Organic vs. Inorganic

- The proper selection of analytical techniques that will allow the forensic scientist to identify or compare matter can best be understood by categorizing all substances into one of two broad groups: organics and inorganics.
- In general, organic substances contain the element carbon, commonly in combination with one or more other elements.
- Inorganic materials encompass all other known chemical substances.

Qualitative vs. Quantitative

- Another consideration in selecting an analytical technique is the need for either a qualitative or a quantitative determination.
- The former relates just to the identity of the material, whereas the latter requires the determination of the percent composition of the components of a mixture.
- Most of the evidence received by crime laboratories requires the identification of organic compounds.

Chromatography vs. Spectrometry

- Chromatography, spectrophotometry, and mass spectrometry are all readily used by a forensic scientist to identify or compare organic materials.
- Chromatography is a means of separating and tentatively identifying the components of a mixture.
- Spectrophotometry is the study of the absorption of light by chemical substances.

What Is Chromatography?

- A family of laboratory techniques for separating mixtures into their component compounds
- Uses some version of a technique in which two phases, one mobile, one stationary, flow past one another
- The mixture separates as it interacts with the two phases

Basic Principles

- Different compounds will stick to a solid surface with different degrees of strength or vary in the efficiency with which they dissolve in a liquid
- When a mixture of compounds flows over a surface, the molecules will stick to the surface
- If a molecule does not stick to the surface too strongly, the molecules stick & unstick many times as it is swept along the surface
- Over time, the molecules will become physically separated from each other
- When the molecules reach the far end of the surface, they are detected or measured one at a time as they emerge
**Basic Principles**

- A mobile phase sweeps the sample over a stationary phase
  - like the wind sweeps the swarm over the flower bed

**Chromatography-Types**

**Planar**
- paper chromatography
- thin layer chromatography (TLC)
- Gel electrophoresis

**Column chromatography**
- Adsorption (SEC, IE Affinity)
- gas-liquid chromatography (GC)
- high-pressure liquid chromatography (HPLC - high-performance liquid chromatography)
- Capillary electrophoresis (CE)

**Types of Chromatographic Attraction**

- Adsorption Chromatography
  - depends on physical forces such as dipole attraction to cause the molecules to "stick" to the stationary phase
  - column, TLC, HPLC

- Partition Chromatography
  - depends on the relative solubility of the mixture's molecules in the stationary phase coating
  - polarity may also have some effect
  - gas chromatography
Types of Chromatographic Attraction

- Ion-exchange
  - depends on the relative strength with which ions interact with an ionic resin
  - less strongly held ions are displaced by more strongly attaching ions
  - one kind of ion is exchanged for another
    • ion exchange chromatography

- Size-exclusion
  - the relative sizes of the molecules determine how fast the molecules move through the stationary phase
  - large molecules flow right through
  - small molecules spend time trapped in the pores of the stationary phase
    • gel filtration chromatography

Chromatography-Types

- Mobile anions held near cations that are covalently attached to stationary phase
- Large molecules are excluded
- Small molecules penetrate pores of particles
- Anion-exchange resin: only anions can be attracted to it

Paper Chromatography

- Stationary phase
  - a sheet or strip of paper
- Mobile phase
  - a liquid solvent
- Sample mixture spotted onto the paper
- Capillary action moves mobile phase through stationary phase

- Components appear as separate spots spread out on the paper after drying
- Can be used for ink analysis

- 2D Chromatography
  - accomplished by running another chromatography with the paper turned 90°
  - Can complete separation of overlapping compounds
TLC

- TLC uses a solid stationary phase usually coated onto a glass plate and a mobile liquid phase to separate the components of the mixture.
- The liquid will slowly rise up the plate by capillary action causing the sample to become distributed between the stationary phase and the moving liquid phase.
- Because most compounds are colorless, the materials must be visualized by placing the plates under ultraviolet light or spraying the plate with a chemical reagent.
- The distance a spot travels up a thin-layer plate can be assigned a numerical value known as the $R_f$ value.

Thin Layer Chromatography

- **Stationary Phase**
  - a thin layer of adsorbent coating on a sheet of plastic or glass
  - usually $\text{Al}_2\text{O}_3$ (alumina) or $\text{SiO}_2$ (silica)
- **Mobile Phase**
  - a liquid solvent
- Sample mixture spotted onto the adsorbent

Thin-Layer Chromatography (TLC)

- Some components bind to the adsorbent strongly; some weakly
- Components appear as separate spots after development

Retention Factor ($R_f$)

- Quantitative indication of how far a compound travels in a particular solvent
- Good indicator of whether an unknown & a known compound are similar, if not identical
  - If the $R_f$ value for the unknown compound is close to or the same as that for the known compound, the two compounds are most likely similar or identical

Retention Factor ($R_f$)

- $R_f = \frac{D_1}{D_2}$
- $R_f < 1.0$
Column Chromatography -
General Process

Column Chromatography -
Output Chromatogram

General Parts of Column
- Column
- copper tubing
- stainless steel tubing
- glass tubing
- Support
  - finely divided solids (packed)
    - ground firebrick
    - alumina, specially treated
- walls of column for capillary columns

Parts of Column
Stationary Phase
- stationary phase evenly dispersed on surface of support
  - column chromatography
    • non-volatile, viscous liquids dispersed evenly on surface of support

Parts of Column
Mobile Phase
- sample mixture carried through stationary phase by mobile phase
- non-reactive gas in glc (gas-liquid chromatography, gc)
- non-reactive liquid in llc (liquid-liquid chromatography, lc)
Introduction Gas Chromatography

- GCs are the most widely used analytical instruments in the world
- Premiere technique for separation and analysis of volatile compounds
  - Organic and inorganic
  - Weights up to 1,000 g/mol
- Often coupled to MS for both qualitative and quantitative analysis

Gas Chromatography

- Stationary phase
  - a solid or very syrupy liquid lines a tube (column)
    - silicone polymers (like Silly Putty) commonly used
- Mobile phase
  - an inert gas
    - nitrogen
    - helium

Advantages of GC

- Fast – minutes
- High Resolution. Record of N~1 x 10^6
- Sensitive detectors (ppm and ppb range)
- Highly accurate quantification (RSD of 1.5%)
- Automated
- Non-destructive
  - Coupled to other techniques, especially MS
- Reliable
- Low cost ($25 K)
- Small sample sizes (microliters)

GC Instrumentation

- Carrier gas: Nitrogen, Helium
- Inlet Pressure: 10-50 psi
- Flow: 1-25 mL/min, 2-50 mL/min, packed 1-25 mL/min, open 2-50 mL/min
- Operating range: 0-400°C, accurate to 1°C
- Many detectors: FID, TCD, ECD, MS

GC Columns

- A packed column
- A capillary column
**Retention Time**

- The time between when the sample is injected & when it exits the column reaching the detector
- Tm is the time taken for the mobile phase to pass through the column

**Analysis Using the GC**

- Retention time can be used as an identifying characteristic of a substance
  - retention times may not be unique
  - GC is not an absolute method of identification
- An extremely sensitive technique
  - area under a peak is proportional to the quantity of substance present
  - allows quantization of sample

**Applications-Solid Phase Microextraction (SPME)**

- Solid Phase Microextraction
  - Syringe barrel
  - Septum piercing needle
  - Fiber attachment tubing
  - Bonded fiber with stationary liquid phase bonding

**Identification of Accelerants**

- unevaporated gasoline
- 90% evaporated gasoline
- unevaporated kerosene
- 90% evaporated kerosene
Disadvantages of GC

- Limited to volatile samples
  - \(<380^\circ C\)
  - Need \(P_{\text{vap}} \sim 60\) Torr at 380\(^\circ\) C
- Not suitable for thermally labile samples
- Some samples and those less volatile require extensive sample prep (extraction, derivatization)
- Requires secondary technique (MS) to confirm peak identity

GC and Mass

- A direct connection between the GC column and the mass spectrometer allows each component to flow into the mass spectrometer as it emerges from the GC.
- The separation of a mixture's components is first accomplished by the GC.
- Then, fragmentation of each component by high-energy electrons in the mass spectrometer, will produce a distinct pattern, somewhat like a "fingerprint," of the substance being examined.

The Mass Spectrometer

- A detector
- Allows the identification of a chemical compound
- In the MS, a compound is bombarded with a stream of electrons
  - compound breaks into fragments
  - each compound gives a unique set of fragments
    - "fingerprint"

Mass Spectrometer (MS)
The sample components are retarded by fine solid particles.

**Liquid Chromatography**

Four types of high performance liquid chromatography (HPLC):
- partition
- adsorption (liquid-solid)
- ion exchange
- size exclusion or gel

**High Pressure Liquid Chromatography (HPLC)**
- Stationary Phase
  - fine solid particles
- Mobile Phase
  - a liquid solvent
- The solvent is pumped through the column
- The sample components are retarded by different amounts by interaction with the column packing
HPLC

- HPLC separates compounds using a stationary phase, a column filled with fine solid particles, and a mobile liquid phase.
- As the liquid carries the sample through the column, different components are retarded to different degrees, depending on their interaction with the stationary phase.
- The major advantage of HPLC is that the entire process takes place at room temperature.
- Substance, such as organic explosives, which are heat sensitive are more readily separated by HPLC.

Partition Chromatography:
- Most popular method
- Low molecular weight (mw<3600) analytes
- Polar or non-polar
- Bonded stationary phase column (liquid chemically bonded to support particles)
  
  \[
  \frac{R_1}{R_2} = \frac{1}{N \cdot \theta}
  \]

Normal phase HPLC: nonpolar solvent/polar column
Reversed phase HPLC: polar solvent/nonpolar column

Stationary Phase Structure

Schematic of HPLC

HPLC Separation Process

TYPICAL HPLC Instrument
Advantages of HPLC
- Separation occurs at room temperature
- Does not decompose heat sensitive materials
  - explosives
  - heat sensitive drugs like LSD

Analysis of Components of Sake
- Sake is composed of several chemical components
  - sugar
  - organic acids
  - amino acids
- Each component relates to the taste of the sake

Sugar & organic acids can be analyzed by HPLC

Advantages and Disadvantages of HPLC
- Advantages
  - Speed
  - High Resolution
  - Sensitivity (ng-fg)
  - Reproducibility (1%)
  - Automated
- Disadvantages
  - Complexity
  - Irreversibly bound species not detected
  - Need secondary detector, not applicable to all analytes
  - Co eluting species difficult to analyze
Size Exclusion Chromatography (Gel Permeation):

- Used for large mw compounds - proteins and polymers
- Separation mechanism is sieving not partitioning
- Stationary phase porous silica or polymer particles (polystyrene, polyacrylamide) (5-10 μm)
  - well-defined pore sizes (40-2500 Å)
  1. Large molecules excluded from pores - not retained, first eluted (exclusion limit - terms of mw)
  2. Intermediate molecules - retained, intermediate elution times
  3. Small molecules permeate into pores - strongly retained, last eluted (permeation limit - terms of mw)

Electrophoresis

- A technique analogous to TLC is electrophoresis.
  - Here, materials are forced to move across a gel-coated plate under the influence of an electrical potential.
- In this manner, substances such as proteins and DNA can be separated and characterized.

Spectroscopy: Basic Terms

- **Wavelength**: the distance between crests of adjacent waves
- **Frequency**: the number of waves that pass a given point per second
- **Electromagnetic spectrum**: the entire range of radiation energy from the most energetic cosmic rays to the least energetic radio waves
- **X-ray**: a high energy, short wavelength form of electromagnetic radiation
- **Laser**: light amplification by the simulated emission of radiation. Light that has all its waves pulsating in unison
- **Photon**: a small pocket of electromagnetic radiation energy. Each photon contains a unit of energy equal to the product of Planck’s constant and the frequency of radiation: \( E = hf \)
- **Monochromator**: a device for isolating individual wavelengths or frequencies of light
- **Monochromatic light**: light having a single wavelength or frequency

**Theory of Light**

- Two models describe the behavior of light.
  - Light is described as a continuous wave.
  - Light is depicted as a stream of discrete energy particles.
- When white light passes through a prism, it is dispersed into a continuous spectrum of colors.
- Visible light ranges in color from red to violet in the electromagnetic spectrum.
- Waves are described in terms such as:
  - Wavelength, the distance between two successive crests (or one trough to the next trough).
  - Frequency, the number of crests (or troughs) passing any one given point per unit of time.

**Wave Nature of Light**

- **Wavelength**: distance between crests
- **Frequency**: number of crest that pass a given point in one second

**Electromagnetic Spectrum**

- Ultraviolet: Invisible long frequencies of light beyond violet in the visible spectrum
- Infrared: invisible short frequencies of light before red in the visible spectrum
- **Ion**: an atom or molecule bearing a positive or negative charge

**Energy**: frequency & energy are proportional
Theory of Light

- Frequency and wavelength are inversely proportional to one another.
- The electromagnetic spectrum is the entire range of radiation energy from the most energetic cosmic rays to the least energetic radio waves.
  - Visible light is only a small part of the electromagnetic spectrum.
- As electromagnetic radiation moves through space, its behavior can be described as that of a continuous wave; however, once radiation is absorbed by a substance, it is best described as discrete particles of light known as photons.

The Electromagnetic Spectrum

- Just as a substance can absorb visible light to produce color, many of the invisible radiations of the electromagnetic spectrum are likewise absorbed.
- Spectrophotometry, an important analytical tool, measures the quantity of radiation that a particular material absorbs as a function of wavelength and frequency.
- The quantity of light absorbed at any frequency is directly proportional to the concentration of the absorbing species. This is known as Beer’s Law.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Frequency (Hz)</th>
<th>Energy (kcal/mol)</th>
<th>Energy (eV)</th>
<th>Color</th>
<th>Wavelength (nm)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>8 x 10^14</td>
<td>1.2 x 10^-15</td>
<td>2.4 x 10^-19</td>
<td>Violet</td>
<td>400</td>
<td>Visible</td>
</tr>
<tr>
<td>450</td>
<td>7 x 10^14</td>
<td>1.4 x 10^-15</td>
<td>2.8 x 10^-19</td>
<td>Blue</td>
<td>450</td>
<td>Visible</td>
</tr>
<tr>
<td>500</td>
<td>6 x 10^14</td>
<td>1.6 x 10^-15</td>
<td>3.2 x 10^-19</td>
<td>Green</td>
<td>500</td>
<td>Visible</td>
</tr>
<tr>
<td>550</td>
<td>5 x 10^14</td>
<td>1.8 x 10^-15</td>
<td>3.6 x 10^-19</td>
<td>Yellow</td>
<td>550</td>
<td>Visible</td>
</tr>
<tr>
<td>600</td>
<td>4 x 10^14</td>
<td>2.0 x 10^-15</td>
<td>4.0 x 10^-19</td>
<td>Orange</td>
<td>600</td>
<td>Visible</td>
</tr>
</tbody>
</table>

The Hydrogen Atom

- The electron structure of an atom is quantized – electrons can only exist in discrete energy levels

Excitation/Absorption

- When a "packet" of energy equal to the energy difference between two energy levels is absorbed – electron is promoted
Excitation/Absorption

**Continuous Spectrum**

**Absorption Spectrum**

Excitation causes wavelengths to be absorbed & removed

---

Deexcitation

[Diagram of atomic de-excitation]

- As the electrons falls back to the ground state
  - an energy "packet" is emitted

---

Deexcitation

**Continuous Spectrum**

**Emission Spectrum**

Deexcitation causes wavelengths to be emitted

---

Absorbance of Light

- Beer's Law
  - Describes the linear relationship between absorbance of light and concentration
  
  \[ A = a \times b \times c \]
  
  - a is the absorptivity in L/g cm
  
  When concentration is molar (mol/L) then the equation is:
  
  \[ A = \varepsilon b c \]

Where \( \varepsilon \) is the molar absorptivity in L/mol cm

Molar absorptivity describes the probability of absorbing a photon
It is wavelength dependent

---

<table>
<thead>
<tr>
<th>Wavelength of maximum absorption (nm)</th>
<th>Color absorbed</th>
<th>Color observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>380-420</td>
<td>Violet</td>
<td>Green-yellow</td>
</tr>
<tr>
<td>420-440</td>
<td>Violet-blue</td>
<td>Yellow</td>
</tr>
<tr>
<td>440-470</td>
<td>Blue</td>
<td>Orange</td>
</tr>
<tr>
<td>470-500</td>
<td>Blue-green</td>
<td>Red</td>
</tr>
<tr>
<td>500-530</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>530-550</td>
<td>Yellow-green</td>
<td>Violet</td>
</tr>
<tr>
<td>550-580</td>
<td>Yellow</td>
<td>Violet-blue</td>
</tr>
<tr>
<td>580-620</td>
<td>Orange</td>
<td>Blue</td>
</tr>
<tr>
<td>620-660</td>
<td>Red</td>
<td>Blue-green</td>
</tr>
<tr>
<td>660-780</td>
<td>Purple</td>
<td>Green</td>
</tr>
</tbody>
</table>

---
Beer's Law

Absorption is proportional to concentration.

The Spectrophotometer
- The spectrophotometer is the instrument used to measure and record the absorption spectrum of a chemical substance.
- The components of a spectrophotometer are:
  - A radiation source
  - A monochromator or frequency selector
  - A sample holder
  - A detector to convert electromagnetic radiation into an electrical signal
  - A recorder to produce a record of the signal
- Absorption spectra can be done in the visible, ultraviolet (UV) or infrared (IR) regions.

Visible Spectroscopy

The components of a spectrophotometer are:
- A radiation source
- A monochromator or frequency selector
- A sample holder
- A detector to convert electromagnetic radiation into an electrical signal
- A recorder to produce a record of the signal

Absorption of Grape Soda

Example
- Determination of the wavelength of light absorbed by a sample of grape soda
- Determination of the amount of dilution of a sample of grape soda
Dilution of Grape Soda

![Sample Calibration Curve](image)

Note how the same molecule displays markedly different spectral features depending upon the environment in which it finds itself.

Typical Organic UV/Vis Spectra

Types of Luminescence

- **Fluorescence**
  - Happens quickly after initial photon absorption (μs to ps lifetime).

- **Phosphorescence**
  - Happens slowly after initial photon absorption (ms to μs lifetime).

- **Chemiluminescence**
  - Excitation arises from a chemical reaction, instead of photoabsorption.

All of these techniques can be used for analytical procedures. Best applied for trace analyses, having detection limits 100 to 1000 times lower than corresponding absorption techniques.

Radiative Decay

Filter Fluorometer Design

A basic fluorometer compares fluorescence intensity while monitoring incident light intensity. The most simple instruments use filters to select specific bands, thus dedicating the instrument to a specific task.

This unit is the AU-10 Field Fluorometer from Turner Designs. Different filters allow it to perform different experiments in the field. For example, its detection limit for chlorophyll in water is 30 parts per million; for crude oil in water it is 10 parts per billion.

Spectra of 9,10-diphenylanthracene

These spectra were taken with cyclohexane as the solvent. This molecule has an extremely high quantum yield: 0.90. 90% of absorbed incident photons show up as fluorescent photons.

Fluorescence Applications

Since fluorescence depends linearly on concentration, it can be used to measure concentration just like absorption experiments. Because the fluorescence can be increased by increasing the incident power, fluorometry can be used to detect very low concentrations – much better than absorption experiments. Applied to a species which fluoresces or chemically attach something that does fluoresce. Fluorescent tags are commonly used in biochemistry. Choose and excitation and emission wavelength, if known. Otherwise, measure their spectra and make appropriate selections. Create a calibration curve for concentration range of interest. Make measurements and employ statistics the same as with other experiments.

Chemiluminescence

Here are two containers of tris(2,2'-bipyridyl)ruthenium (II). Sodium hydroxide is being added to the one of the left and codeine is being added to the one on the right. This produces orange light (at 610 nm). The reaction is used to detect and monitor the presence of certain amines, alkaloids, and oxalates.

Chemiluminescence Detector Design

Chemiluminescence instrumentation is considerably simpler than fluorescence; it needs no excitation source – the chemical reaction provides the input energy – and it needs no wavelength selection device. Essentially one only needs a light detector and a sample holder/introduction system.

This unit is the PMT-FL from FIAlab Instruments. It has a detection limit of 2 parts per trillion for fluorescein. An example application uses a compound called Luciferase Photinus pyralis. The bioluminescent reaction of this enzyme with luciferin, ATP, and O₂ results in the emission of light. Luciferase can be used to detect trace amounts of ATP.

IR

- Probes different molecular vibrations
  - absorption occurs when the frequency of the IR wave matches a vibrational frequency of the molecule
- Most molecules have numerous vibrations
  - bond stretching vibrations
    - detect different kinds of A-B bonds
  - bond bending involves several atoms at once

IR Radiation

- Exposing molecules to the correct frequencies of infra-red light will result in some of that light being absorbed
  - gives that molecule more energy
  - results in more energetic motion of the atoms in the molecule
Types of Vibrations

- Molecular dipole moment must change during a vibration to be IR active.
- This oscillating dipole interacts with the oscillating E-M field of the photon, leading to absorption.

**Stretching Vibrations**
- Symmetric
- Anti-symmetric
- Rocking
- Scissoring
- Twisting
- Wagging

**Bending Vibrations**
- In-Plane
- Out-of-Plane

Changes in bond length
Changes in bond angle

Types of Vibrations

Molecule must have change in dipole moment due to vibration or rotation to absorb IR radiation.

Absorption causes increase in vibration amplitude/rotation frequency.

Molecules with permanent dipole moments (p) are IR active.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>IR Active</th>
<th>IR Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl, H₂O, NO</td>
<td>Yes</td>
<td>No dipole moment</td>
</tr>
<tr>
<td>Ar, O₂, H₂, Cl₂</td>
<td>Yes</td>
<td>IR active</td>
</tr>
</tbody>
</table>

p measured in debyes (D)

1 D = 3.3 x 10⁻²² C m

10 D equivalent to +1 and -1 charge separated 1 Å

IR Spectrophotometer

Typical Spectra

- Spectra are usually presented as % transmittance against wavenumber. Because we are plotting transmittance, the spectrum dips down when there is a strong absorption. This is the opposite when plotting absorbance.

Qualitative Analysis

IR Spectroscopy is widely employed as an identification technique.
- Many narrow peaks
- Subtle shifts are measurable with good resolution

- Analyze wavenumber shifts in specific group:
  - CH stretches: 3000 - 2800 cm⁻¹
  - OH stretches: 3600 - 3200 cm⁻¹
  - CO stretch: 1690 - 1760 cm⁻¹

- Fingerprint region: 1200 - 700 cm⁻¹

- Computer search libraries: >100,000 spectra
Sample Prep

Most time-consuming part is sample preparation.

Gases: dry, pure
(a) transparent windows (No CIKBr)
(b) long path length (10 cm) - few molecules

 Liquids: 6H liquid cell
(a) water in transparent solvent - not water (ethanol, ethanol)
(b) short path length (0.015-0.1 mm) - solvents absorb
(Fig. 17-4, 17-5)

Solids
(a) make area-transparent pellet with KBr
(b) grind and mix with Nujol (hydrocarbons oil) in form and put on cold plate.

Raman Spectroscopy

Another spectroscopic technique which probes the rotational and vibrational structure of molecules.

C.V. Raman discovered in 1928; received Nobel Prize in 1931.

Can probe gases, liquids, and solids.

Must use a laser source for excitation.

Resurgence in recent years due to the development of new detectors with improved sensitivity and diode lasers that are stable and cheap.

How It Works

Excited electronic state

Rayleigh Scattering

Raman Scattering

Virtual electronic states

Ground electronic state

The Spectrum

A complete Raman spectrum consists of:

- a Rayleigh scattered peak (high intensity, same wavelength as excitation)
- a series of Stokes-shifted peaks (low intensity, longer wavelength)
- a series of anti-Stokes shifted peaks (still lower intensity, shorter wavelength)
- spectrum independent of excitation wavelength (488, 632.8, or 1064 nm)

Some Raman Advantages

Here are some reasons why someone would prefer to use Raman Spectroscopy.

- Non-destructive to samples (minimal sample prep)
- Higher temperature studies possible (don’t care about IR radiation)
- Easily examine low wavenumber region: 100 cm\(^{-1}\) readily achieved.
- Better microscopy; using visible light so can focus more tightly.
- Easy sample prep: water is an excellent solvent for Raman. Can probe sample through transparent containers (glass or plastic bags).
Sources

Raman intensity is weak and the excitation source must be strong to generate sufficient signal.
Source must be monochromatic so that spectrum is sufficiently uncomplicated.
Intense lamps work, but when monochromatized, have very little power.
Scattering efficiency increases as $\nu^4$: the bluer the light, the more the scattering.
The bluer the light, the greater the chance of producing fluorescence.
Lasers are used almost exclusively.
Ar$^+$: 488.0 and 514.5 nm
Kr$^+$: 530.9 and 647.1 nm
He:Ne: 632.8 nm
Diode Lasers: 782 and 830 nm
Nd: YAG: 1064 (532 when doubled) nm

Here is a 500 mW Ar ion laser

Experiment used to require considerable excitation power
Ion lasers, 40 W cw
HeNe, 10 W cw
YAG, 1 J/10 ns pulse (100 MW average pulse)
But detectors have improved so much, the source power requirements have been decreased.
Diode laser, 25 mW
other lasers can be made correspondingly smaller.

Multichannel Raman Spectroscopy

Focus laser to small spot. Tune spectrometer to particular Raman transition peak.
Raster scan the sample under the laser beam, record intensity changes. Resultant map correlates with substance. Acquire an entire spectrum at every point, then choose the feature with which to key the image.

Chemical Imaging

Mixture of cocaine and sugar. Bright spots are cocaine.

Chemical Mapping

This is a drug tablet. The yellow corresponds to the active ingredient. Particles are in the 10’s of µm range.

Applications - Art Restoration

This 12 century fresco on a church wall in Italy needed to be restored. What paints to use?
Raman analysis clearly identified the paints and pigments that were originally present, permitting a correct choice of cleaning materials and subsequent repainting to restore its original condition.
Applications - Paint Chips

Forensic analysis of paint chips in vehicle accidents. Often multiple layers. Can analyze with IR by stripping successive layers. Image edge with micro-Raman.

Layers 1 and 3 turned out to be rutile phase TiO$_2$ - a white paint. Layer 2 was a Goethite, a red pigment and corrosion inhibitor. Layer 4 was molybdate orange, a common red paint in the 70’s in North America and still used in the U.K. today. Layer 5 was a silicate based paint. Data arising from a case investigated by LAPD.

Applications - Gem Forgery

In 1999 a new process was developed – called GE POL – whereby brown type IIa diamonds could be treated to become indistinguishable from naturally clear diamonds. Raman presented way to distinguish them.

Applications - Bullet Proof Glass

Identify poly(carbonate) from poly(methylmethacrylate). Both used for shatter-proof glass.

Applications - Sunscreen Formulations

Here are the spectra of 5 common sunscreen ingredients. Raman is able to determine from a spectrum on the arm the nature of the sunscreen being used:

A: ODPABA (octyl N,N-dimethyl-p-aminobenzoic acid)
B: OMC (octyl p-methoxycinnamate)
C: BZ3 (oxybenzone)
D: OCS (octyl salicylate)
E: DBM (dibenzoylmethane)