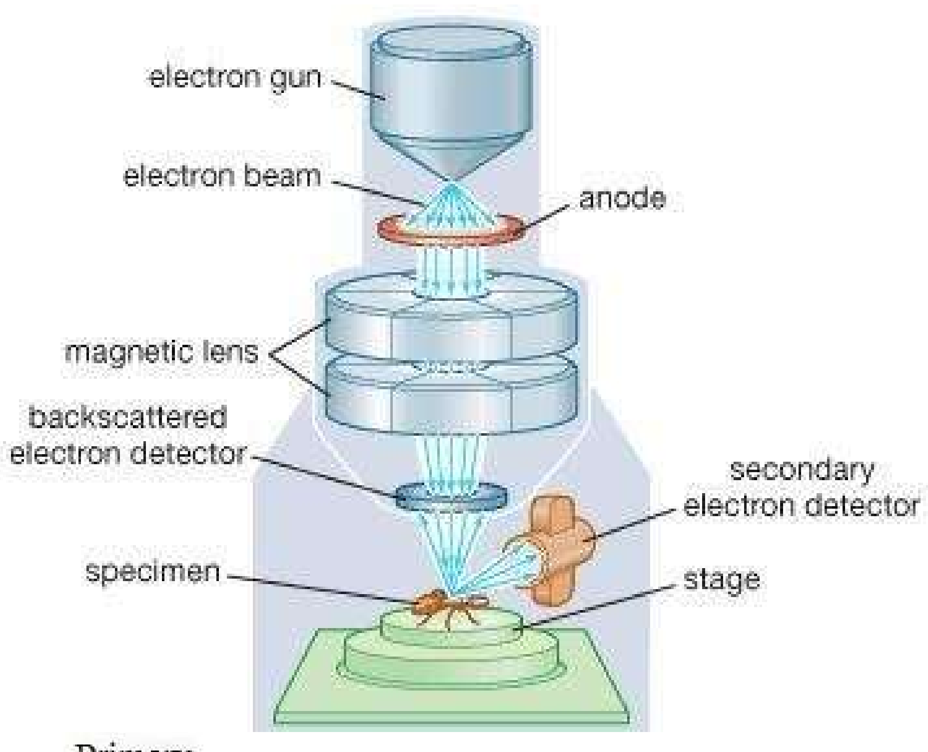
Gas chromatography-mass spectrometry (GC-MS)

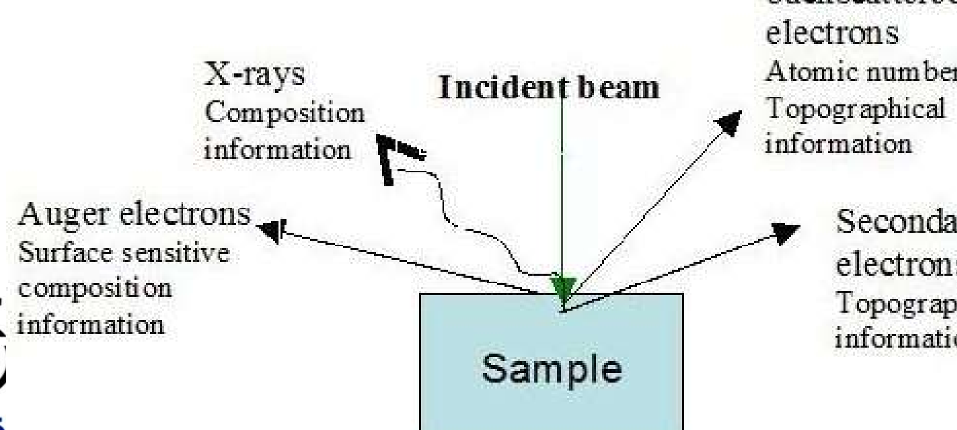
In MS, gaseous samples are ionized by high energy that break the resulting ion to smaller ions. The latter ions have different mass to charge ratios (m/z), which can be separated by applying electrical or magnetic fields. This process would produce a mass spectrum which provide qualitative and quantitative information of the sample.



- MS peaks are representative of fragments from the parent compound induced by the instrument during detection.
- Every compound gives a unique mass spectrum, thus allowing for structural assignments to be made.

Scanning electron microscope (SEM)





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FEG-SEM instrument has several components, they are:

- a **vacuum and a control unit** to prevent the contamination of the electron gun and prohibit the scattering of the electron beam.
- an **electron column** – which has electron gun and electromagnetic lenses – to focus an electron beam on the specimen. The gun emits electrons beam and speed up their energies while the lenses monitor the beam diameter and centre a specific electron probe on the sample. The beam is scanned in a rectangular raster and electrons undergo interaction with the specimen's atoms.

- a **scanning system and a detector** to capture the secondary electrons that would generate different signals، intensities, that resulted from the sample upon the interaction mentioned in previous part, showing the sample's surface structure on the display mean.

The resolution of the image depends on various factors, such as the size, the energy and the location of the electron beam, and the sample structure.

- SEM can be used for general forensic investigation and analysis as well as in specific areas such as ballistics. Many types of microscopic evidences can be obtained from fabrics, metals, textiles and glass. Moreover, SEM can also be used to identify scratches and indents on objects.

References

Basic Gas Chromatography-Mass Spectrometry: Principles and Techniques. F. W. Karasek and R. E. Clement. 2003. Elsevier Science B.V.

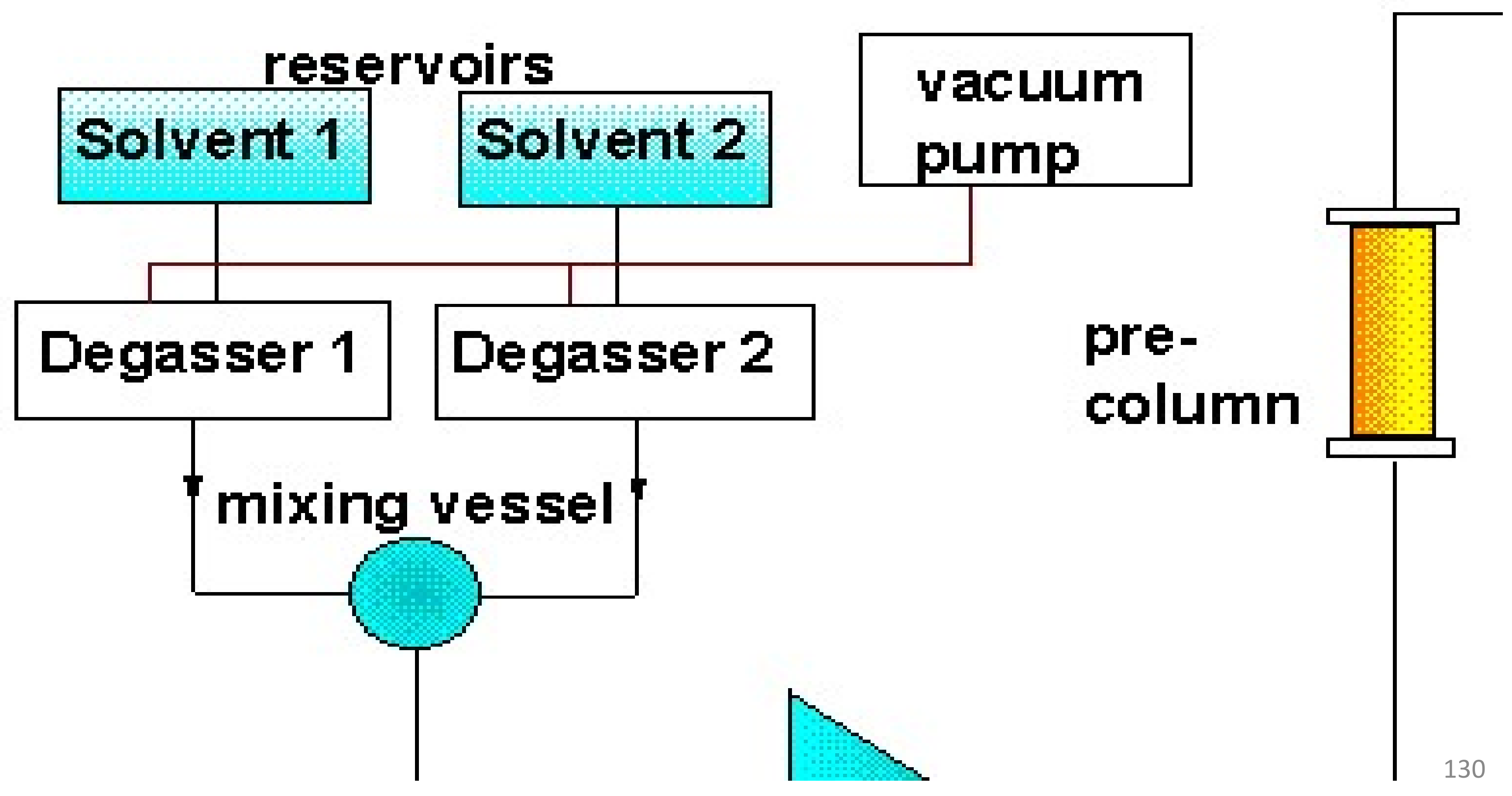
Lecture title: **Video Comparative spectrum device , A highly efficient liquid chromatography,atomic absorption spectrometry, Ultraviolet and visible spectrometer ,)**

High Performance Liquid Chromatography (HPLC)

- HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.

- Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase. The mobile phase must be degassed to eliminate the formation of air bubbles.

HPLC system



FOUR TYPES OF LIQUID CHROMATOGRAPHY

- Partition chromatography
- Adsorption, or liquid-solid
- chromatography
- Ion exchange chromatography
- Size exclusion, or gel, chromatography

COMPOSITION OF A LIQUID CHROMATOGRAPH SYSTEM

- Solvent
- Solvent Delivery System (Pump)
- Injector
- Sample
- Column
- Detectors (Diode Array)
- Waste Collector
- Recorder (Data Collection)

Uses of HPLC

- This technique is used in chemistry and biochemistry research for analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation.
- In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.

HPLC Chromatograph injectors

- The function of the injector is to place the sample into the high-pressure flow in as narrow volume as possible so that the sample enters the column as a homogeneous, low-volume plug. To minimize spreading of the injected volume during transport to the column, the shortest possible length of tubing should be used from the injector to the column.

- When an injection is started, an air actuator rotates the valve: solvent goes directly to the column; and the injector needle is connected to the syringe. The air pressure lifts the needle and the vial is moved into position beneath the needle. Then, the needle is lowered to the vial.

HPLC columns

- The column is one of the most important components of the HPLC chromatograph because the separation of the sample components is achieved when those components pass through the column. The High performance liquid chromatography apparatus is made out of stainless steel tubes with a diameter of 3 to 5mm and a length ranging from 10 to 30cm.
- Normally, columns are filled with silica gel because its particle shape, surface properties, and pore structure help to get a good separation. Silica is wetted by nearly every potential mobile phase, is inert to most compounds and has a high surface activity which can be modified easily with water and other agents. Silica can be used to separate a wide variety of chemical compounds, and its chromatographic behavior is generally predictable and reproducible.

Picture of an HPLC column

WHAT AFFECTS SYSTEM

Column Parameters

- Column Material
- Deactivation
- Stationary Phase
- Coating Material

Sample Parameters

- Concentration
- Matrix
- Solvent Effect
- Sample Effect

Instrument Parameters

- Temperature
- Flow
- Signal
- Sample Sensitivity
- Detector

Several column types (can be classified as)

- ***Normal phase***
- ***Reverse phase***
- ***Size exclusion***
- ***Ion exchange***

EVALUATION PARAMETERS

- EFFICIENCY
- RESOLUTION
- INERTNESS
- RETENTION INDEX
- COLUMN BLEED
- CAPACITY FACTOR

Types of Detectors

- Absorbance (UV with Filters, UV with Monochromators)
- IR Absorbance
- Fluorescence
- Refractive-Index
- Evaporative Light Scattering Detector (ELSD)
- Electrochemical
- Mass-Spectrometric
- Photo-Diode Array

Atomic absorption spectrometry (AAS)

- Atomic absorption spectrometry, AAS, and atomic emission spectrometry, AES, are two widely used atomic spectrometry techniques in forensic analysis.
- In AAS, the light source will emit radiations, a fraction of it is absorbed by atoms while the rest will reach a detector.
- In AES, atoms that are in thermally excited states will lose energy by emitting light, the light will reach a detector and measured.
- So, AAS and AES techniques work by the absorption or the emission of light at specific wavelengths.

- AAS and AES found applications in the analysis of elements and they can detect them at very low concentrations; down to ppm and ppb.
- AAS consist of atomization source like a flame, e.g. a graphite furnace, injection system, optical filters and photodetectors.

Ultraviolet and visible spectrometer (UV-Vis)

- Spectrophotometric techniques are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer.
- The spectrophotometer can measure the amount of light or electromagnetic radiation (of certain frequency) transmitted or absorbed by the solution.

The UV-Vis absorption spectrometry has the following components:

- **A light source**. It could be deuterium or hydrogen lamps – for UV spectrum range between 160to 375 nm – or tungsten filament lamp – for visible and near to IR spectrum region from 350 to 2500 nm –. It produces radiation which bombards the sample, causing the transition of atoms or molecules into higher energy levels – called absorption –. Possible incidents could occur upon the light beam touching the solid sample depending on the angle between the sample and the light beam.

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- **A monochrometer**. Its function is to select wavelengths of interest from the radiation coming from the light source.
- **A sample compartment,** also known as cuvette. It should be made from substances that would allow the radiation to pass through them in the required spectral region. The cuvette is usually of 1 cm length and could be made from quartz for UV regions or from silicate glass for visible or near IR regions.
- **A light detector**.
- **A display system**. Electronic equipment to record and display the result absorption spectra.

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UV-Vis spectrometer can measure areas of electromagnetic spectrum near the ultraviolet and infrared regions. Accordingly, the absorbance, reflectance and transmittance measurements.

Ultraviolet and visible spectrometer (UV-Vis)

spectrophotometer

Ultraviolet and visible spectrometer (UV-Vis)

The absorption of the light follows Beer's law; $A = \varepsilon l c$

A = absorbance (abruptly unit).

ε = molar absorptivity.

l = cell pathlength (cm)

c = molar concentration of the analyte

Calculation the concentration of unknown sample

1) From calibration curve

Conc of Std µg/mL	Absorbance
0.154	0.094
0.422	0.251
0.615	0.370
0.843	0.504
1.233	0.740

y = 0.601

Absorbance

1.000
0.800
0.600
0.400
0.200

2) From the following equation

$$\frac{C_s}{A_s} = \frac{C_x}{A_x}$$

Where:

C_s and C_x: The concentration of standard solution and unknown solution, respectively

A_s and A_x: The absorbance od standard solution and unknown solution, respectively

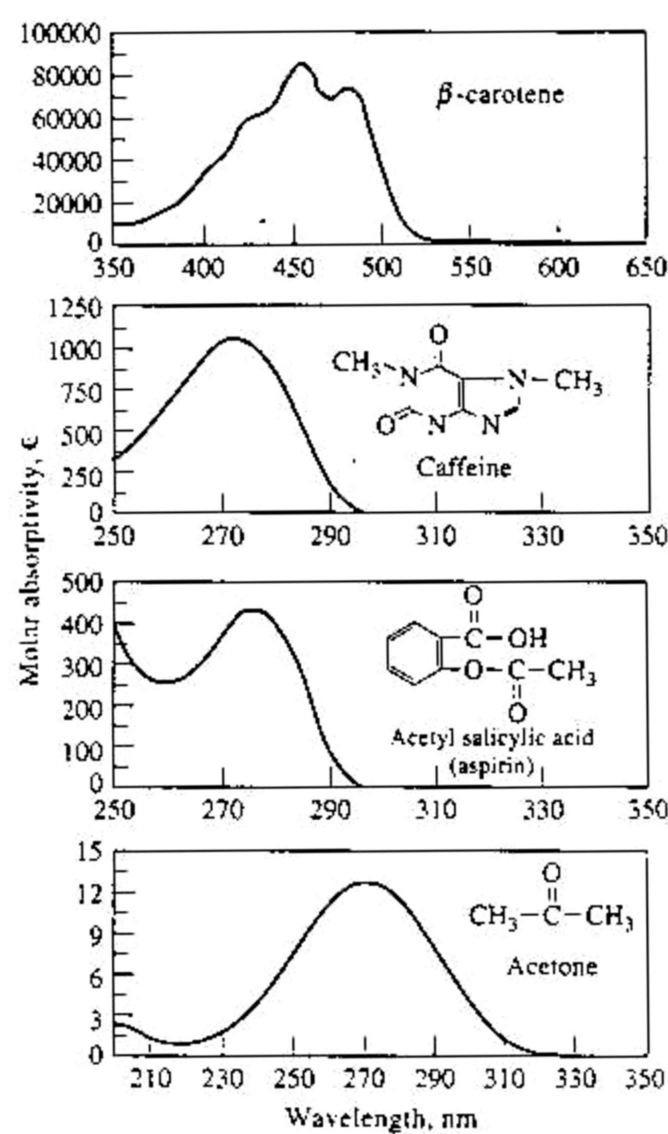
Absorption Spectrum

Most organic compounds and many inorganic ions and complexes absorb radiation in the UV - VIS region. A plot of this absorption by a compound against wavelength is called its *absorption spectrum*

- Different chemicals have different energy levels
- different ground vs. excited electron states

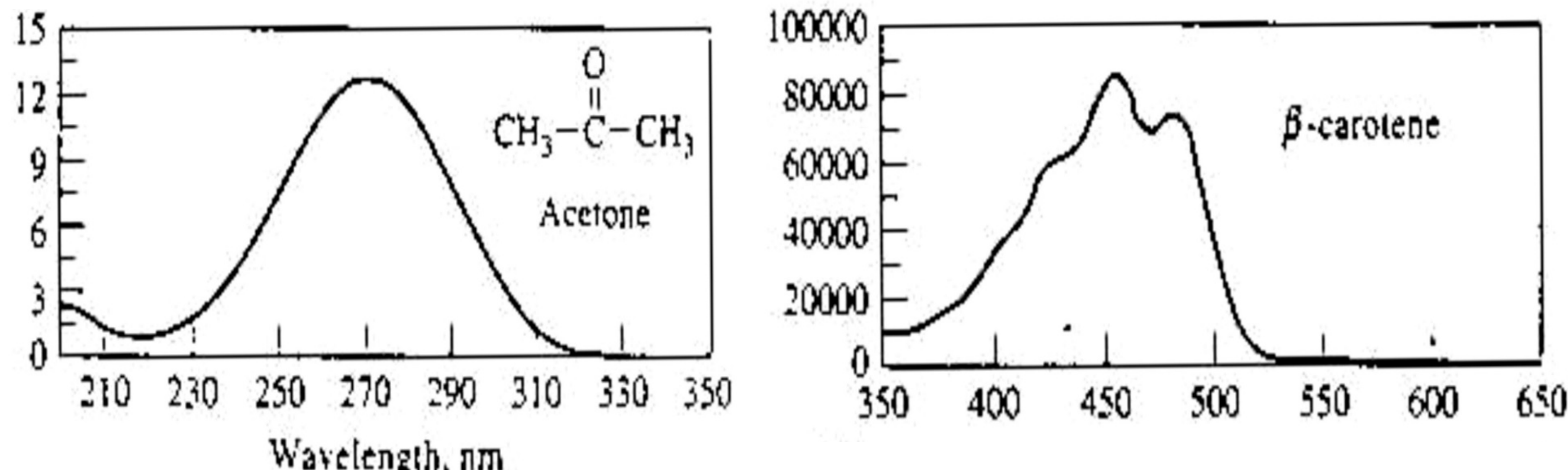
- will have different abilities to absorb light at any given wavelength

- The greater the absorbance of a compound at a given wavelength (high e), the easier it will be to detect at low concentrations



Absorption Spectrum

By choosing different wavelengths of light (l_A vs. l_B) different compounds can be measured



Selection of wavelength

Absorbance measurements are always carried out at fixed wavelength (using monochromatic light). When a wavelength is chosen for quantitative analysis, three factors should be considered

Wavelength should be chosen to give the highest possible sensitivity. This can be achieved by selecting λ_{max} or in general the wavelengths at which the absorptivity is relatively high.

λ_{max} - wavelength where maximum absorbance occurs

References

Forensic Analytical Techniques. Barbara H. Stuart. 2013. John Wiley & Sons Ltd.

Lecture title: **Infrared device , Automated fingerprint system - Genetic Analysis System- Light microscopes**

References:

1- John M. Chalmers, Howell G. M. Edwards, Michael D. Hargreaves (Infrared and Raman Spectroscopy in Forensic Science) John Wiley & Sons, 2012.

2- John M. Chalmers and Peter R. Griffiths ,(Handbook of Vibrational Spectroscopy)John Wiley & Sons Ltd, Chichester, 2002.

3-Norah Rudin, Keith Inman,(An Introduction to Forensic DNA Analysis) CRC Press, 2001.

1- General Use of Infrared Spectroscopy in Forensic Analysis

-With the development of **Fourier transform infrared (FT-IR)** spectroscopy, the application of FT-IR to forensic analysis became more prevalent **because of the increased speed, sensitivity and** lower cost.

The development of **diffuse reflection (DR)** accessories provided ease of sample introduction for several forensic applications.

-Samples with matte-finished surfaces could be analyzed with no sample preparation.

-Samples such as illicit drugs that previously required extensive grinding to make KBr pellets required less preparation.

Suzuki was the first to apply the DR method to forensics with the analysis of **drugs, polymers, wood and solvents** and **paints**.

Document analysis by DR has been reported for copy toners and inks.

It was not until the 1990s that the use of FT-IR became more regularly applied in forensic laboratories.

Infrared Spectroscopy

Features of infrared spectrophotometer:

*a source of infrared rediation

*sample and reference cell

*a wavelength selector

*an infrared detector

Fig (1): IR Spectroscopy instrument

FT-IR microscope development is considered a milestone achievement for forensic analysis and is considered the most significant recent advancement for microsample analysis.

Sample preparation to introduce specimens into microscopes is frequently easier, because only a small portion of the sample is required for placement in the IR beam for transmission spectroscopy.

For example, to analyze **pain**t from a surface, all that is required is a sliver sliced from the surface with a scalpel.

Therefore, standard sized samples are often reduced in size and analyzed in microscopes because of convenient sample preparation.

The reflection techniques, reflection-absorption (R-A), specular reflection, diffuse reflection (DR), and internal reflection spectroscopy (IRS), frequently referred to as attenuated total reflection (ATR), provide additional ease of sampling in IR microscopes. **Because reflection methods require little or no sample preparation, they are used at least as frequently as traditional transmission methods.**

Polymeric materials such as fibers, paints and adhesive tapes are frequently analyzed to identify characteristic information regarding their composition.

Physical and chemical information on these materials is stored in computer databases to help determine the manufacturer or, supplier, or simply to discriminate between many similar samples of material.

Some of the available databases will be described as part of the analyses mentioned in the forthcoming pages. Other general polymeric materials found as evidence do not fall into a particular category and must be studied on a caseby-case basis without the aid of comparison with similar material in a database.

Copy toners

Questioned documents involving fraud and threatening let- ters are often produced on printers, copy machines and facsimile machines. The machine model identification of this common office equipment has been achieved through comparison of the resins of the toners used as ink. These "copy toners" have been studied for forensic analysis as a class of polymeric material.

In the laboratory, the samples were prepared for IR analysis using a heat transfer technique to remove the toner from the documents. The preparation technique involves heating the back of the paper with a soldering iron at a specified temperature and smearing the toner onto aluminum foil attached to a glass microscope slide. Spectra were obtained with an FT-IR microscope by R-A. With this method, the IR beam passes through the sample and is reflected from the aluminum foil to the detector via the microscope optics.[22] Figure 2(a) shows the original spectrum of the toner from the bomb pack- age label. This spectrum is sloped due to scattering from the carbon black particles used for the copy image.

The baseline flattened spectrum in Figure 2(b) is typical of a styrene/acrylate copolymer. Significant variations in the IR spectra are produced by the polymeric resins which contain numerous additives that vary in type and quantity

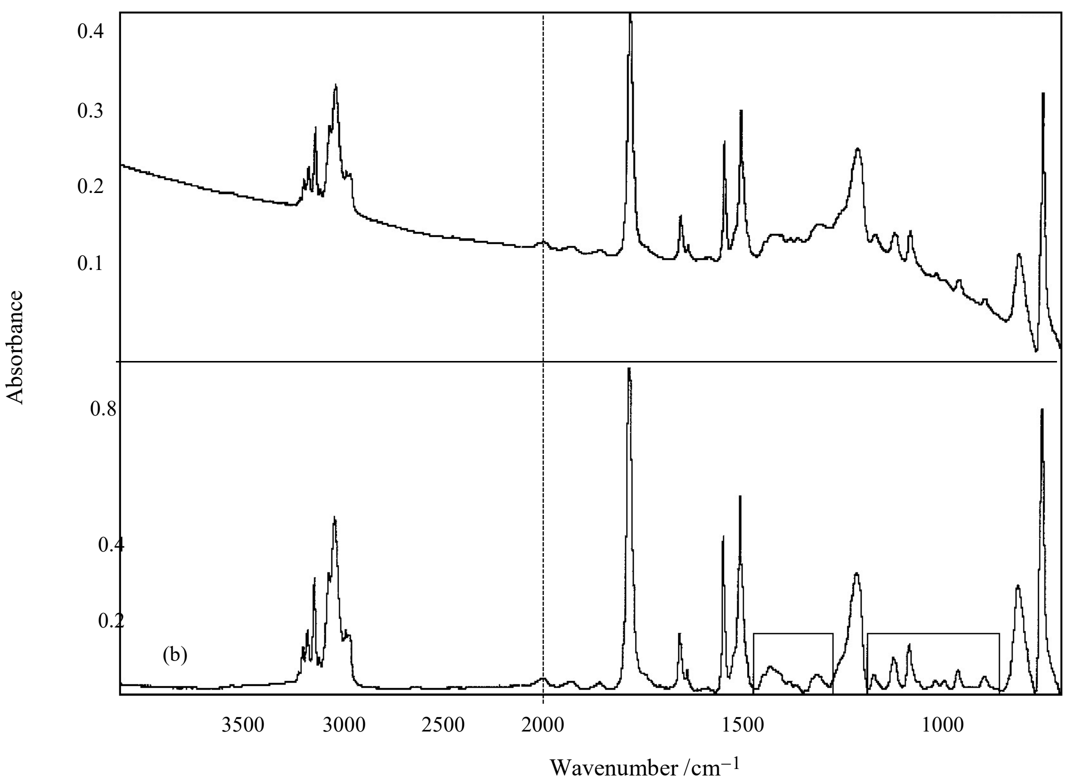


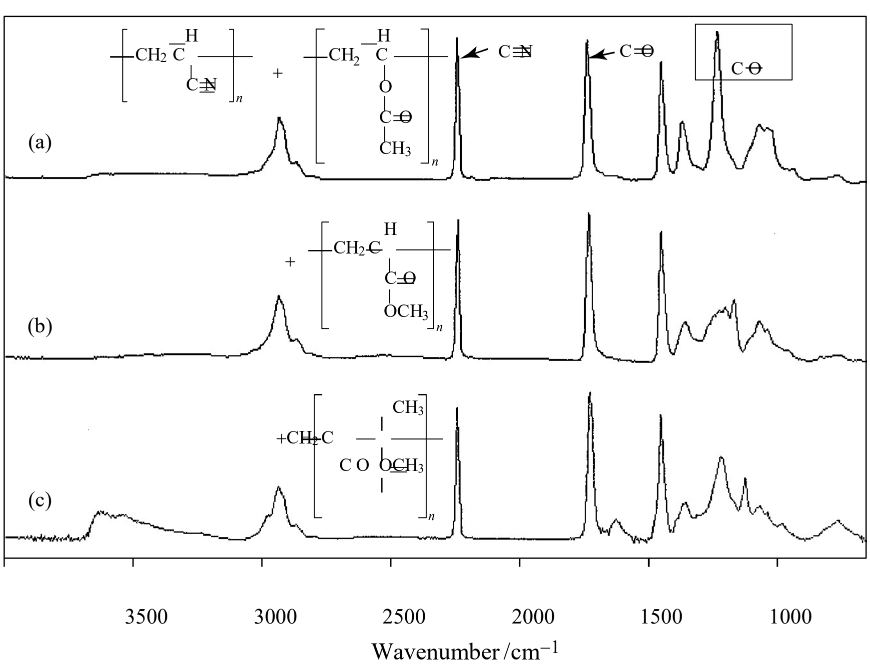
Figure (2). Poly(styrene:acrylate) resin copy toner spectrum from an address label on a bomb package: (a) original uncorrected spectrum; (b) flattened spectrum showing boxed regions where additive absorptions can be observed.

Fibers

Polarized light microscopy is used to determine the generic classification of the polymer type, and IR microscopic analysis plays an important role by identifying subclasses of synthetic fibers

Polarized light microscopy is used to determine the generic classification of the polymer type, and IR microscopic analysis plays an important role by identifying subclasses of synthetic fibers position or environment within the comonomer structure as a methacrylate, methyl methacrylate or vinyl acetate.

Over 20 variations of acrylics can be identified by IR. Thus, IR is a very useful tool in providing information that further discriminates fiber types to enhance the evidential value of a sample.



Figur(3) e . IR spectra of acrylic copolymer fibers: (a) poly(acrylonitrile:vinyl acetate); (b) poly(acrylonitrile:methyl acrylate); (c) poly(acrylonitrile:methyl methacrylate).

Paints

IR spectroscopy of paints has been useful in forensic analysis since the 1960s.

After visual light microscopy, IR analysis offers the most information in forensic paint examination. The organic binders are frequently identified with IR, and both organic and inorganic pigments can often be identified.

Scientists from the RCMP have been classifying automotive paints based on chemical composition since the 1970s.

The original analysis was performed with the use of high-pressure diamond-anvil cells in beam condensers on dispersive IR spectrometers. Since then, the RCMP and other analysts have changed to using the less cumbersome low-pressure compression diamond cells with beam condensers in FT-IR systems.

Inorganic pigment components in paints have revealing spectral features at the lower wavenumbers. Beam condensers are used rather than FT-IR microscopes to overcome the limited frequency range of mercury cadmium telluride (MCT) detectors used in IR microscopes.

For paint analysis, the extended range to near 200cm $^{-1}$ is obtained with CsI optics and a standard deuteratedtriglycine sulfate (DTGS)detector in the spectrometer bench.

Figure 5 compares IR and Raman paint spectra of a yellow acrylic melamine enamel automotive paint. The IR spectrum in Figure 5(a) clearly shows the resin binder features. The N–H stretch near3350 cm^{-1}, the C–H stretches near 3000 cm^{-1}, the CDO stretch near 1730 cm^{-1}, the C–N stretch near 1540 cm^{-1}, and the typical C–O envelope from 1300 to 1000 cm^{-1} are observed in the IR.

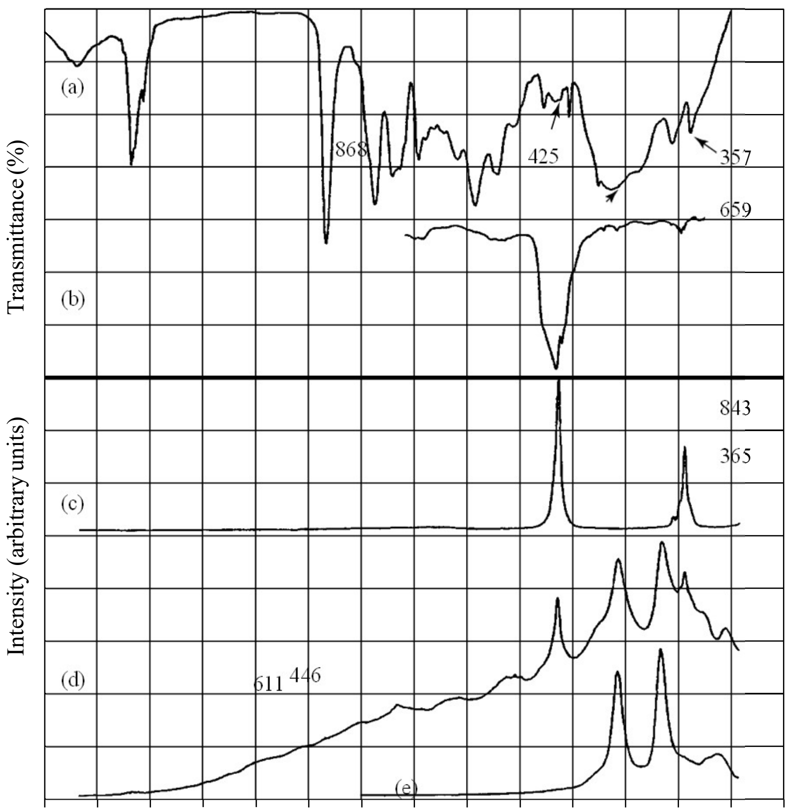


Figure 5. IR and Raman spectra of yellow acrylic melamine enamel auto paint with pigments: (a) IR spectrum of the auto paint; (b) IR spectrum of chrome yellow pigment; (c) Raman spectrum of chrome yellow pigment; (d) Raman spectrum of the yellow auto paint; (e) Raman spectrum of rutile. (Spectra provided by E. Suzuki, Washington State Patrol, Forensic Laboratory, Seattle, WA.)

DRUGS

- IR has been used for the analysis of both licit and illicit drugs for many years.
- Samples prepared in standard 13-mm KBr pellets have been used for inclusion of drug spectra in the library. However, recently the GSCL successfully applied ATR to drug analysis.
- Horizontal ATR sample compartment accessories with three reflections provide sufficient sensitivity to acquire spectra of approximately 400 ng of lysergic acid diethylamide (LSD) as a film cast from chloroform (Figure 6).
- The region between 2400 and 1800 cm^{-1} was blanked to remove the uncompensated diamond absorption produced by the IRE.
- The ATR spectra of drugs can be successfully searched in the original transmission spectral library in spite of the intensity differences in the peaks.
- GC combined with IR (GC/IR) simplifies the analysis of drug mixture samples typical of those associated with clandestine laboratories and is a standard procedure of the Drug Enforcement Administration (DEA) laboratories.
- For the analysis of methamphetamine and related compounds, the DEA is required to identify the optical

Ephedrine

Reduction

Methamphetamine

+ OH^-

Figure 6 . Reduction reaction of ephedrine to methamphetamine.

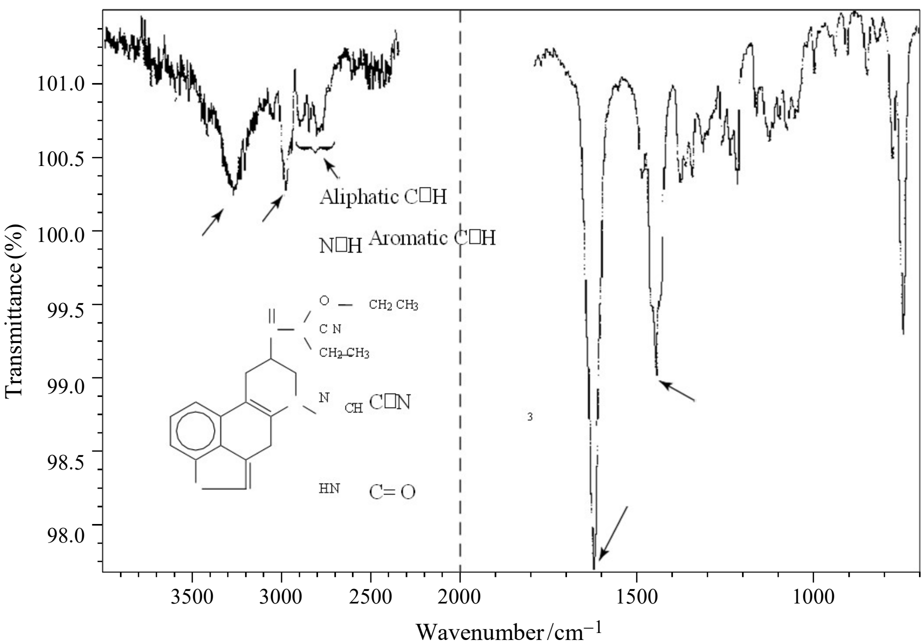
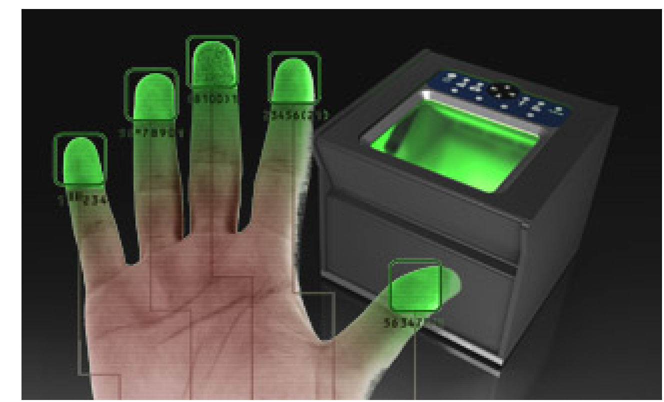


Figure 7. Spectrum of 400 ng of LSD by ATR. (Spectrum provided by Robert Ollis, Georgia Bureau of Investigation Crime Laboratory, Decator, GA.)

2- Automated fingerprint system



What Are Fingerprints?

- All fingers, toes, feet, and palms are covered in small ridges.
- These ridges are arranged in connected units called *dermal,* or *friction, ridges.*
- These ridges help us get or keep our grip on objects.
- Natural secretions plus dirt on these surfaces leave behind an impression (a print) on those objects with which we come in contact.

Formation of Fingerprints

- An animal's external tissue (skin) consists of (a) an inner dermis and (b) an outer epidermis.
- The creation of fingerprints occurs in a special layer (the **basal layer**) in the epidermis where new skin cells are produced.
- Fingerprints probably begin forming at the start of the 10th week of pregnancy.
- Because the basal layer grows faster than the others, it collapses, forming intricate shapes.

Principles of Fingerprints

First Principle: A fingerprint is an individual characteristic; no two fingers have yet been found to posses identical ridge characteristics.

Second Principle: A fingerprint will remain unchanged during an individual's lifetime.

Third Principle: Fingerprints have general ridge patterns that permit them to be systematically classified.

How to collect fingerprint data?

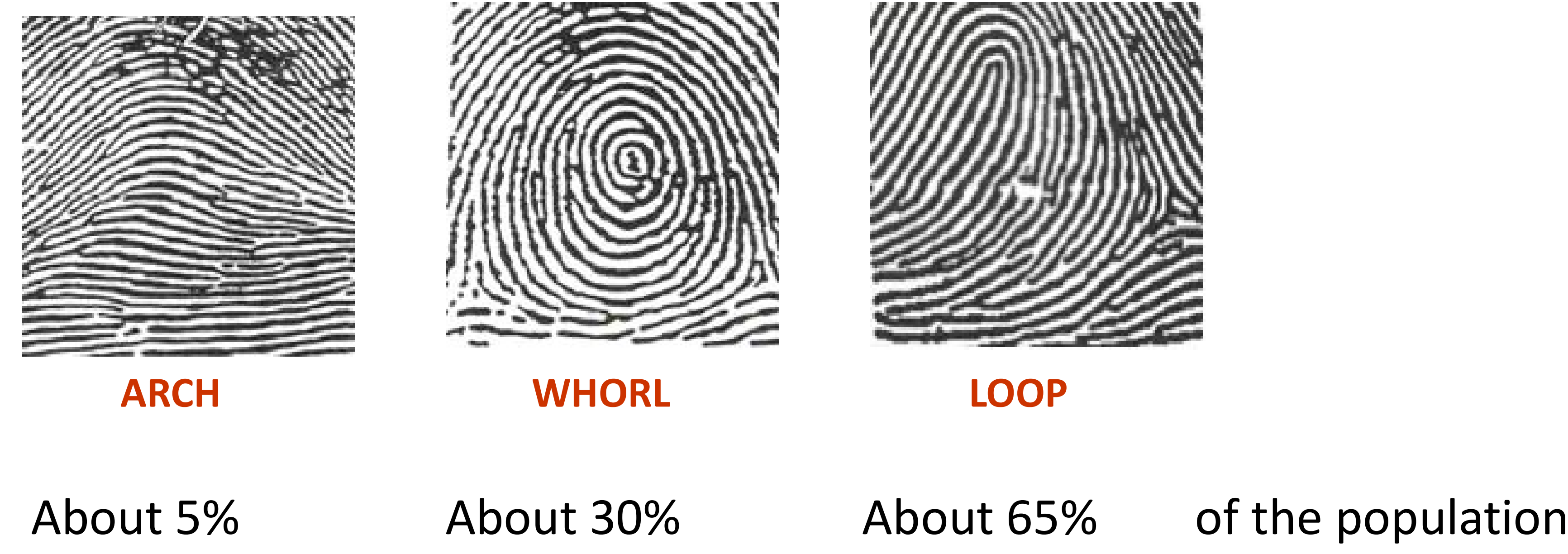
1- By applying a thin coating of ink over a finger and rolling the finger from one end of the nail to the other end of the nail while pressing the finger against a paper card.

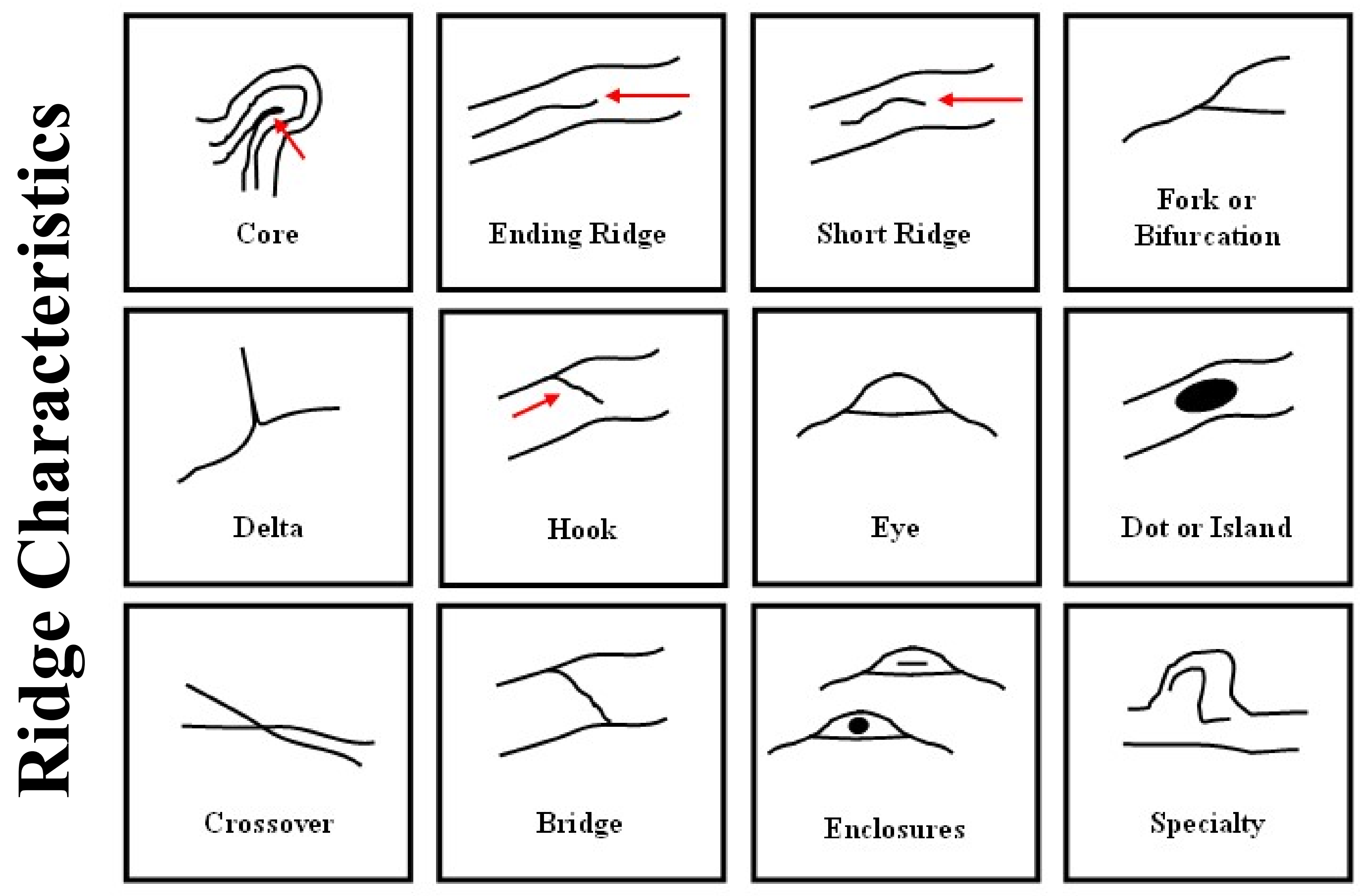
2- If the finger was simply pressed straight down against the paper card instead of rolling, the resulting fingerprint impression would only contain a smaller central area of the finger rather than the full fingerprint, resulting in an inked "flat" or "plain" fingerprint impression.

3- The perspiration and contaminants on the skin result in the impression of a finger being deposited on a surface that is touched by that finger. These "latent" prints can be chemically or physically developed and electronically captured or manually "lifted" from the surface by employing certain chemical, physical, and lighting techniques.

Characteristics of Fingerprints

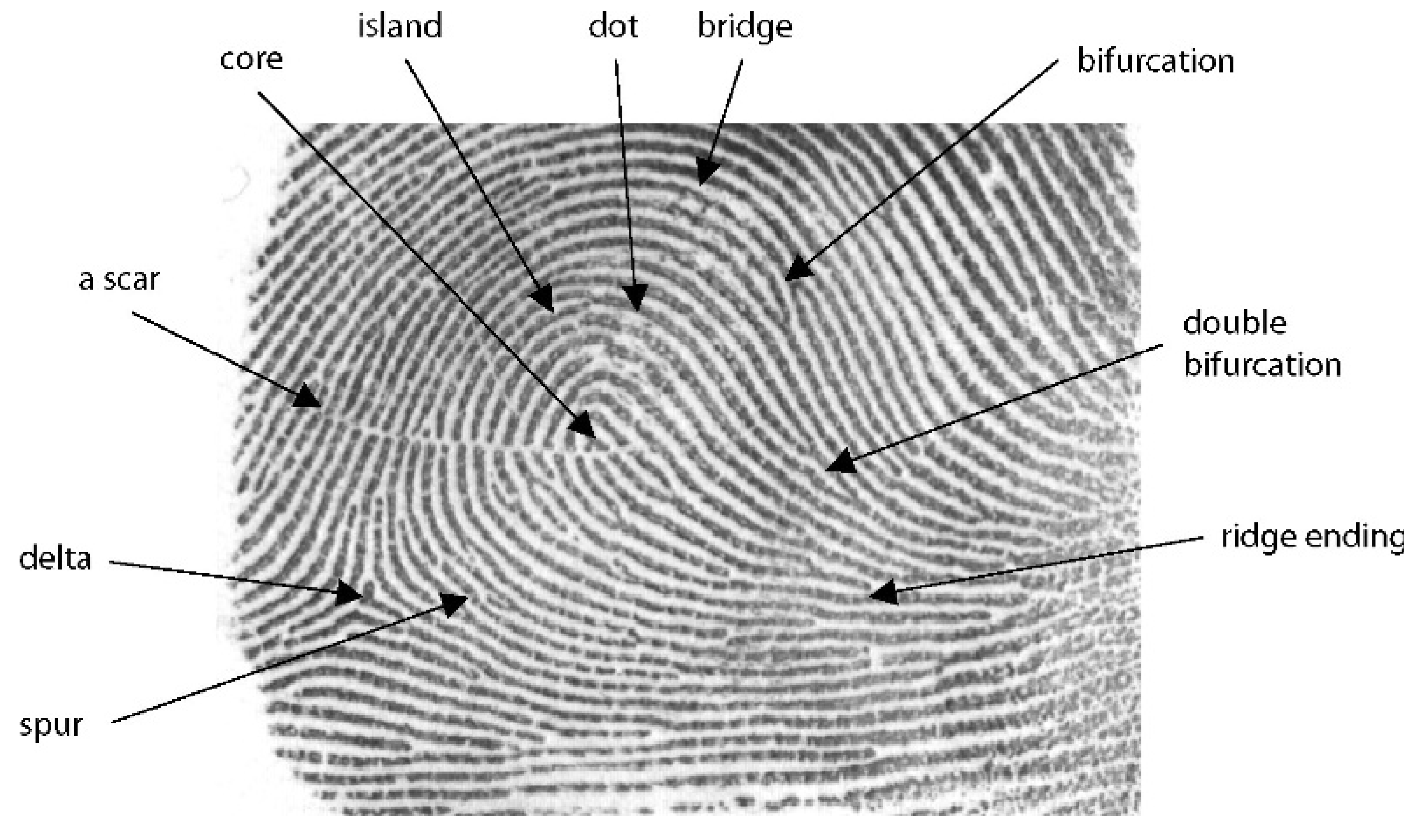
- There are 3 general fingerprint distinctions:



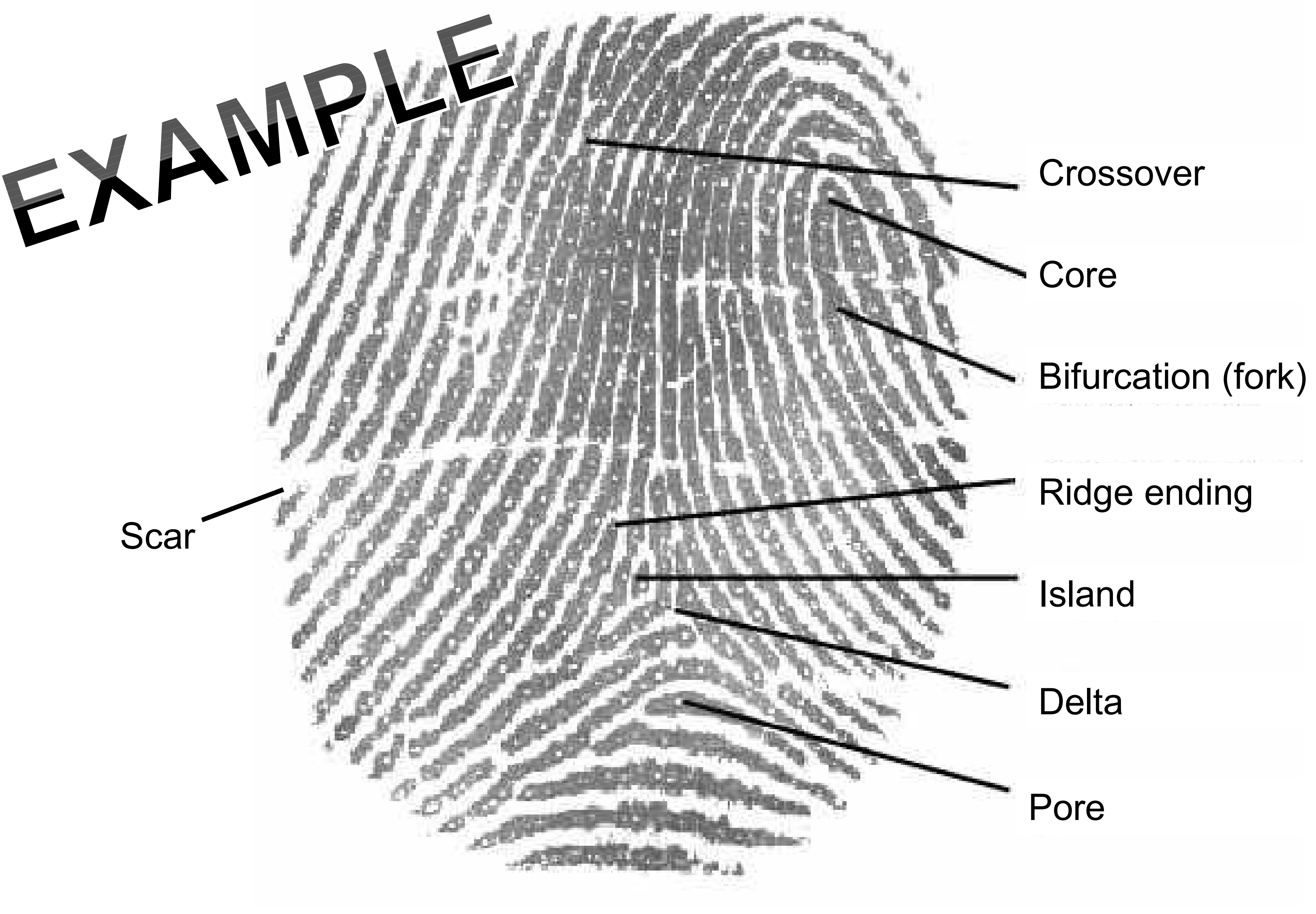


Use these characteristics as points of identification when comparing fingerprint samples. The more points you can find in common, the better the match!

Fingerprint Minutiae- characteristics of ridge patterns



Ridge Characteristics



http://cnx.org/content/m12574/latest/properties.jpg

Fingerprint Identification

When minutiae on two different prints match, these are called points of **similarity** or points of **identification**. At this point there is **no** international standard for the number of points of identification required for a match between two fingerprints. However, the United Kingdom requires a minimum **sixteen** points while Australia requires **twelve**. There are no legal requirements in the United States on the number of points. Generally, criminal courts will accept **8** to **12** points of similarity.

Types of Fingerprints

There are 3 types of prints that investigators look for at crime scenes:

1. **Patent fingerprints** are visible prints transferred onto smooth surfaces by blood or other liquids.

1. **Plastic fingerprints** are indentations left in soft materials such as clay or wax.

1. **Latent fingerprints** are not visible but made so by dusting with powders or the use of chemicals.

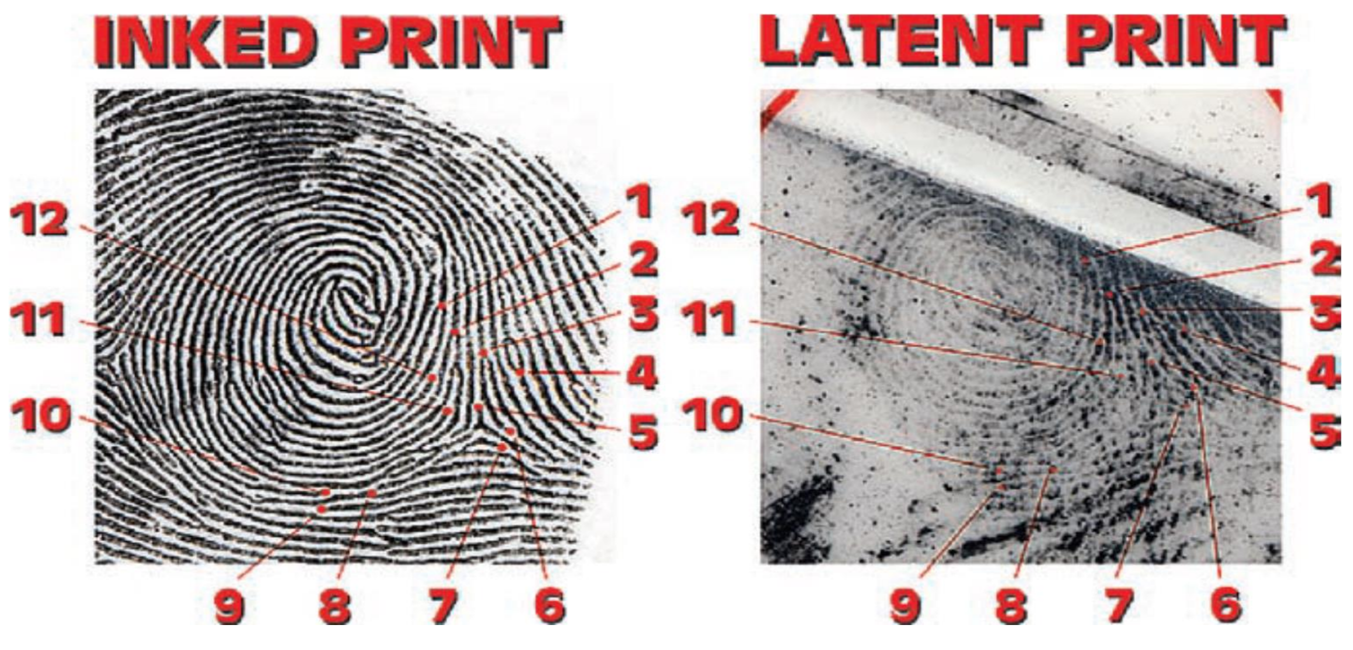


FIGURE (8) …. Individual characteristics link this fingerprint found at a crime scene to a known origin (person).

Latent Prints

- Latent fingerprints are those that are not visible to the naked eye. These prints consist of the natural secretions of human skin and require development for them to become visible.
- Most secretions come from three glands:
  - **Eccrine**—largely water with both inorganic (ammonia, chlorides, metal ions, phosphates) and organic compounds (amino acids, lactic acids, urea, sugars). Most important for fingerprints.
  - **Apocrine**—secrete pheromones and other organic materials.
  - **Sebaceous**—secrete fatty or greasy substances.

Developing Latent Prints

- Developing a print requires substances that interact with secretions that cause the print to stand out against its background. It may be necessary to attempt more than one technique, done in a particular order so as not to destroy the print.
  - **Powders**—adhere to both water and fatty deposits. Choose a color to contrast the background.
  - **Iodine**—fumes react with oils and fats to produce a temporary yellow brown reaction.

Investigators use many different types of powders.

Most black powders are made from fine carbon or iron. Light-colored gray or white powders can be made of any number of substances, such as finely divided aluminum.

There are also **fluorescent powders** in red, green, yellow, or orange, some of which may also contain iron particles.
Any powder containing iron may be applied with a magnetic applicator.

Once the powder has been applied and contrast can be seen, the fingerprint can be lifted and preserved using fingerprint tape, a high-quality transparent tape typically at least an inch wide.

The lifted fingerprint can then be placed onto a fingerprint lift card that offers the greatest contrast (black for white-powder lifts and white for black-powder lifts).

Identifying information such as the name of the investigator, date and time of collection, location of fingerprint, and case number all should be recorded on the card. Figure 9 shows a fingerprint lift of a black powder impression

Fig (9) Dusting for fingerprints at a crime scene.

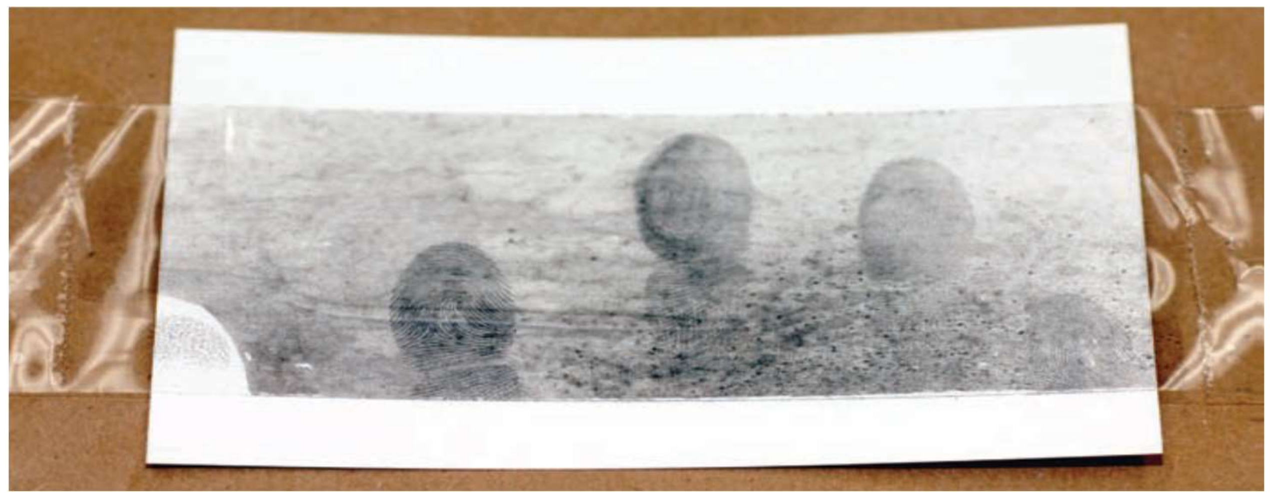


Fig (10) A fingerprint lift of a black powder impression

4-Iodine Fuming

Iodine, in much the same way as solid carbon dioxide, undergoes a phase transition from solid to gas, skipping the liquid phase. This phenomenon is known as **sublimation**.

Iodine is a purple solid under ambient temperature and pressure. When iodine crystals are heated, they will sublime, producing iodine vapors.

These vapors are thought to be absorbed by the fingerprint residue so that they produce a transient amber-colored product, shown in Figure 13a.

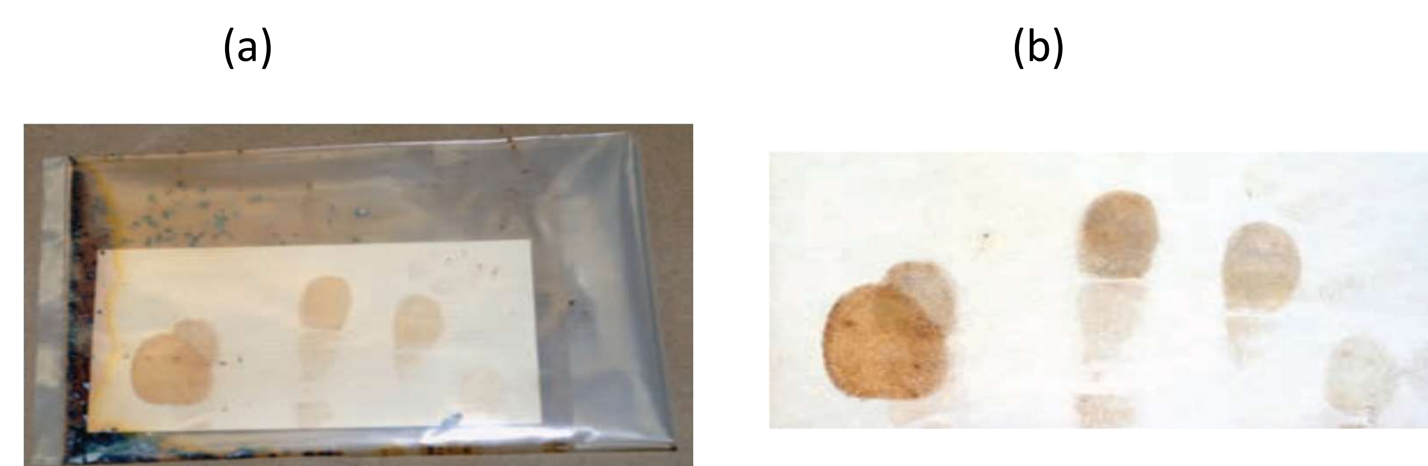


FIGURE 13 Fingerprints (a) during iodine fuming and (b) after being treated with a starch fixative.

Developing Latent Prints

- **Ninhydrin**—reacts with amino acids to produce a purple color.
- **Silver nitrate**—reacts with chloride to form silver chloride, a material which turns gray when exposed to light.
- **Cyanoacrylate**—"super glue" fumes react with water and other fingerprint constituents to form a hard, whitish deposit.

In modern labs and criminal investigations, lasers and alternative light sources are used to view latent fingerprints. These were first used by the FBI in 1978. Since lasers can damage the retina of the eye, special precautions must be taken.

Iodine Fingerprint

Ninhydrin Fingerprint

Cyanoacrylate Fingerprints

Fingerprint Forensic

- ***How are latent fingerprints collected?***

Chemical	Uses	Application	Theory	Print
Dust	Glass, metal, plastic, hard non-porous surfaces	Dusted with powder	Will be attracted to oils in sebaceous secretions	Will be the color of the dust
Cyanoacrylate vapor	Household itesm, plastic, glass, metal, skin	Heat sample in vapor tent	Vapors attracted to amino acids in eccrine secretions	White, crystalline print
Ninhydrin	paper	Object dipped in ninhydrin. Wait 24 hours.	Attracted to amino acids in eccrine secretions	Purple-blue
Silver nitrate	Wood, Styrofoam, cardstock	Dip, spray, or paint surface	Reacts with chlorides in eccrine secretions	Black, or red brown under UV light
Iodine vapors	Paper, cardboard, unpainted surfaces	In vapor tent, heat solid iodine crystals, use starch solution to fix print	Reacts with oils and fats in sebaceous secretions	Brownish print

Other Prints

Palm

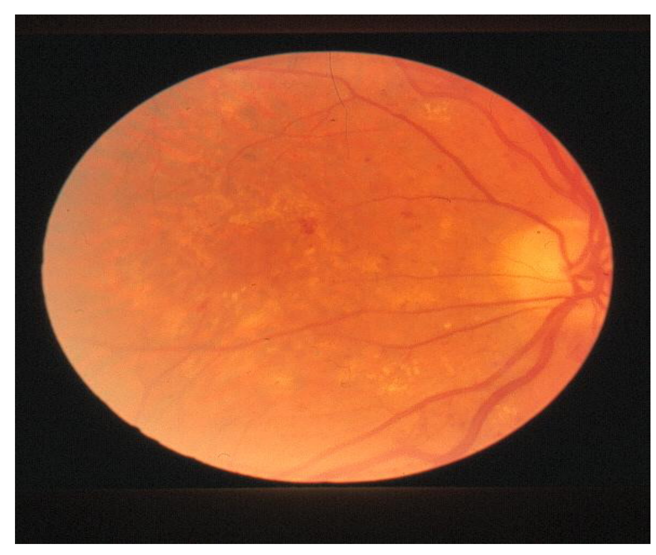
Foot

teeth

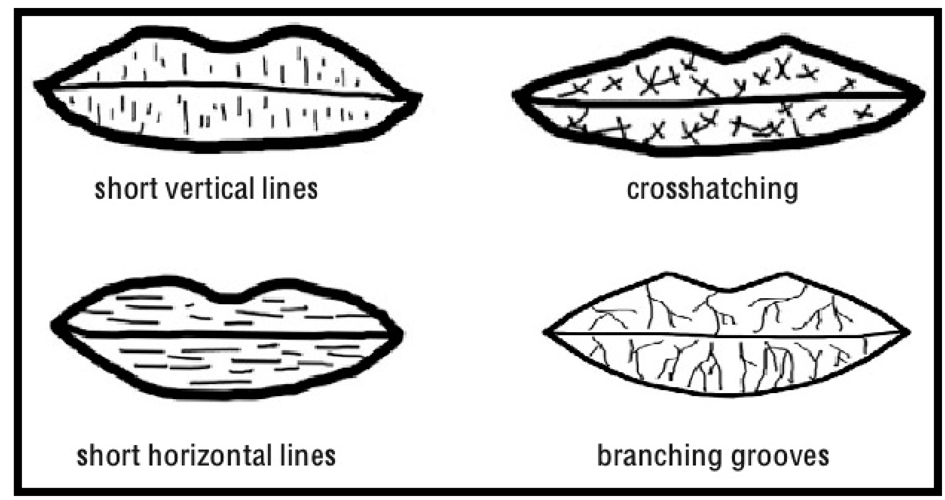
Other Prints

- **Ears**—shape, length and width
- **Voice**—electronic pulses measured on a spectrograph
- **Foot**—size of foot and toes; friction ridges on the foot
- **Shoes**—can be compared and identified by type of shoe, brand, size, year of purchase, and wear pattern.

Other Prints



The blood vessel patterns in the eye



Lips

- **Genetic analysis system**

DNA FINGERPRINTING

- Human DNA can be analyzed by this method to identify individuals at the genetic level with a far greater degree of certainty than previous forensic methods such as blood group determination or traditional fingerprint analysis.
- DNA fingerprinting (also called DNA profiling, DNA typing or genetic fingerprinting) can also be used to determine the relationship between individuals (e.g. paternity testing).
- DNA fingerprinting quickly became now a major resource that is used routinely in the detection and conviction of criminals, and produces hundreds of matches to DNA found at crime scenes every week.
- The analysis of short tandem repeats **(STRs**) forms the basis of forensic DNA profiling systems used throughout the world.

Short tandem repeats

Certain regions of our DNA contain more differences than others, and short tandem repeats are an example of a region of DNA that exhibits large variations between individuals.
DNA fingerprinting depends on the analysis of short tandem repeats (STRs), short repeating patterns of two or more nucleotides (e.g. (**CA**)$_n$ or (**ACGT**)$_n$, where *n* is several hundred).

For example, in the sequence CGTCAG**CACACACACACACACACACACACA**TGGCGTG, the dinucleotide **CA** is repeated 13 times (*n* = 13).

Tens of thousands of different short tandem repeats, or *microsatellites* have been identified in the human genome.

STRs are observed at the same positions on chromosomes (loci) in different members of the population, but the number of repeats (*n*) varies between individuals.

This variation in number of repeats is an example of *polymorphism*.

STR Analysis

STR analysis uses PCR to measure the number of repeats at specific loci. Primers bind to the DNA at specific STR loci and, are extended by PCR. The length of the PCR product depends on the number of repeats.

If the PCR primers are labelled, the PCR products will be labelled, allowing the products to be detected at the end of the reaction. For each STR locus, there will be two PCR products (one for each of two alleles).

The simultaneous analysis of multiple different STR loci enables a unique profile of an individual to be built up. Several PCR reactions are carried out simultaneously in a single tube at different STR loci, giving several products (two for each locus).

The following components are required:

- **A DNA sample**, e.g. a single human hair from the scene of a crime, or buccal cells from a mouth scrape of a suspect
- **Two oligonucleotide PCR primers:** one primer labelled at the 5′-end with ^{32}P, and one unlabelled reverse primer
- **A thermostable DNA polymerase**
- **Four deoxynucleoside triphosphates**: dATP, dGTP, dCTP, dTTP.

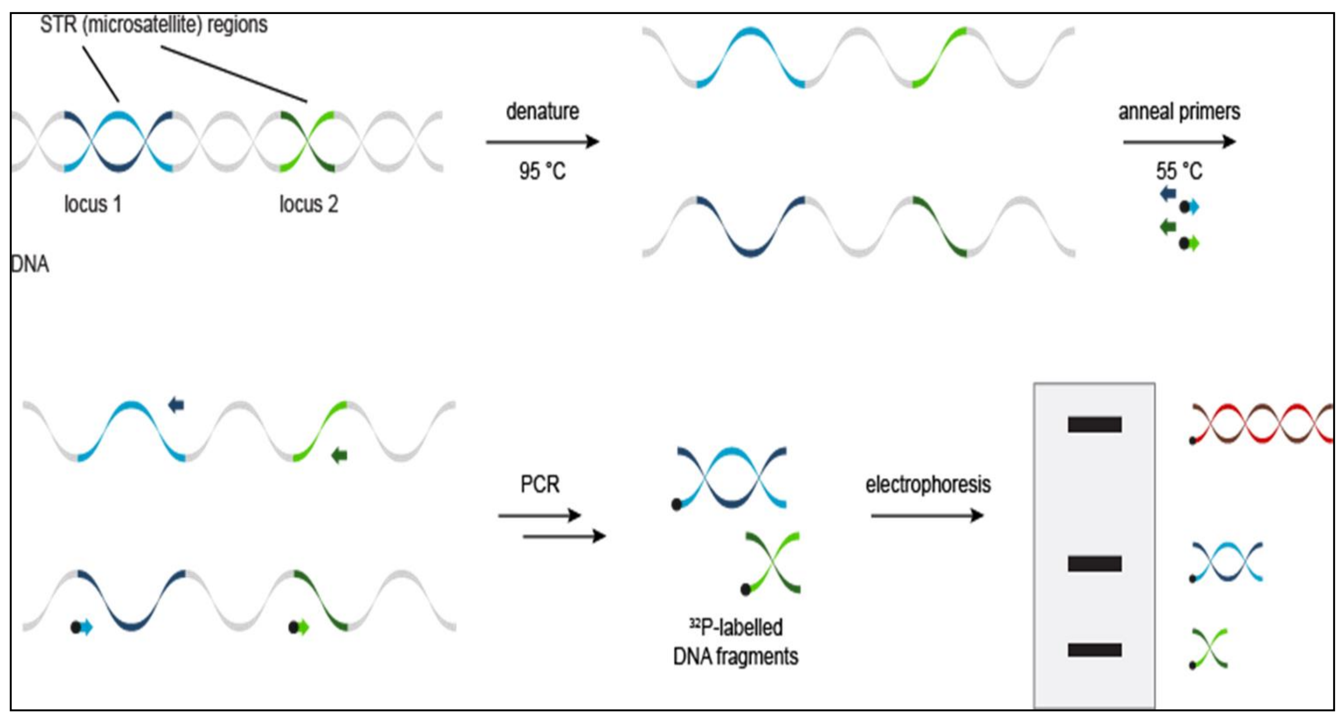


Figure : DNA Fingerprinting by STR analysis Short tandem repeats (STRs), di-, tri- or tetranucleotide units that are repeated several times, exist in everyone's genome. When amplified by PCR using labelled primers, labelled DNA fragments of different lengths are generated. These fragments, separated by gel electrophoresis or capillary electrophoresis, give a unique DNA "barcode" of an individual.

DNA microarrays

Oligonucleotides can be chemically attached to the surface of materials such as glass or silicon, on which they form small "spots" of around 100 µm in diameter.

Large numbers of oligonucleotides can be laid down on a single slide to form a microarray, and single strands of fluorescently-labelled DNA (labelled PCR products or cDNA) can be captured by hybridization.

(cDNA is single stranded DNA complementary to the RNA from which it is synthesized by reverse transcription.

It gives indirect information on the nature of the various RNA messages expressed in a cell (expression analysis)). If such a microarray contains 1000 spots then in theory it is possible to hybridize a unique complementary nucleic acid sequence to each spot. The identity of the DNA sequence is deduced from the location of the spot to which it hybridizes using a fluorescence scanner.

The fluorescent label attached to the captured nucleic acid strand can be added by a number of different methods. PCR products can be labelled at the 5′-end simply by using a PCR primer containing a 5′-fluorescent dye. PCR primers can be labelled with multiple fluorophores, but these tend to quench each other and also inhibit the PCR reaction. A better way to introduce multiple labels into the PCR product is to use fluorescently labelled deoxynucleoside triphosphates in the PCR or reverse transcriptase reaction. However, the efficiency of the PCR reaction may be compromised by the chemical modification on the heterocyclic base, which can inhibit the Taq polymerase. A carefully determined mixture of unlabelled and labelled deoxynucleotide triphosphates must therefore be used, and it is rare to achieve labelling densities greater than one fluorophore per 30 nucleotides.

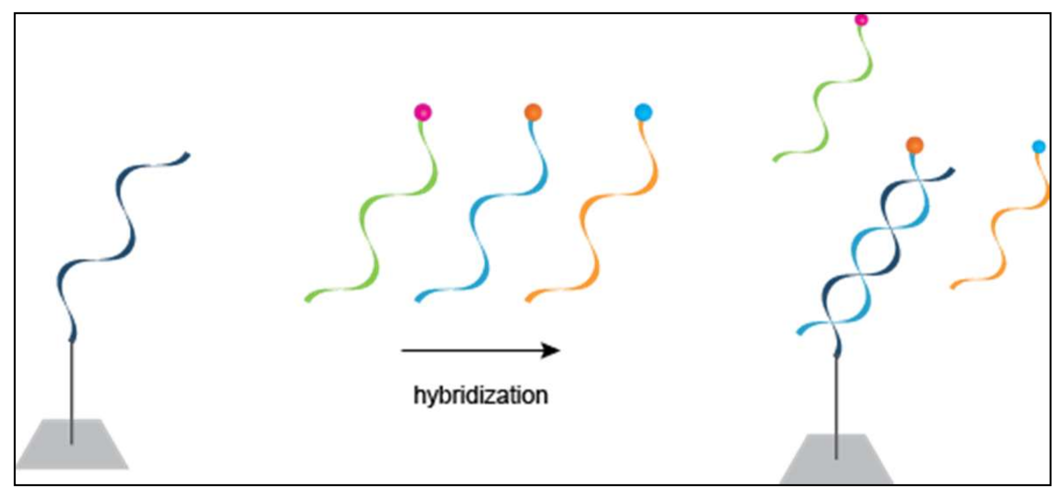


Figure 18 | DNA microarrays

Fluorescent STR analysis

In a more modern variant of STR analysis, the PCR primers are labelled with fluorescent dyes. Primers for different STR loci are labelled with different fluorescent dyes, adding a second dimension to the assay . As it has so far been possible to develop only a limited number of fluorescent dyes with well-resolved spectral characteristics, three different fluorescent dyes are typically used.

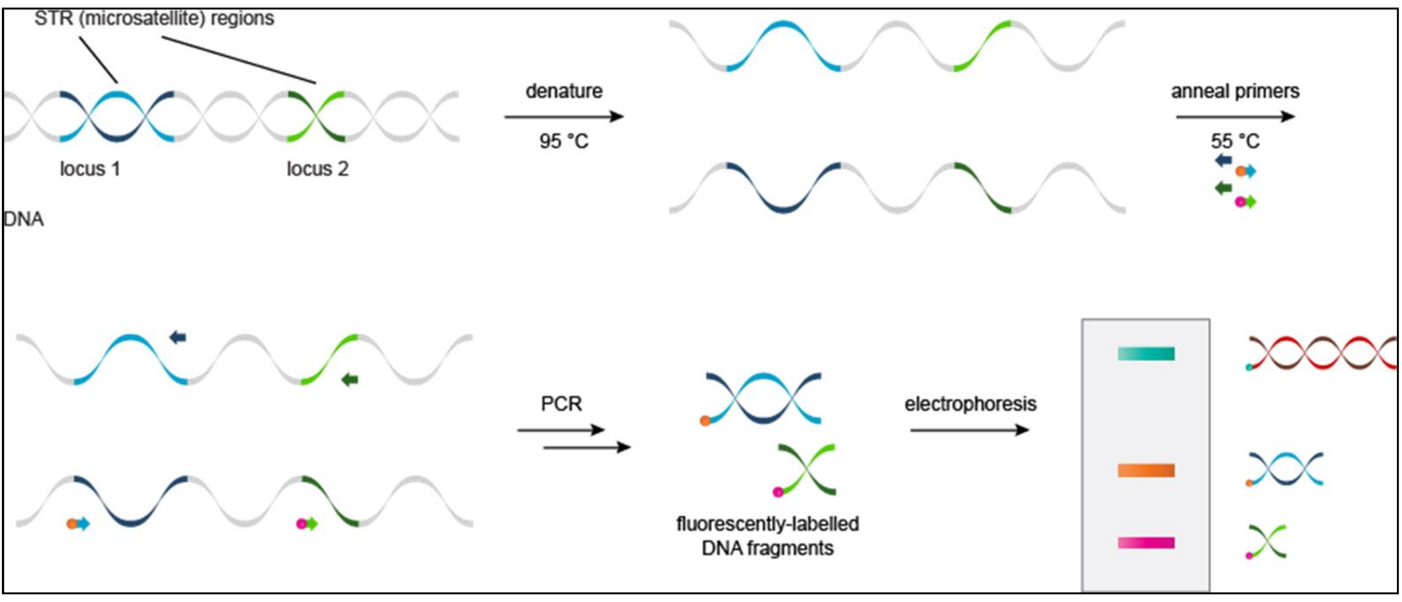


Figure | Fluorescent STR analysis In fluorescent STR analysis, the PCR primers are fluorescently labelled, which leads to fluorescently-labelled PCR products. The use of different fluorescent labels means that the products and bands originating from different STR loci can be more easily distinguished.

Light microscopes

Aplication on SOIL ANALYSIS

A body is found in a mountain canyon next to a riverbank. The victim is covered with mud and appears to have been dragged several yards.

Soil is a complex mixture of both organic and inorganic material.

A forensic soil analysis could include a wide range of substances from potting soil to safe insulation.

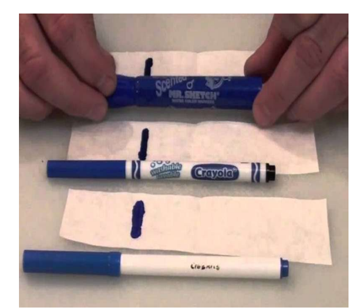
Regardless of its actual identity, most techniques used for soil analysis are similar. All techniques require an understanding of common chemistry principles such as pH, heterogeneous mixtures, and density.

Most soil analyses are performed by forensic chemists because geologists and mineralogists are rarely associated with crime laboratories.

Lecture title: **Analysis of Forensic Samples - Drug Analysis - Inks, Paints, Pigments, Blood Alcohol Analysis.**

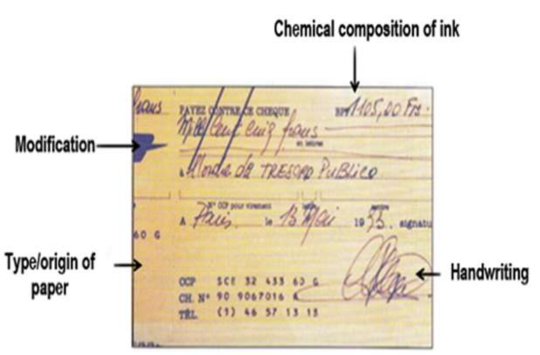
INK analysis

Forensic analysis and identification of writing and printing ink is connected with document examination.



Ink analysis may answer the flowing forensics' questions :

- Are these two writings made in the same ink?
- Are documents are original?
- What is the source of t manufacturer?
- Were documents made at the sa time ?
- How old is the analyzed writting?



The scientific appraisal of a suspect cheque. The analyses carried out take different approaches. The analysis of the ink brings to light the fraudulent reinsertion of the number 1 in the amount issued.

INK analysis

The analysis involves:

- predicting the type of device used to produce the document such as pens, pencils, photocopiers, printers, seals, stamps, etc.
- physical and chemical analysis of ink.

Ink analysis revolves around classifying and identifying
the various **components of ink**.

INK analysis

Ink Composition

inks, in their basic form, are composed mainly of :

1- colorant(s)

two types of colorants can be used: **dyes** and/or **pigments**.

- **Dyes** are generally compounds with highly conjugated resonance structures. Their molecular weights can vary from the hundreds to the thousands. Dyes can be classified according to their chemical structure or how they are applied to material. ***The Colour Index*** divides dyes into groups such as: acid dyes, azoic dyes, basic dyes, developers dyes, food dyes, ingrain dyes, natural dyes, oxidation bases, and vat dyes……..etc.

- **Pigments** consists of fine particles of insoluble material, it usually more stable and long-lasting than dyes. There are five major categories of pigments: organic pigments, toners, lakes, extended pigments, and inorganic pigments.

2- solvents

This is a fluid portion of ink that carry the color from the cartridge to the paper. Once on the paper, the solvent undergoes a series of changes (such as polymerization, evaporation, oxidation, or photodecomposition) causing the colorant to dry onto the paper.

3- resins

Resins can be natural or synthetic, such as Phthalates, ketone resins, styrene-type resins, phenol-type resins. They provide ink with a desired viscosity and a means to bond the ink and the substrate as the ink dries.

4- other organic and inorganic ingredients

these may be present in inks in traces , which can include antioxidants, preservatives, wetting agents, lubricants, and trace elements.

Ink analysis procedure

1- Physical Examinations :

-determination of the color and type of ink by evaluation of morphological characteristics using a stereomicroscope.

- This is used to identify the writing instrument used to make a questioned entry, as this dictates the extraction solvent necessary and how to proceed with chemical methods of analysis.

Example:

Morphological Characteristics in the Written Line can help in a distinction between absorbent and nonabsorbent ink. Inks that are water based will absorb into the paper fibers, providing nearly uniform coverage. Semiabsorbent inks such as ballpoint inks generally rest on top of the paper fibers.

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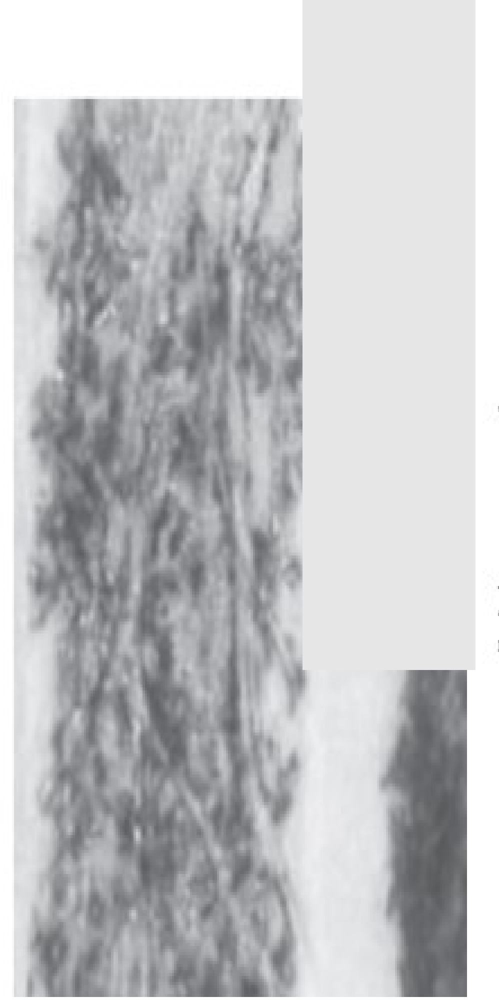

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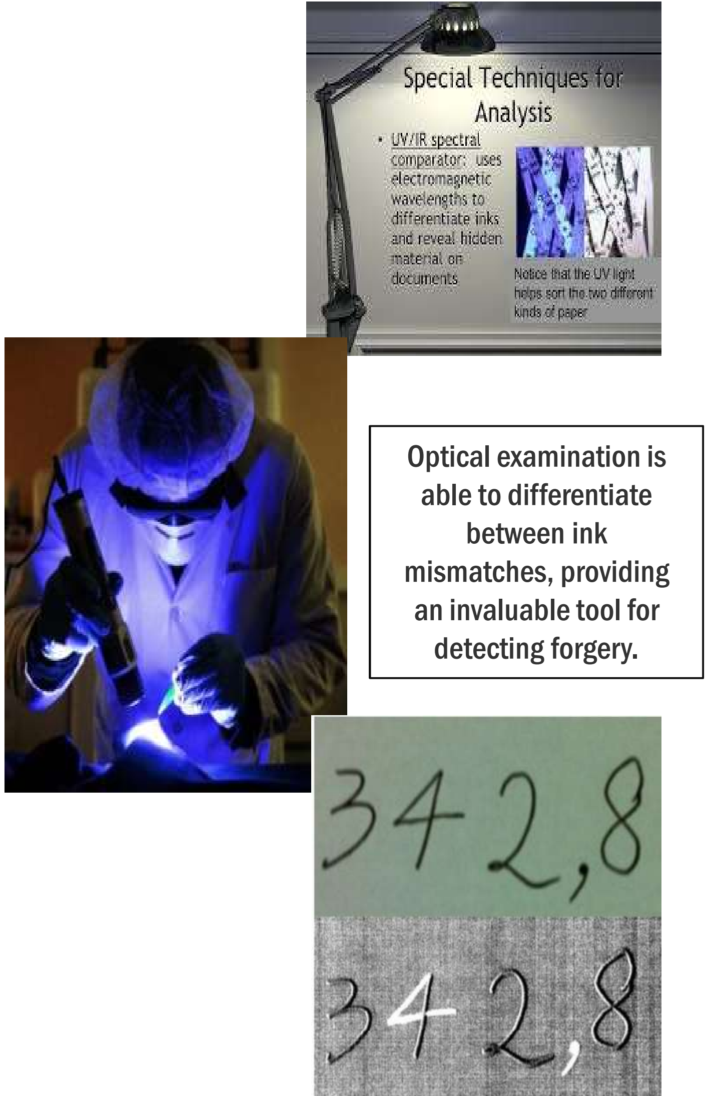
INK analysis

2- Optical Examinations

-This can provide valuable insight regarding the overall composition of an ink. The presence of colorants and other materials will directly affect the manner in which an ink absorbs, reflects, and transmits electromagnetic radiation.



INK analysis

-Ultraviolet (**UV**) and infrared (**IR**) radiation are forms of energy that can be used to examine inks on a document. Near-infrared reflectance (**IRR**) and infrared luminescence (**IRL**) properties can help significantly when evaluating properties of ink.

Optical examination is able to differentiate between ink mismatches, providing an invaluable tool for detecting forgery.

INK analysis

3- Chemical Examinations

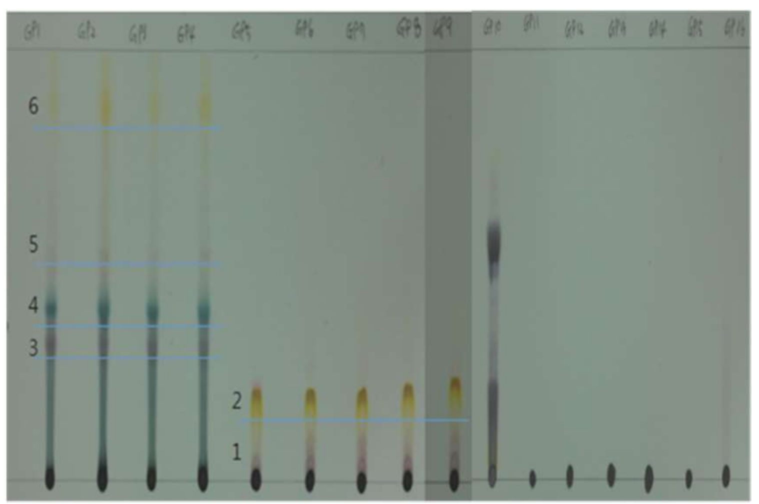
a)extraction of the ink

This achieves using appropriate solvents. inks with different extractabilities will have different components. However, it is necessary to maintain a single solvent or solvent system across a reference collection for searching purposes.

Ink Dye Chromatograms

b) Thin-Layer Chromatography (TLC)

TLC is one of the most widely used and generally accepted scientific methodologies employed to characterize ink formulations. It is an effective and efficient method for separating and identifying colorants. For example, two or more questioned inks can be compared using TLC to determine if they are the same.



c) Other Instrumentation

- gas chromatography–mass spectrometry (**GC–MS**)
- high-performance liquid chromatography (**HPLC**)
- spectroscopic techniques such as Fourier transform–infrared (**FT–IR**) and Raman spectroscopy have been studied for the characterization of inks.

Combination of all results is necessary to draw relevant and useful conclusions

Numerous research papers have been published regarding to the use of instrumental methodologies for the analysis of ink. **Search about some of them, and Then write a short assay.**

contaminate or destroy the analyzed sample. If the
about the examined document the further examinatio

Table 1: Analytical methods used for ink analysis.

Method	Destructiv
Vsible and polarizing light microscopy (PLM)	no
Thin Layer Chromatography (TLC)	yes
Infrared spectroscopy (IR) and microspectrophotomery	no
Raman spectroscopy	no
X-ray analysis	

Paint analysis

What is the relationship between forensic me and paint?

Tools and objects used in breaking and entering, vehicles that are involved in accidents or ram-raids, have some form of coating that acts as a protector; usually these coatings are **paint**. This paint may transfer or leaves tool marks ….. etc. on the items of forensic interest.

Paint analysis

Forensic investigations usually involved:

a. identifications/classifications of the paint sample
b. - comparison of samples taken from the crime scene and the suspected object.

Forensic paint analysis uses comparison tasks; these usually require specialist techniques

Components of paint

Automotive paint

Typical new automotive paint consists of at least four layers:

a. The first layer is The **ELECTROCOAT PRIMER**. It is electroplated onto the steel body to provide corrosion resistance. Usually black or Wight.
b. The **PRIMER SURFACER** is applied over the first layer. It is help to smooth out and hide seams.
c. The **BASECOAT** is the layer that provides the color and appearance of the finish.
d. The **CLEARCOAT** has no color and is used to provide gloss and add durability.

Each layer of the coating is a mixture of different components:

See the next slide

Paint analysis

Components of paint’s layers

1- Binders

Provides support medium for pigments and additives; usually polymers.

2- Pigments / dyes

They are used to import color to paint, Pigment is any material that is colored, black or white, organic or inorganic that retains a crystalline form in paint. however dyes dissolve in the paint.

3- Solvents

Suspension of binders and pigments for application

Paint analysis procedure

1) Physical examination of paint

- Color assessment.
- Number and sequence of layers.
- Surface texture of the paint and thickness of layers

This can be examine using a optical microscopy .

2) chemical examination of paint

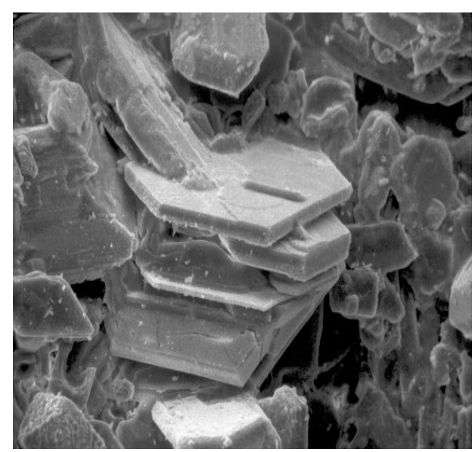
a) Solubility

different samples may show different solubility in various solvent.

b) Infrared spectroscopy (IR)

Is a very popular technique for the examination of paint, and justifiably . In this technique the absorption spectrum of paint is scanned and this will help in the differentiation of paints originating from different polymer classes.

Paint analysis (chemical examination of paint)



c) Scanning electron microscopy-energy dispersive X-ray spectrometry (SEM-EDX)

this technique is useful for imaging the specimen, individual pigment granules can be located and examined. It also important for the elemental analysis of paint specimens of “forensic” size.

d) Pyrolysis gas chromatography

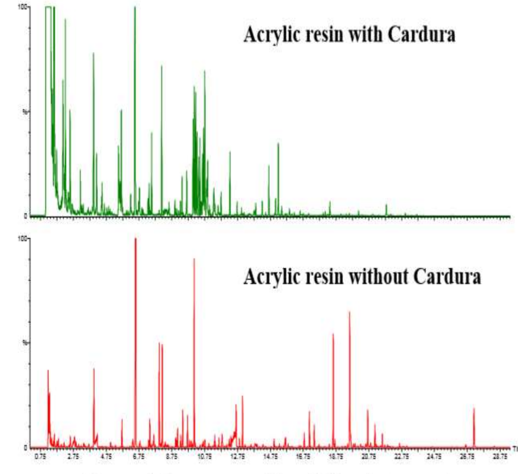


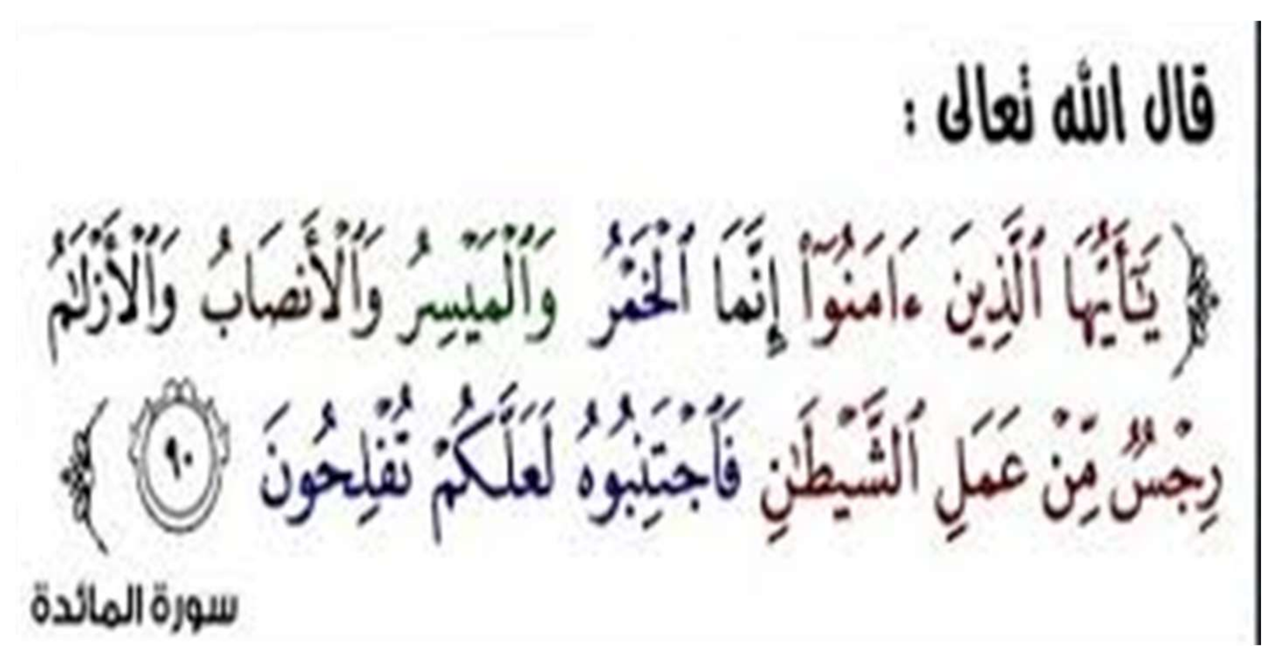
Figure 4. Pyrograms of two different clearcoats

small samples of organic complex molecules are decomposed by heat to gaseous products, later separated on a chromatographic column and detected in a mass spectrometer.

***pyrolysis** is a perfect method for breaking apart large molecules into smaller through a short application of heat*

Reference : FORENSIC EXAMINATION OF CAR PAINTS (Jakub M. Milczarek1, Grzegorz Zadora2, Janina Zięba-Palus2, Paweł Kościelniak)

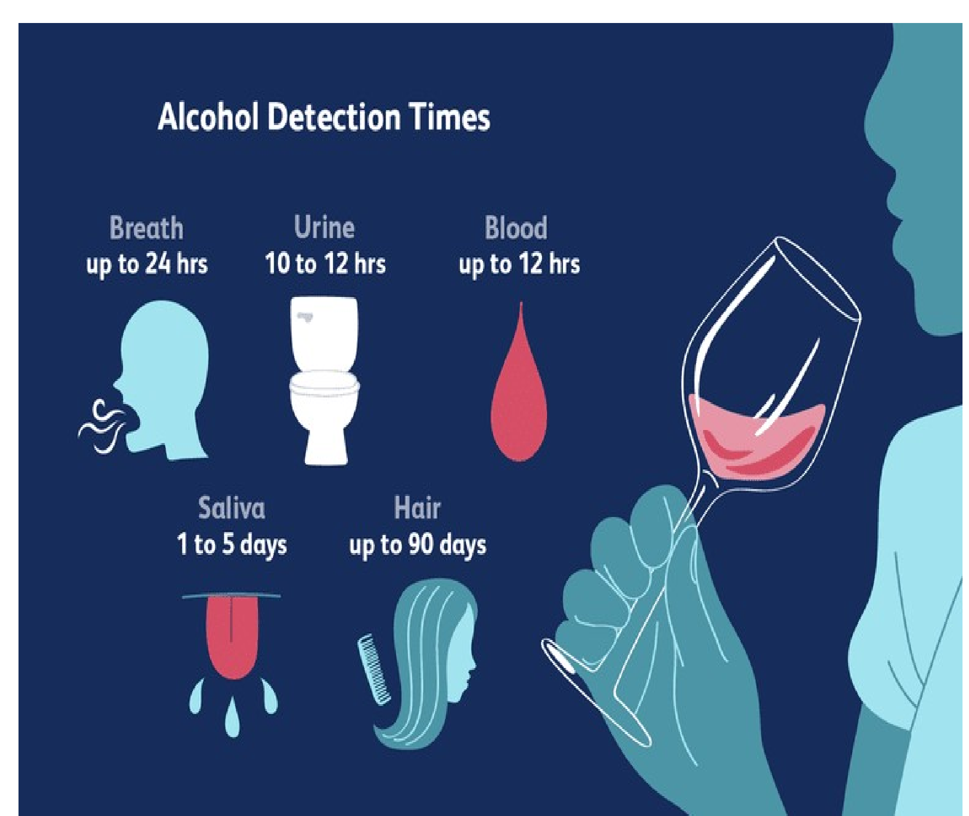
Blood alcohol analysis



Blood alcohol analysis

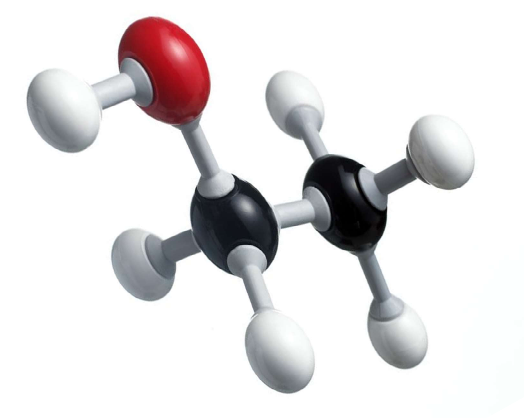
- Ethanol is a central nervous system (CNS) depressant and causes most of its effects on the body by depressing brain function.

- CNS depression correlates directly to the concentration of alcohol in the blood.



Blood alcohol analysis

The estimation of blood alcohol concentration must be based on important parameters such as body weight, ETOH concentration of the beverage consumed and length and pattern of the drinking



Blood alcohol analysis

BLOOD ALCOHOL MEASUREMENTS method

a)Enzymatic Methods -

- enzymatic oxidation can be an acceptable forensic method of determining BAC.
- they rely on the enzyme-specific oxidation of ethanol to acetaldehyde with alcohol dehydrogenase.
- This oxidation requires reduction of the cofactor oxidized nicotinamide–adenine dinucleotide (NAD+) to NADH, which is accompanied by a change in absorbance that can be monitored spectrophotometrically.

Blood alcohol analysis

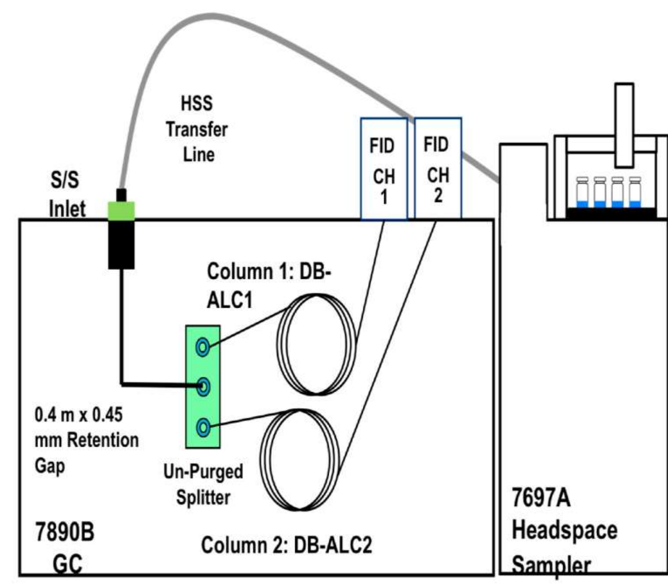
b) Headspace Gas Chromatography

- It is the most popular, precise, and accurate method of determining blood alcohol.
- packed column and capillary column procedures can be used for forensic blood alcohol analysis.
- Direct injection of biological samples into GC columns has been used in the past. However, this leads to column contamination.
- incorporation of headspace sampling into the method prevents the contamination.

Blood alcohol analysis

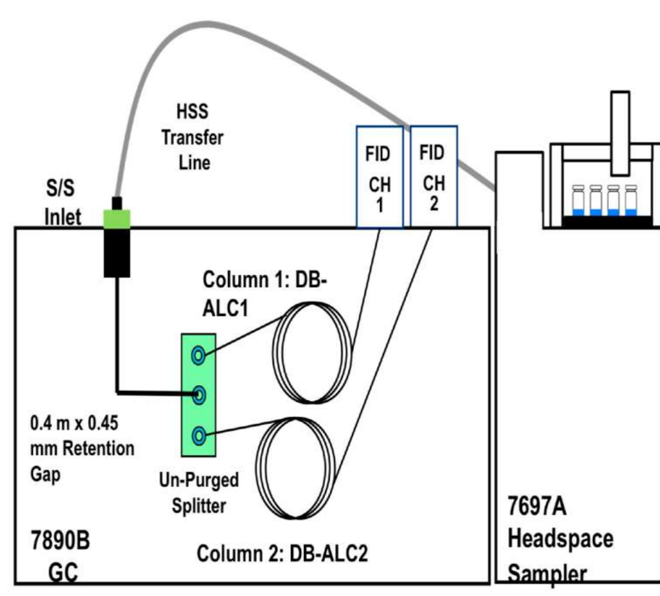
c) Headspace Gas Chromatography method

- mixing the blood with 2 mL of a 3 M NaCl solution that contains 1.0 g/L of 1-propanol as the internal standard.
- The mixed sample is dispensed into a 10-mL septum-topped vial and sealed.
- The vials are loaded into an automated headspace autosampler attached to a GC equipped with flame ionization detection (FID) in duplicate.



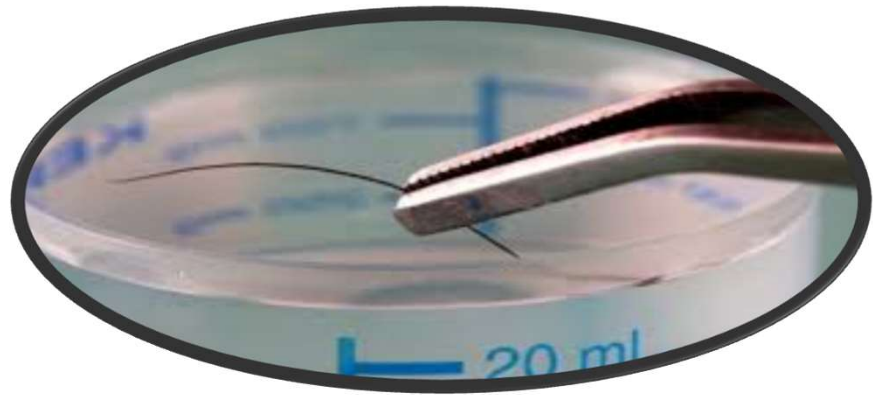
Blood alcohol analysis

- use of complementary GC columns for duplicate testing is highly desirable, since the retention times obtained as a result of the different selectivity of the two columns provide an added dimension in the identification of a compound.



Lecture title: **Applications of analytical chemistry in the hair analysis - fingerprinting**

The Forensic Analysis of Hair



History

- 1891 - Han Gross published the first description of the uses of physical evidence to help solve crimes

- 1897 - Rudolph Virchow became the first person to do an in depth study of hair.

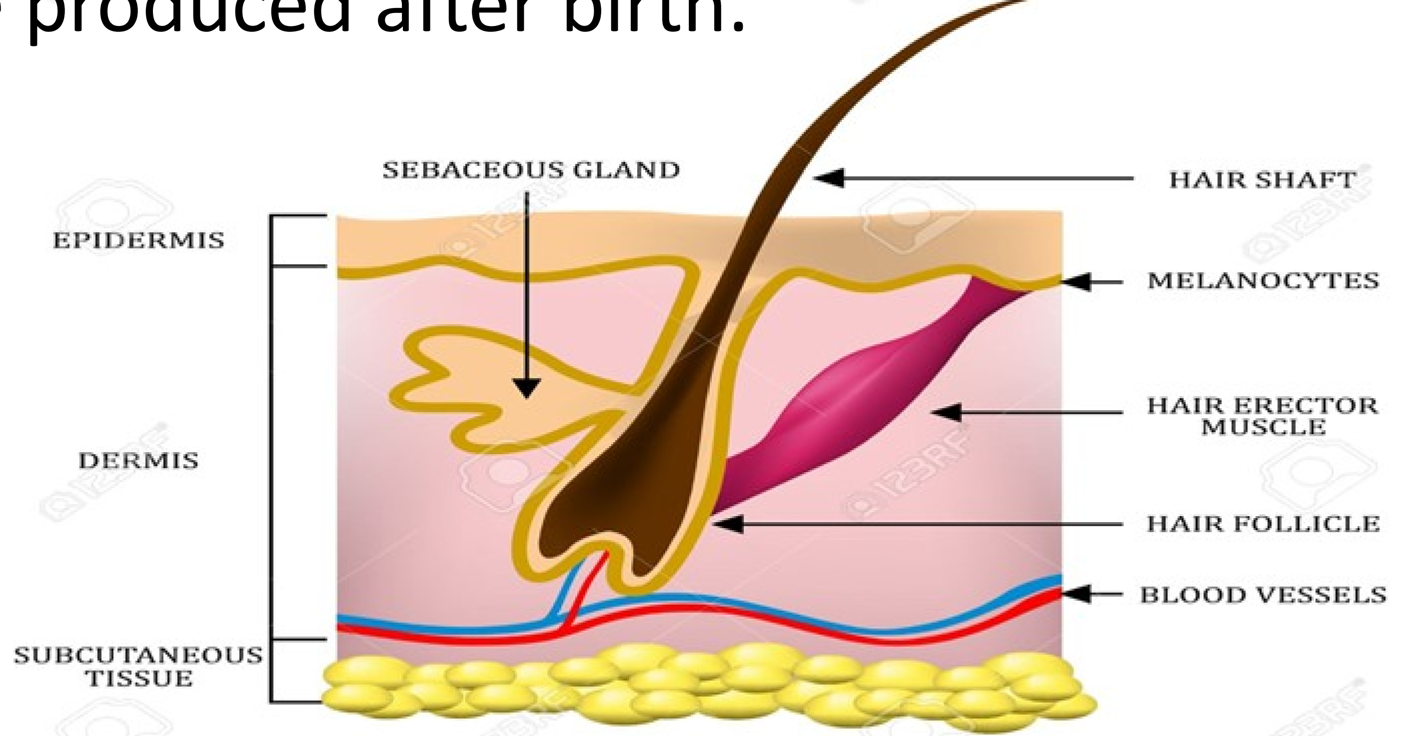
- 1906 - Hugo Marx wrote a paper on the use of hair in forensic investigations to determine identity.

Hair Morphology

- The most basic components of hair are keratin, a very strong protein that is resistant to decomposition, and melanin, a pigment.
- The keratins form groups that interact and interconnect to form very stable fibrils. It is this property of hair that makes it such a prime example of physical evidence.
- The portion existing above the epidermis is called the shaft

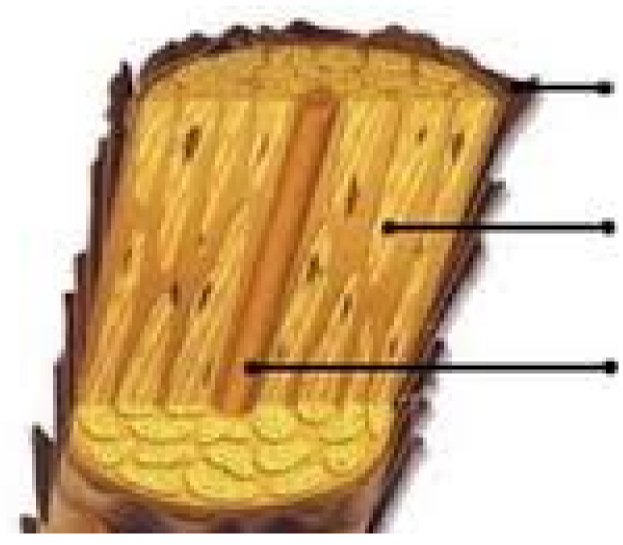
Hair Morphology

- Hair is produced from a structure called the hair **follicle**. Humans develop hair follicles during **fetal** development, and no new follicles are produced after birth.



The Function and Structure of Hair

- Hair on mammals helps to regulate body temperature, decrease friction, and protect against sunlight.
- A hair has three layers :the inner medulla, the cortex, and the outer cuticle.



Hair structure

The hair shaft is composed of three layers:

- Cuticle

  It is the outermost layer made of over-lapping scales that protect the inner layers of the hair.

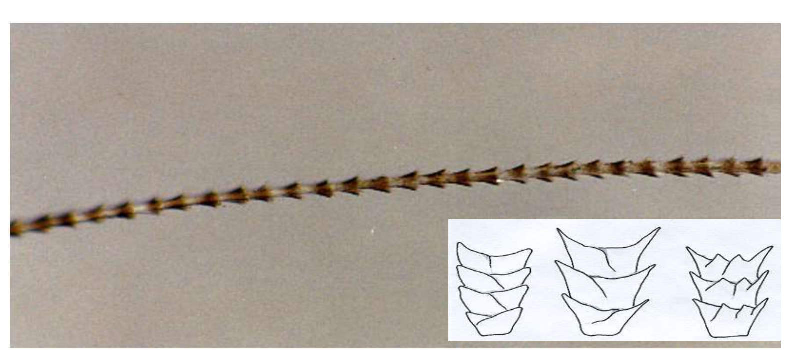
- Cortex

  It is the thickest layer containing most of the pigment giving hair its color.

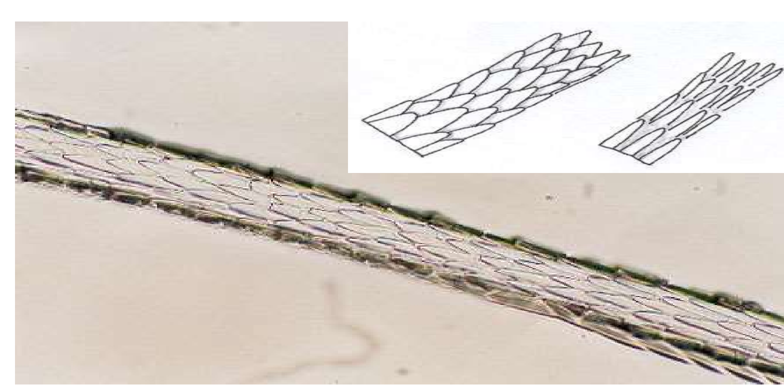
- Central medulla

  The medulla is a central core of cells that runs through the center of the cortex.

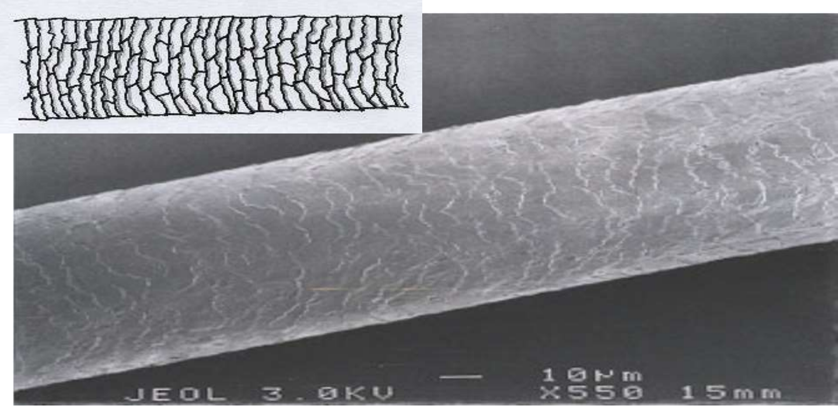
Types of cuticle



Coronal



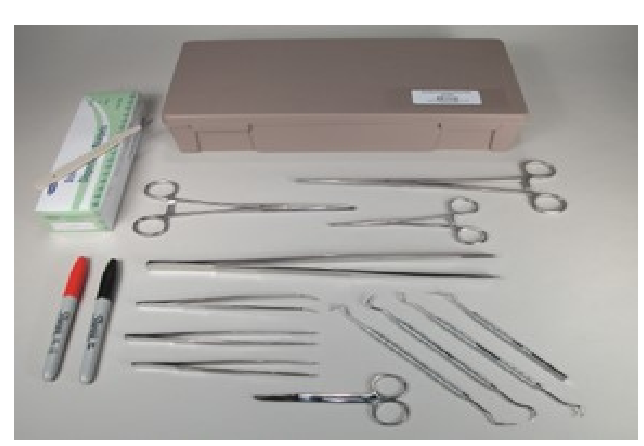
Spinous



Imbricate

Collection of Hair Evidence

- A special filtered vacuum cleaner may be used to collect hairs and fibers from carpet, bedding, etc.
- Once collected, the hair evidence should be packaged into paper packets.

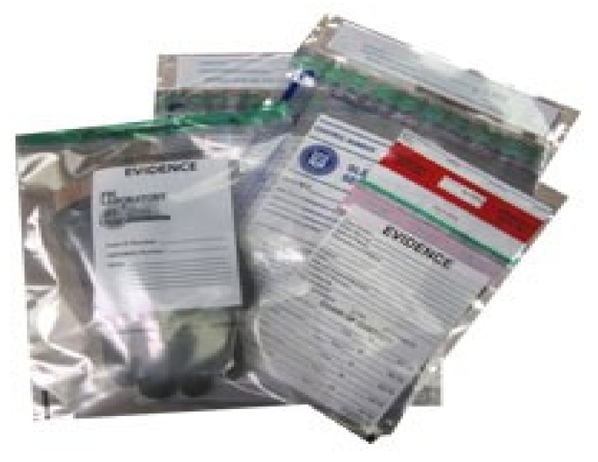


Evidence Collecting Kit

Evidence Collecting Vacuum

Collection of Hair Evidence

- Collection should be done by hand if the location of the hair is important, which is usually the case. Sticky tape and lint rollers may be used to assist.
- If sticky tape or a lint roller are used, the entire surface used should be packed into a polyethylene storage bag .
- Control samples need to be collected from the victim, suspect, and other individuals who could have left evidence at the scene.



Identification and Comparison

The examiner must have an adequate number of known hair samples that are representative of all its features

Hair from any part of the body exhibits a wide range of characteristics

Microscopic examination

- Animal or human
- Species of animal

Identification and Comparison

Compare

Length

Color

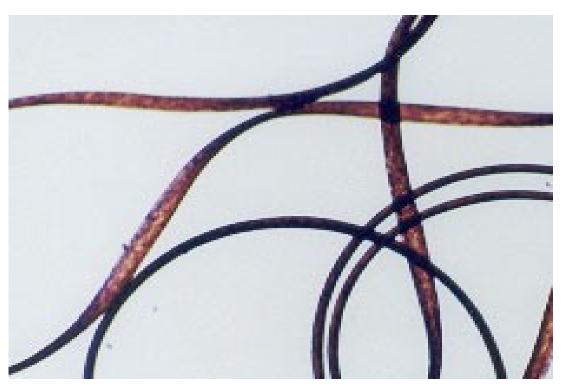
Diameter

Presence or absence of medulla

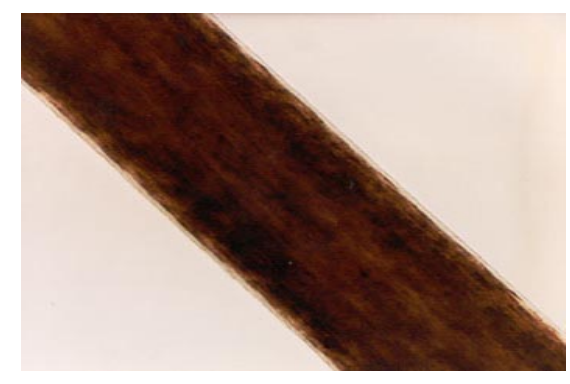
Distribution, shape and color intensity of the pigment granules present in the cortex

Dyed, bleached or natural hair

Types of hair



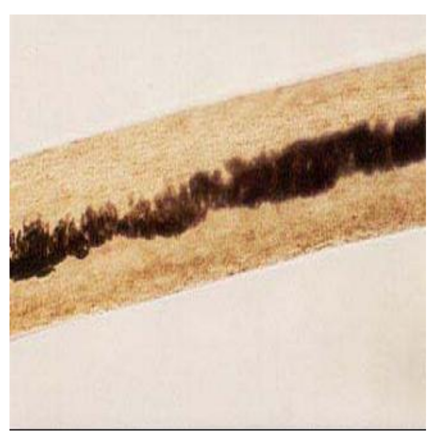
Photomicrograph of a Negroid Head Hair



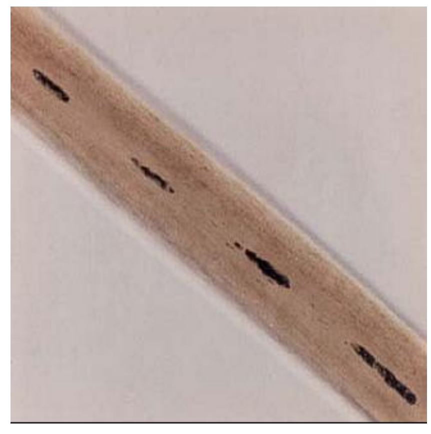
Photomicrograph of a Mongoloid Head Hair

Photomicrograph of a Caucasian Hair

Types of hair

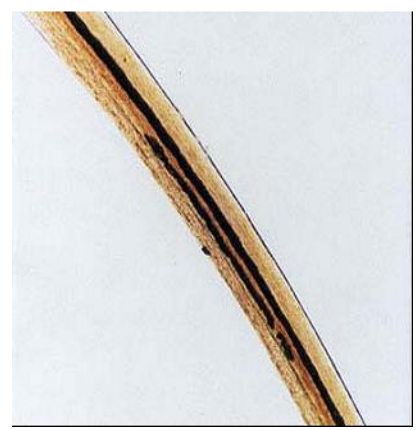


Pubic Hair



Head Hair

Limb Hair



Beard Hair

Types of hair

Scissor Cut

Razor Cut

Broken Hair

Burned Hair

What can be determined

- Using a microscope (SEM), forensic scientists can typically determine the species, race, and somatic origin of a hair. They may use comparative microscopy to do one of the following:
  - Link the suspect to a crime scene, meaning that a control hair matches the evidential hair.
  - Exclude the suspect from a crime scene, meaning that a control hair does not match the evidential hair.
- In addition to comparing hairs in with a microscope, the scientists may test for DNA on the follicular tag, and run a number of tests for drugs and environmental toxins.

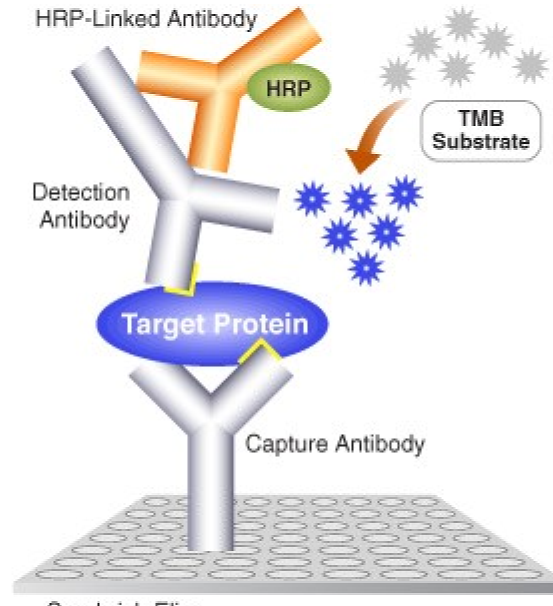
Scanning Electron Microscope

What can be determined

- Hair analysis is used in forensic toxicology to test and determine whether a drug was used.
- When a drug is ingested, it enters the blood stream and is broken down to a specific metabolite.
- Hair strands normally grow at an average rate of 1.3 centimeters every month; they absorb metabolized drugs that are fed to the hair follicle through the blood stream.
- The drug will only disappear if exposure to the drug is ceased, and the hair containing the drug is cut.
- Hair analysis can be used for the detection of many therapeutic drugs and recreational drugs, including cocaine, heroin, benzodiazepines (Valium-type drugs) and amphetamines.

What can be determined

- The radioimmunoassay and enzyme-linked immunosorbent assay are two common assays that are used by forensic toxicologists to detect substances such as drugs in the hair.
- Recall that the immunoassays function on the basis of an antigen-antibody interaction. The analyte, or drug, is added and binds to the solid phase, typically producing a color change, fluorescence, etc. that can be measured to determine the amount of drug present.
- Forensic toxicologists also look for toxic metals in the hair to explain poor mental and physical health.



Testing the Hair Follicle

Microscopic assessment of the follicle is performed first because it is cost effective and quick.

- If a microscopic match is found, the follicle can be **blood tested** and perhaps show the blood type.
- If a microscopic match is found, the follicle can be **DNA analyzed** to provide identification with a high degree of confidence.

Lecture title: **Applications of analytical chemistry in the analysis of forged banknotes and documents**

Forgery of banknotes and documents

Document Analysis

Document Analysis is the examination and comparison of questioned documents with known material.

Common Questioned Documents

Checks ,Certificates ,Wills, Passports, Licenses, Money, Letters and Contracts

Handwriting Analysis

- Like Fingerprints, every person's handwriting is unique and personalized
- Handwriting is difficult to disguise or forge
- Questioned documents are compared to exemplars(prewritten handwriting samples from a suspect) to determine matches

Handwriting Analysis

forensic science
forensic science

The Right of the People
The Right of the People
The Right of the People

Technology Used in Handwriting Analysis

Biometric Signature Pads

a new research tool, has been designed for identity authentication. This computer pad recognizes your signature based on speed, pressure and rhythm of signing your name.

Computerized Analysis

this can be faster and more objective. Pressure is subjective, but when placed in a computer, the pen pressure can be objectively analyzed by the shading in the pixels. The Forensic Information System for Handwriting (FISH) database is maintained/used by the Secret Service.

Documents are scanned in and it can be determined that no two writers pen their words the same nor do they have the same combination of handwriting characteristics.

Forgery

Forgery- making, altering, or falsifying a person’s signature or any other aspect of a document with the intent to deceive another

Preventing Check Forgery

- Chemically sensitive paper
- Large font size requires more ink and makes alterations more difficult
- High resolution borders that are difficult to copy
- Multiple color patterns on paper
- Embed fibers that glow under different light
- Use chemical wash detection systems that change color when a check is altered

Currency (banknote)

- Security features are added to paper currency that scanning cannot reproduce
- Regular printer paper contains starch.
- Paper currency contains rag fiber instead of starch.
- People usually first suspect money as fake because its texture does not feel right

Hidden numbers
and letters

Written above
Hamilton's name

CHARACTERISTICS OF HANDWRITING

Letter form

includes the shape of letters, curve of letters, the angle or slant of letter, proportional size of letters, and the use and appearance of connecting lines between letters. (Are "i"s dotted and "t"s crossed?)

Line form

includes the smoothness of letters and the darkness of the lines on the upward compared to the downward stroke. Line form is influenced by speed and pressure and occasionally the choice of writing utensil.

Formatting

includes the spacing between letters, the spacing between words and lines, the placement of words on a line, and the margins.

PREVENTING CHECK FORGERY

These are some methods used to prevent check forgery:

- Print checks on chemically s
- Use a large font size that req
  and makes alterations more c
- Use high resolution borders c
  that are difficult to copy.
- Print checks in multiple colo
- Embed fibers in checks that c

DETECTING COUNTERFEIT CURRENCY

- Counterfeit detecting pens are inexpensive special pens/markers containing the element iodine. When they come in contact with a counterfeit bill, the paper marked with the pen will change color to a bluish-black. This is because of starch, found in regular printer paper. Real money does not contain starch but has a different fiber. When the pen is marked on real money, it'll leave a pale yellow-brown line that fades quickly.

DETECTING COUNTERFEIT CURRENCY

- Pen manufacturers claim the pen to have a 98% accuracy, but some counterfeiters will bleach $1 bills to have the correct paper to print off larger bills. There is currently a global movement to change to polymer money, a type of plastic money because it’s much more difficult to counterfeit and less expensive to make.

METHODS OF HANDWRITING FORGERY

- **Simulated forgery**—one made by copying an exemplar
- **Traced forgery**—one made by tracing an exemplar
- **Blind forgery**—made without an exemplar

Handwriting Characteristics

Despite minor variations due to type of writing **instrument**, **mood**, age or stress, everyone's handwriting has their own unique **style**.

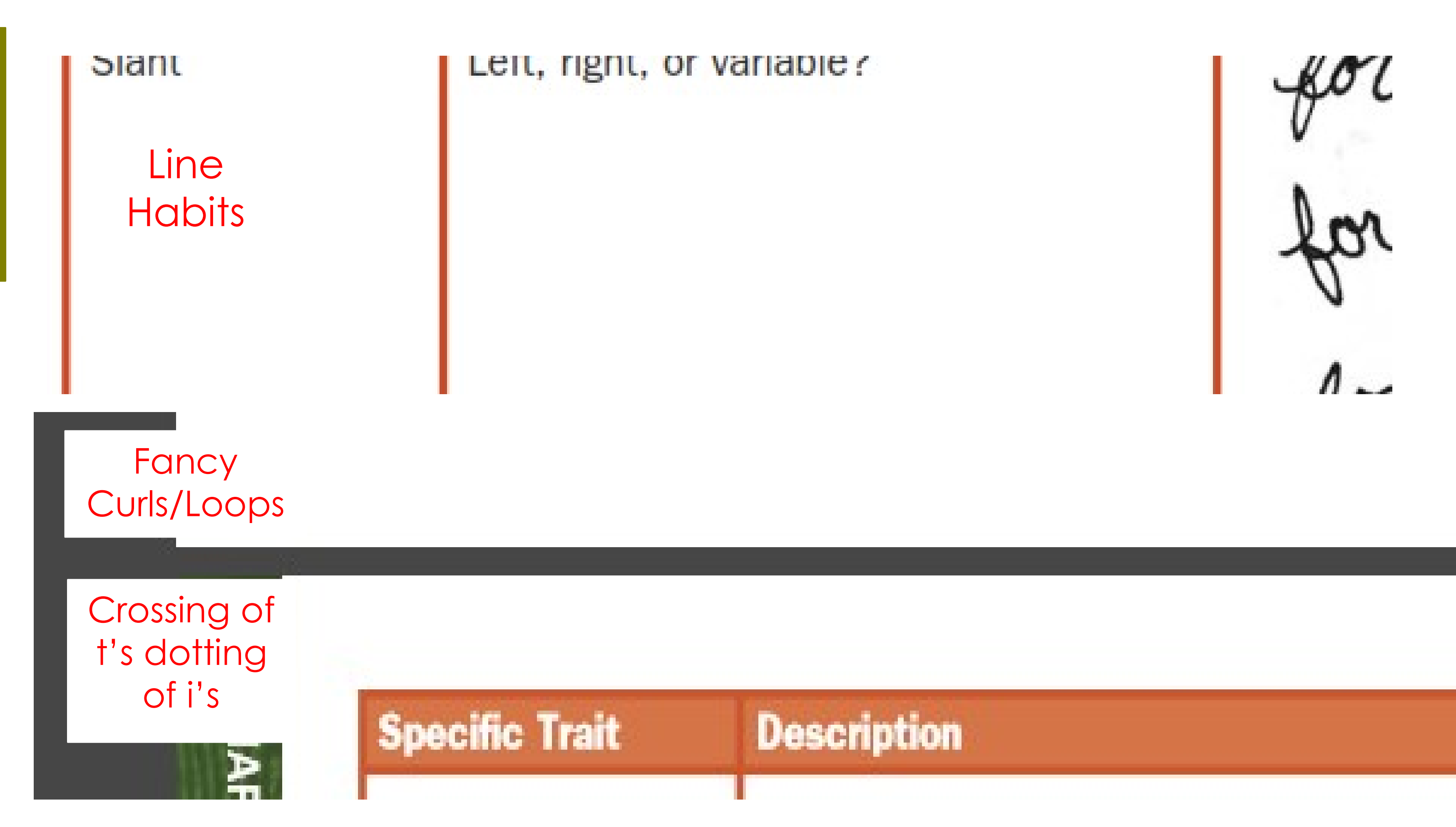
Experts examine 12 major characteristics of handwriting:

Specific Trait	Description	Example
Line Quality	Do the letters flow or are they erratic or shaky?	

Spacing	Are letters equally spaced or crowded?	
Size Consistency		

Specific Trait	Description	Example
Continuous Connecting Letters	Is the writing continuous or does the writer lift the pen?	
Connecting letters Letters Complete	Are capitals and lower-case letters connected and continuous?	

Cursive or Printed Letters	Are there printed letters, cursive letters, or both?
Pen Pressure	Is pressure equal when applied to upward and downward strokes?
Slant	Left, right, or variable?


Slant
Left, right, or variable?
Line Habits
Fancy Curls/Loops
Crossing of t's dotting of i's
Specific Trait
Description

Types of Forgery

Freehand simulation- attempt to copy a signature or handwriting sample

Tracing-placing a new document over the original and tracing it

Disguised writing- Attempting to **alter** writing so it cannot be traced back (Example: Ransom note or threat)

Alteration- Removing, adding, or changing a portion of the original.

Four ways to alter:

1. **Erasures-** Using an **eraser**, knife, **sandpaper**, or sharp tool to erase part of a document
2. **Obliterations-** Burning, **bleaching**, or using a **laser** to get rid of a document
3. **Alterations-** Adding or changing words, dates, and numbers
4. **Overwriting-** Overwrites a portion of the document, for example changing a **4** to a **9**

Counterfeiting

- Counterfeiting is the production of an **imitation** of **currency**, **artwork**, documents, and **name-brand** knock-off items for the purpose of deception.
- Common counterfeited items include currency, traveler's checks, **food** stamps, bonds, **postage** stamps, and **birth** certificates.

Lecture title: **The use of analytical chemistry in the analysis of toxins (drug analysis) - Chemical - abusive drugs.**

This lecture will satisfy the following internal leaning outcomes of the course :

1- The terms toxins, Forensic Toxicology, Analytical Toxicology

,

2- drug analysis

.

Analytical Toxicology

Analytical toxicology identifies the toxicant through analysis of body fluids, stomach content, excrement, or skin.

Forensic Toxicology

Establish cause and effect relationships between exposure to a drug or chemical and the toxic or lethal effects that result from that exposure.

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What Is A Dose In Toxicology?

The actual amount of a chemical that enters the body

• Acute (short) exposure: a very short period of time,usually 24 hours

• Chronic (long-term) exposure: long periods of time such as weeks,

months, or years

Toxicant, Toxin, Or Poison?

Toxicants	substances that produce adverse biological effects of any nature may be chemical or physical in nature effects may be of various types *(acute, chronic, etc.)*
Toxins	specific proteins produced by living organisms *(mushroom toxin or tetanus toxin)* most exhibit immediate effects
Poisons	toxicants that cause immediate death or illness when experienced in very small amounts

A **toxic agent** is anything that can produce an adverse biological effect. It may be chemical, physical, or biological in form.

For example, toxic agents may be chemical (such as cyanide), physical (such as radiation) and biological (such as snake venom).

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Principles of Forensic Drug Chemistry

- Forensic drug chemistry is simply chemistry as it is applied to the identification of illegal substances within the criminal justice system. Like all other chemistry disciplines, it examines the way the atoms and molecules in matter interact and bond with each other.
- All matter has a chemical signature, or set of characteristics that are unique to only that substance.
- Chemists use these characteristics to identify substances using scientific methods that can be replicated by other chemists and thus are presentable as fact in court.

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Principles of Forensic Drug Chemistry

- Forensic chemistry covers illegal drugs, explosives and poisons
- Forensic drug chemists analyze samples of unknown materials including powders, liquids and stains to determine the chemical identity or characteristics of the compounds that make up the sample.
- Samples submitted as evidence in a drug-related case can contain one compound or a mixture of many compounds

Drug Scheduling and Classification

Schedule I - no medical usage, high potential for abuse. Examples include Heroin, LSD, peyote, MDMA

Schedule II - severely restricted medical usage, high potential for abuse, but slightly less than Schedule I drugs. Examples include cocaine, methamphetamine, methadone, oxycodone

Schedule III - currently accepted medical usage, moderate potential for abuse, and moderate to low risk of dependence. Examples include barbiturates, steroids, ketamine

Drug Scheduling and Classification

Schedule IV - widely used for medical purposes, low potential for abuse and low risk of dependency. use such as darvon, phenobarbital, and some tranquilizers such as diazepam (valium) and chlordiazepoxide (librium).

Schedule V - widely used for medical purposes, very low potential for abuse, contain limited quantities of narcotics. Examples such as opiate drug mixtures that contain nonnarcotic medicinal ingredients.

Presumptive & Confirmatory Testing for Drugs

There are two main types of tests used to determine whether an illegal drug is present in a substance: presumptive tests and confirmatory tests.

Presumptive tests are less precise and indicate that an illegal substance may be present.

Confirmatory tests provide a positive identification of the substance in question

Presumptive testing

- may be conducted in the field by law enforcement officers or in the laboratory once the seized material is accepted.
  - is usually colorimetric, meaning the test will indicate that the suspected substance is present or not present by changing color. If the substance is present, the test kit will turn one color, if not, it turns a different color.
  - Presumptive testing by law enforcement is typically followed up with laboratory tests that confirm with certainty the presence of the suspected substance.
  - Is also performed in the laboratory as part of the analysis process

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Confirmatory tests

- involve a battery of instrumental tests using techniques such as Gas Chromatograph-Mass Spectrometry (GC-MS) or infrared spectroscopy that separate individual compounds in the substance and positively identify the chemical signature of the illegal substance(s) within the material.

Presumptive & Confirmatory Testing for Drugs

- Most confirmatory analyses employed for drug identification are moderately time-consuming and require the use of expensive instrumentation such as a gas chromatograph-mass spectrometer or a Fourier transform infrared spectrophotometer. To save time and money, before conducting a confirmatory analysis (potentially resulting in inconclusive information), quick and inexpensive presumptive drug analyses are performed.
- These analyses direct the forensic scientist toward an appropriate confirmatory analysis that will yield the desired results the first time.

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Presumptive testing or screening

determines the general characteristics of the sample material and allows analysts to narrow down the field of confirmatory tests that will be used.

Presumptive laboratory tests may include:

- **Color tests**, sometimes called spot tests, are examples of presumptive drug tests used to probe questioned drug samples for their chemical properties. If chemical properties consistent with a known drug are discovered, a questioned drug sample can be *presumptively* identified.

Presumptive testing or screening

- When conducting a color test, chemicals known to produce a colored product in the presence of a suspected drug are added to a small amount of the questioned sample. If the questioned sample contains the suspected drug, a colored product, having a color representative of the suspected drug, will be produced.
  - The measured chemical property for drug identification is both its reactivity with the chemicals and its ability to produce the color-indicative product

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Presumptive testing or screening

- **Microcrystalline tests**, a special class of presumptive drug analyses, produce solid products. Typically the solid forms slowly, producing representative crystalline structures that may be viewed under the microscope with transmitted illumination

Presumptive testing or screening

- **Ultraviolet spectroscopy** works by exposing the material to UV light and measuring the way the material absorbs the light. Different chemicals absorb light differently and can give clues to analysts about what might be present in the material.

- **Gas chromatography** may provide presumptive identification of materials, but is commonly used to separate components prior to confirmatory testing.

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Confirmatory testing

includes separation and identification of the individual components of the material. Confirmatory testing is usually a two-step process by which the analyst first separates the compounds using a suitable method such as gas chromatography (GC), capillary electrophoresis, or wet chemistry. Once the components are separated, instruments such as a mass spectrometer (MS) or infrared spectrometer (IR) are used to identify each component by comparing its chemical signature against reference materials.

Confirmatory testing

- These processes may be combined depending on laboratory policies. The most common is gas chromatography/mass spectroscopy (GC/MS); however gas chromatography can also be combined with infrared spectroscopy (GC/IR) among others.
- The most common techniques used to separate the compounds in a sample include:

Confirmatory testing

- **Gas chromatography** - an instrument that separates substances into individual components by dissolving the material in a liquid solvent, injecting the liquid into a superheated oven, vaporizing the liquid and pushing it through a very small, very long, glass capillary tube using a carrier gas such as helium or hydrogen. The mixture separates into individual chemical components inside the tube. As each component travels through the tube at a slightly different speed, the analyst can measure the time it takes each component to emerge and compare that to reference materials.

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Confirmatory testing

- **Liquid chromatography** - is similar to gas chromatography, however the evaporation phase using the superheated oven is removed. The material is dissolved into a liquid and injected into a wider, shorter stainless steel capillary tube at a high pressure. The components will then separate inside the tube and emerge at different times. This method is used when the material may be sensitive to the high temperatures required in gas chromatography.

Confirmatory testing

- **Capillary electrophoresis** - uses an electrical field to separate the components inside a capillary tube. Molecules move toward the positive or negative charges placed at either end of the tube and analysis can measure the speed and direction at which they move to compare against reference materials.

Instruments most often used to identify compounds include:

Mass spectrometry - once the components of a material are separated, mass spectrometry uses a beam of electrons that causes them to break apart. Chemicals always break apart in the same way due to their chemical structure and this can be mapped into a spectrum and compared against a database of known spectra.

Instruments most often used to identify compounds include:

Infrared spectroscopy - uses infrared (IR) light to decipher the chemical signature of materials, specifically the bonds between atoms. Different components in a sample will absorb IR radiation differently and analysts can use this information to compare against reference materials.