Laboratory Instruments

Principle, Construction, Working and Applications

BSc – III (2016 – 17)

Institute of Forensic Science, Nagpur

INDEX

Electrostatic Detection Apparatus (ESDA)

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Electrostatic Detection Apparatus

ESDA stands for Electrostatic Detection Apparatus. It is an instrument used for questioned document examination to reveal the indentations or impressions which may be present on paper. ESDA was manufactured by Foster and Freeman. It is a sensitive technique capable of detecting the indentations on paper.

Document is a piece of written or printed matter that provides information or evidence or that provides as an official record.

Questioned Document is a document where any signature, handwriting, typewriting or other marks whose source or authenticity is in dispute.

Indentations: Indentations are created when two or more sheets of paper are resting atop one another during writing. The underlying pages contain the latent impression which can be developed by ESDA.

Construction

ESDA is a specialized piece of equipment made up of various parts which are ESDA machine , a thin base on which the document is to be kept, a cellophane which is a plastic film kept on the document with the help of vacuum, electrostatic charge device and an toner. The toner is more similar to the toner used in electro photographic printing device.

Principle

The ESDA uses the principle that indented areas of the document carry less negative charge than surrounding areas. This causes the toner used in the Electrostatic Detection Device (EDD) to be attracted to these areas, revealing indentations that are present. Using this technique, indented impressions have been recovered from upto seven layers of paper beneath the original writings. The impressions can be successfully visualised from documents upto sixty years old. ESDA provides a method of detecting and permanently recording latent writing impression on the surface of the paper. Virtually all materials, including conductors can be triboelectrically charged. The amount of charge is affected by material type, speed of contact and separation, humidity and several other factors. The EDD is able to visualize indentations because the surface is differently charged depending upon whether or not an indentation is present. The negatively charged toner particles are attracted to areas where there are indentations on paper surface.

Fig. A1: ESDA

FBN MEDICAL CENTER Patient Number 96-0424

Patient's Name ELENA OSSEN wF 45 Phone (954) 221-0774

Address 636 CHERRY CREEK LANE PEMBROKE PINES

DATE	PROGRESS NOTES
	ALLERGIES: TETRACYLIN, PCN
8/14	PT HAS CHEST PAIN + SOB
	LUNGS- NO RHoni
8/15	2230 ADMITTED, PLACED ON MONITOR.
	VITALS RECORDED. ADVISED OF
	OPERATION OPTIONS.
	I RECOMMEND AGAINST THE OPERATION
8/16	0210 T 100.2 TYLENOL SUPP.
8/17	0800 EXPLORATORY - PT EXPIRED 09:12
	(RENAL ATTEMPTED - NEGATIVE)

Fig. A2: Writing on Paper

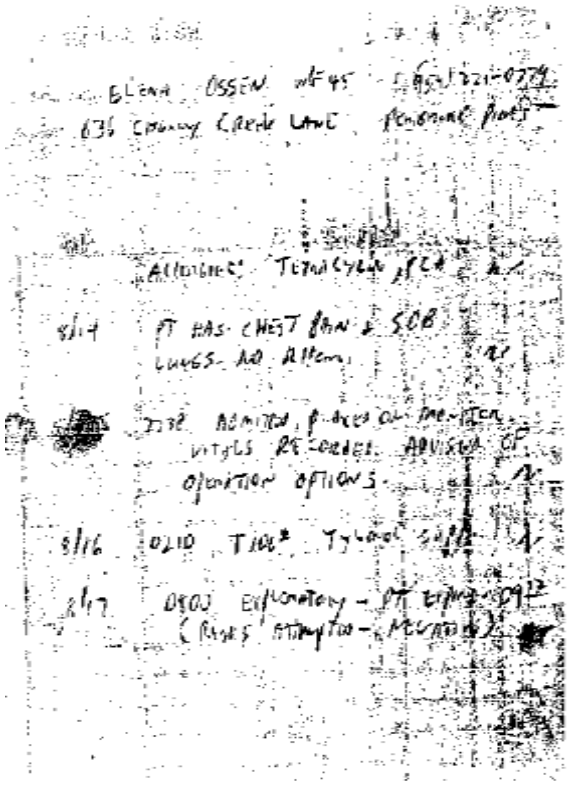

Fig. A3: Indented Writing on Paper Developed by ESDA

Theory

ESDA works by stretching a Mylar film (like Clingfilm) over the document being examined. This Mylar film is then electrostatically charged using a "wand" (a long thin stick or rod) containing a fine wire charged to 7 kV. Where the paper is smooth, the charging is generally uniform. However where the fibres of paper have been disturbed by paper-paper contact caused by indentations, the electrostatic charge is different to the background. This creates a latent image.

In order to visualise this latent image, black toner similar to that used in laser printers is applied to the surface of the Mylar. The toner sticks where the electrostatic charge congregates (i.e. in the areas of indentations). The result is an image with a grey toner background and the darker toner traces in the areas where there are indented impressions.

The image is made permanent as a 'lift' by placing a clear 'sticky backed plastic' over the toner producing a fixed transparent image. Toner can be put onto the Mylar film using three methods; by cascading tiny glass beads coated with toner over the surface, by puffing an aerosol of toner powder over the surface or by using a device similar to the powder puff to apply the toner.

There are large numbers of variables which affect the quality of ESDA lifts. This includes the type of paper, the type of pen or stylus used, the number of sheets of paper between the writing and the sheet holding the impressions, the humidity at the time of impressions. The paper sandwiched between the grounded plate and the Mylar charging film acts as a type of a capacitor with the charge in capacitance being due to differing compensation of the paper.

The page suspected of bearing indentations is covered with a cellophane material which is then pulled into firm contact with the paper by a vacuum drawn through a porous bronze plate. This serves to fasten the document and cellophane covering, to the plate. The cellophane covering prevents damage to the original document. The document and cellophane are than subjected to a repeated high voltage static charge.

Applications of ESDA

1. ESDA is used for visualising traced forgery.
2. A questioned document such as ransom note may exist which can be determined to be the source of indentation detected on another piece of paper.
3. Decipherable indentations may also provide valuable information even when a second document is not present or cannot be located.
4. Documents that don't contain visible identifiable marks may contain valuable impression evidence if they were underneath other documents when the writing was performed.

Advantages and Disadvantages of ESDA

Advantages

1. ESDA has the advantage of being non-destructive so that the paper under examination remains in exactly the same state and is still available for other examination.
2. It is extremely sensitive that means indentations found up to 7 sheets below the page where the original writing was made may be visualised.

Disadvantages

1. It is not suitable for the examination of loose paper such as newspaper or very glossy such as magazine covers.
2. If a document gets wet by any liquid, it will completely destroy the ESDA impressions.

Video Spectral Comparator (VSC)

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Video Spectral Comparator

Video Spectral Comparator (VSC) is a machine used in forensic sciences to examine all types of documents by choosing various types of illuminations and viewing conditions. VSC is mainly built for law enforcement and is often used to evaluate if a document has been altered or forged. As the name suggests, the machine uses images and spectra of sample to compare with the images and spectra of another.

VSC is a preferred tool as it supports non destructive examination of documents. VSC uses multiple parameters like IR, UV, and White light providing accurate results. VSC6000 is the new version of VSC which is available for use for the forensic and general purposes.

Construction

The VSC consists of a main unit and a PC system. The main unit is a square box with 3 flaps. The main unit has a high resolution CCD firewire colour camera with sensitivity from $360\ nm$ to $1100\ nm$, different light sources (incandescent filament lamps, LED, vapour discharge tube, flash tube etc), optical filters as well as a translight panel.

The panel is situated in the center of the document platen. Underneath the panel are light sources which can illuminate the document from below. The document platen in itself is $650\ mm\ x\ 650\ mm$ while the translight panel is $235\ mm\ x\ 175\ mm$. In the VSC there is also a high resolution grating spectrometer. This can analyze light from a small region in the document. As part of the VSC machinery is a 30″ screen and a PC system which is Windows based.

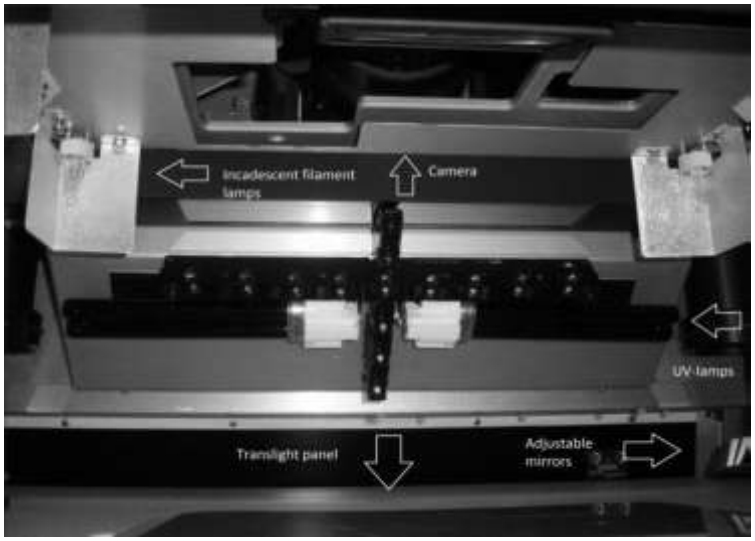

Fig. B1: Front Panel of VSC

Principle

Video Spectral Comparator works in the basic principle of light as we know. Light is a form of electromagnetic radiation and consists of different wavelengths. When the eye sees different colours, it actually perceives different wavelengths of light. When light directed towards an object any of the one of five things usually occurs depending on the emitted wavelengths and the composition of the object –

1. Reflection: Whole of the light or part of it can be reflected off making the object making it appear white or lighter.
2. Absorption: All or most of the light can be absorbed by the object. Object appears black or darker.
3. Reflection and Absorption: A part of the light can be reflected and part can be absorbed. It produces colours in the visible portion of the spectrum.
4. Transmission: Light can be transmitted through the object.
5. Luminescence: Light can strike the object, be absorbed and then reemitted at a longer wavelength.

The visible region to humans is in the range of $400nm - 700nm$ in the electromagnetic spectrum. Infrared (IR) and Ultraviolet (UV) radiations are not visible to humans. However the above mentioned effects occur in IR and UV portions. The same object that absorbed light in the visible spectrum and appeared black can now transmit radiant energy in the IR spectrum. Hence a VSC camera operating in the IR portion of the spectrum can capture an image lying underneath an opaque blue ink similar to the way an X-ray captures images of bones through skin.

Different Light Sources used in VSC 6000

The VSC has four different light sources. These are used to help bring out specific types of features in the document examined.

The first light source is **incandescent filament lamps**. These have a range from $400\ nm - 1000\ nm$ and encompass visible and IR light. These lights are used in the VSC when one uses the functions of flood, transmitted, spot and side lighting. Flood lighting uses broadband illumination. Spot lighting is a more high intensity illumination which only uses one lamp, which is located directly above the document platen.

The **LED lamps** which have a wavelength from $400 - 700\ nm$ are used with coaxial lighting and diffracted lighting. Coaxial light is a light that is shone perpendicularly on the document. Coaxial light reveal retro-reflective features in security documents, which are often used to prevent forgeries. Diffracted lighting is a function which illuminates the document from different directions, and is usually used to see Optically Variable Devices (OVD).

The UV lamps are **Vapour discharge tubes**. The VSC offers three ranges of UV light. This is with $365\ nm$ (UV-A), $312\ nm$ (UV-B) and $254\ nm$ (UV-C) peak wavelengths. All of these can illuminate the document from above. With transmitted lighting, the VSC only offers UV light with $365\ nm$.

The last of the light sources is a **flash tube**. This lamp has a range of $850 - 1100\ nm$ and is used for the Anti-Stokes flash function. Anti-Stokes flash is a function which uses narrow-band illumination to excite fluorescence. This illumination is in the IR region above $800\ nm$ and lack in visible light.

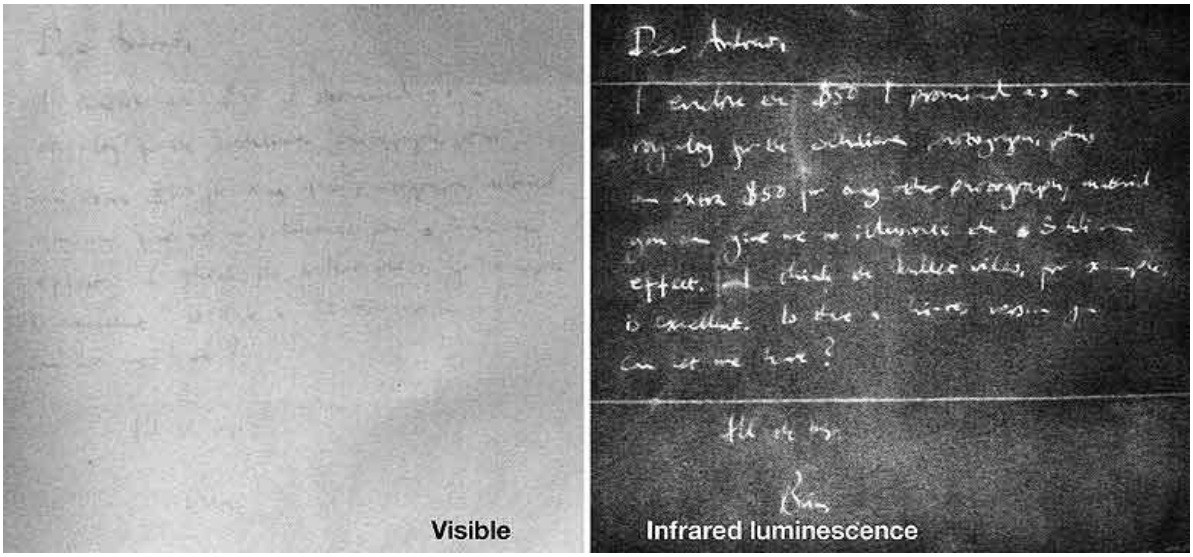

Fig. B2: A Letter Observed Under IR Light.

Hyperspectral Imaging

Hyperspectral imaging is a non-invasive, non-destructive method to enhance the spectral differences of inks, toners and pigments. It can be used for the detection of forgery, alterations and page substitution of questioned documents as well as for the analysis of paintings and artwork. By examining a document under a bandpass filtered light source at progressively longer wavelengths, it is possible to capture a sequence of images of the

document to create a hyperspectral datacube - a dataset that includes all of the spatial and spectral information for each captured image across a predetermined waveband.

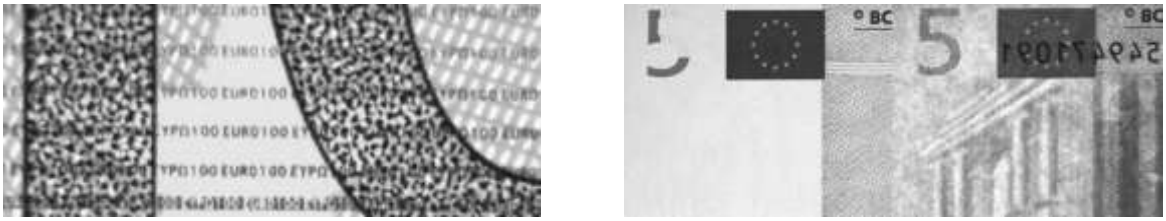

Fig. B3: Hyperspectral Imaging

Applications

1. **Banks**: Currency forgery has always been an issue of concern for the banks. Fraudsters are finding new ways to produce fake currency. Banks have started using VSC to monitor currency in circulation and spot the fake ones. With VSC each security feature of bank notes can be observed like ink, images, paper gradient and security thread. It can be used to examine suspected fake cheques.
2. **Government Offices**: A growing concern these days is fake official documents. Fraudsters create fake certificates, notarized documents, license agreements, degree certificates, birth certificates, etc. VSC can be very useful to examine such documents and establish authenticity of it. It can detect alterations made to original documents.
3. **Airpor**t: VSC is used to examine passport and visa documents at airports. Examination of documents under high magnification can quickly reveal evidence of tampering.
4. **Forensic Examination**: VSC is one the most used tool in questioned document analysis. With its wide array of features it saves a lot of time and produces accurate results. Objects such as currency, stamps, certificates, legal documents, artwork, and identification cards can be examined with the help of Video Spectral Comparator.

Cathode Ray Oscilloscope (CRO)

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Cathode Ray Oscilloscope

The Cathode Ray Oscilloscope or mostly called as CRO is an electronic device used for giving the visual indication of a signal waveform. It is an extremely useful and the most versatile instrument in the electronic industry. **Cathode Ray Oscilloscope** is a very useful laboratory instrument used for display, measurement and analysis of waveforms and other phenomena in electrical and electronic circuits. CRO is very fast X-Y plotter, displaying an input signal versus another signal versus time. CRO operates on voltages.

Principle

Cathode ray oscilloscope uses luminous spot which is produced by striking the beam of electrons and this luminous spot moves in response variation in the input quantity. The general form of CRO operates on voltages.

Construction of CRO

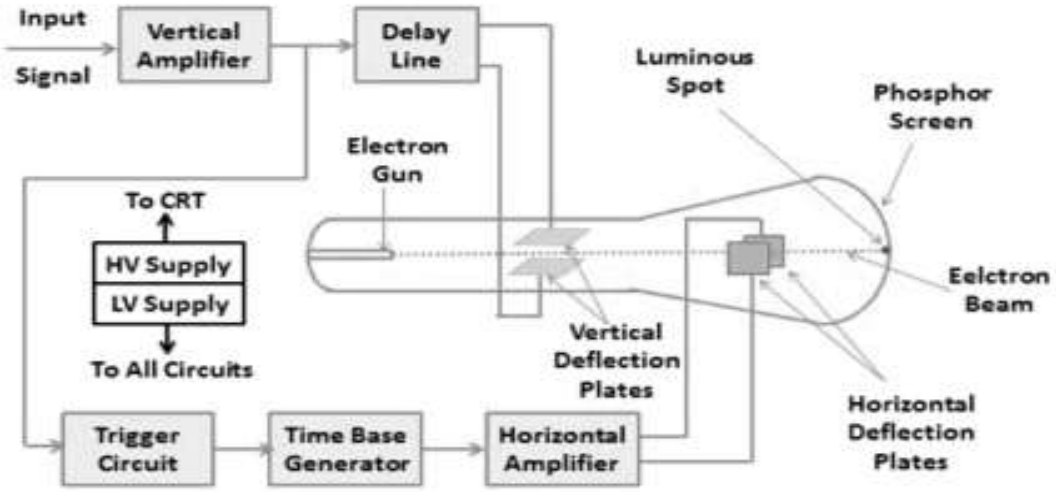


Fig. C1: Block Diagram of CRO

Cathode Ray Tube: It is the heart of the oscilloscope. When the electron is emitted by the phosphor screen, a visual signal is displayed on CRT.

Vertical Amplifier: The input signals are amplified by the vertical amplifier.

Delay Line: As the name suggests, this circuit is used to delay the signal for a period of time in the vertical section of CRT. The input signal is not applied directly to the vertical plates because the part of the signal gets lost, when the delay time is not used.

Time Base (Sweep) Generator: The time base circuit uses a uni-junction transistor, which is used to produce the sweep. The sawtooth voltage produced by the time base circuit is required to deflect the beam in the horizontal section. The spot is deflected by sawtooth voltage at a constant time dependent rate.

Horizontal Amplifier: The sawtooth voltage produce by the time base circuit is amplified by the horizontal amplifier before it is applied to the horizontal deflection plate.

Trigger Circuit: The signals which are used to activate the trigger circuit are converted to trigger pulses for the precision sweep operation whose amplitude is uniform. Hence input signal and the sweep frequency can be synchronized.

Power Supply: The voltages required by CRT, horizontal amplifier, vertical amplifier are provided by the power supply block.

Working

In an oscilloscope, the CRT generates the electron beam which is accelerated to a high velocity and brought to focus on a fluorescent screen. This screen produces a visible spot where the electron beam strikes it. For accomplishing these tasks various electrical signals and voltages are needed, which are provided by the power supply circuit of the oscilloscope.

Horizontal and vertical deflection plates are fitted between the electron gun and the screen so that these can deflect the beam according to the input signal. To deflect the electron beam on the screen in horizontal direction i.e. X-axis with constant time dependent rate, a time base generator is provided in the oscilloscope. The signal to be viewed is supplied to the vertical deflection plate through the vertical amplifier, so that it can amplify the signal to a level that will provide usable deflection of the electron beam. As the electron beam is deflected in X-axis with constant time dependent rate, a time base generator is provided.

As the electron beam is deflected in X-axis as well as Y-axis, a triggering circuit is provided for synchronizing these two types of deflections so that horizontal deflection starts at the same point of the input vertical signal each time it sweeps. Since CRT is the heart of the oscilloscope, we are going to discuss its various components in detail.

Cathode Ray Tube

The Cathode Ray Tube or CRT is a vacuum tube of special geometrical shape which converts an electrical signal into a visual one. A CRT makes available a large number of electrons which are accelerated to high velocity and are brought to focus on a fluorescent screen where it produces a spot when it strikes it. The electron beam is deflected during its journey in response to the applied electrical signal. As a result, the electrical signal waveform is displayed visually. The figure below shows various parts of a cathode ray tube (CRT).

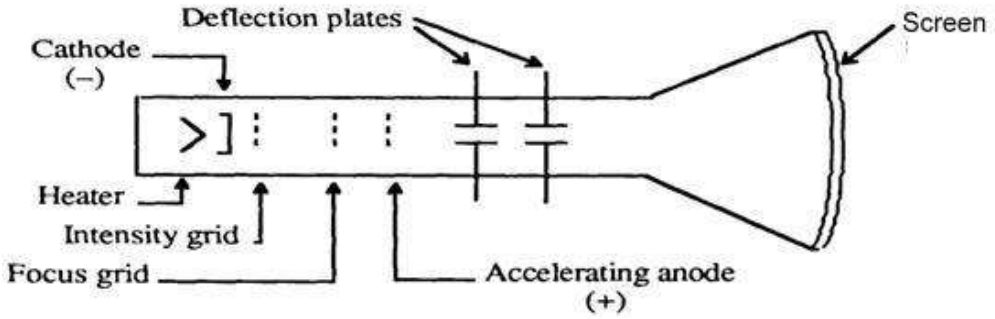


Fig. C2: Block Diagram of CRT

Now we will discuss each part of the CRT in detail.

Glass Envelope: It is a conical highly evacuated glass housing which maintains vacuum inside it and supports various electrodes.

Electron Gun Assembly: The electron gun assembly consists of an indirectly heated cathode, a control grid, a focusing anode and an accelerating anode and it is used to produce a focused beam of electrons. The control grid is held at negative potential w.r.t. cathode. However, the two anodes are held at high positive potential w.r.t. cathode. The control grid encloses the cathode and consists of a metal cylinder with a tiny circular opening to keep the electron beam small. By controlling the positive potential on it, the focusing anode focuses the electron beam into a sharp pin point. Due to the positive potential of about 10,000 V on the accelerating anode which is much larger than on the focusing diode, the electron beam is accelerated to a high velocity. In this way, the electron gun assembly forms a narrow, accelerated electron beam which produces a spot of light when it strikes the screen.

Deflection Plate Assembly: It consists of two sets of deflecting plates within the tube beyond the accelerating anode and is used for the deflection of the beam. One set is called as vertical deflection plates and the other set is called horizontal deflection plates. The vertical

deflection plates are mounted horizontally in the tube. On application of proper potential to these plates, the electron beam can be made to move up and down vertically on the screen. The horizontal deflection plates are mounted vertically in the tube. On application of proper potential to these plates, the electron beam can be made to move right and left horizontally on the screen.

Screen: The screen is coated with some fluorescent materials such as zinc orthosilicate, zinc oxide etc and is the inside face of the tube. When high velocity electron beam strikes the screen, a spot of light appears at the point of impact. The colour of the spot depends upon the nature of fluorescent material.

Working of Cathode Ray Tube

As the cathode is heated, it produces a large number of electrons. These electrons pass through the control grid on their way to the screen. The control grid controls the amount of current flow as in standard vacuum tubes. If negative potential on the control grid is high, fewer electrons will pass through it. Hence the electron beam will produce a dim spot of light on striking the screen. Reverse will happen when the negative potential on the control grid is reduced. Therefore, the intensity of the light spot on the screen can be controlled by changing the negative potential on the control grid.

After leaving the control grid, the electron beam comes under the influence of focussing and accelerating anodes. Since, the two anodes are at high positive potential, they produce a field which acts as electrostatic lens to converge the electron beam at a point on the screen.

After leaving the accelerating anode, the electron beam comes under the influence of vertical and horizontal deflection plates. When no voltage is applied to these deflection plates, the electron beam produces a spot of light at the centre as shown by point O in the figure.

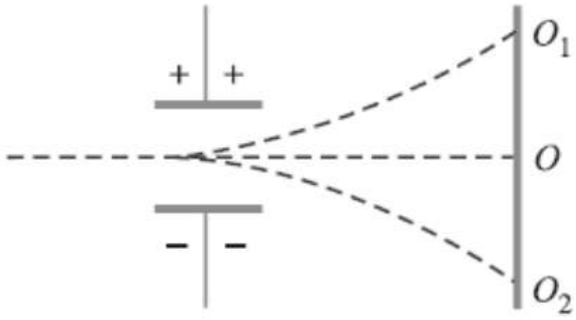


Fig. C3: Deflection Trajectories of Electron Beam

If the voltage is applied to the vertical deflection plates only, the electron beam and so as the spot of light will be deflected upwards i.e. point O1.

And if the potential on the plates is reversed, the spot of light will be deflected downwards i.e. point O2. Similarly, the spot of light can be deflected horizontally by applying voltage across the horizontal deflection plates.

Applications of CRO

1. **Measurement of voltage:** Voltage waveform will be made on the oscilloscope screen of the CRO, the voltage can be measured by seeing its amplitude variation on the screen.

2. **Measurement of current:** Current waveform will be read from the oscilloscope screen in the similar way in the case of voltage. The peak to peak, maximum current value can be measured from the screen.

3. **Measurement of phase:** Phase measurement in CRO can be done by the help of Lissajous pattern figures. Lissajous figure can tell us about the phase difference between two signals. Frequency can also be measured by this pattern figure.

4. **Measurement of frequency:** Frequency measurement in CRO can be made with the help of measuring the time period of the signal to be measured.

C.R.O. Operation

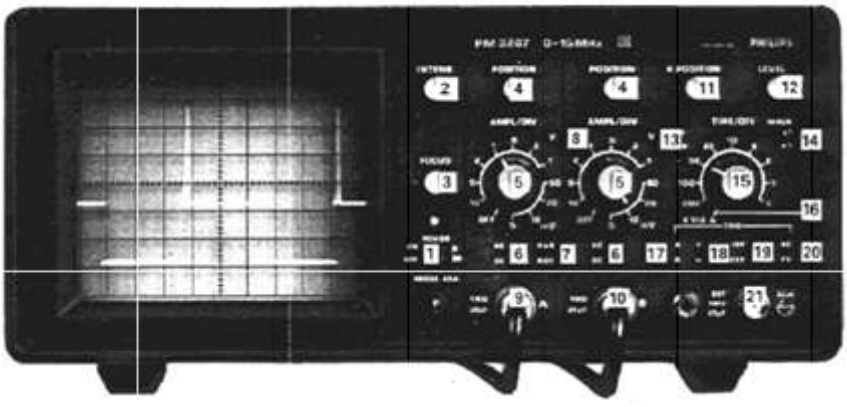


Fig. C4: Typical Front of a CRO

1. On–Off switch.
2. **INTENS:** This is the intensity control connected to the grid G to control the beam intensity and hence the brightness of the screen spot. The intensity should not be made too high. It should be just bright enough for clear visibility. The spots should always weep left to right or the beam may “burn” a hole in the screen.

3. **FOCUS:** It allows obtaining a clearly defined line on the screen.
4. **POSITION:** It allows adjusting the vertical position of the waveform on the screen. (There is one of these for each channel).
5. **AMPL/DIV:** It is a control of the Y (i.e. vertical) amplitude of the signal on the screen. (There is one of these for each channel).
6. **AC/DC Switch:** This should be left in the DC position unless one cannot get a signal on-screen otherwise. (There is one of these for each channel).
7. **A&B/ADD Switch:** This allows to display both input channels separately or to combine them into one.
8. **+/- Switch:** This allows to invert the B channel on the display.
9. Channel A input
10. Channel B input
11. **X POSITION;** These allow to adjust the horizontal position of the signals on the screen.
12. **LEVEL:** This allows determining the trigger level i.e. the point of the waveform at which the ramp voltage will begin in time base mode.
13. **ms/µs:** This defines the multiplication factor for the horizontal scale in time base mode.
14. **MAGN:** The horizontal scale units are to be multiplied by this setting in both time base and XY modes. To avoid confusion, it should be left at X1 unless it is really need to change it.
15. **Time/Div:** This selects or controls the frequency at which the beam sweeps horizontally across the screen in time base mode, as well as whether the oscilloscope is in time base mode or XY mode. This switch has the following positions:
    (a) **XY Mode:** In this position, an external signal connected to input A is used in place of the internally generated ramp.
    (b) **-5, 1, 2, 5, etc.:** Here the internally generated ramp voltage will repeat according to the multiplier and magnitude settings.
16. **A/B Selector:** This allows choosing which signal is to be used for triggering.
17. **-/=:** It will force the ramp signal to synchronize its starting time to either the decreasing or increasing part of the unknown signal being studied.
18. **INT/EXT:** It will determine whether the ramp will be synchronized to the signal chosen by the A/B switch or by whatever signal is applied to the EXT SYNC input.

Digital Camera

Aishwarya Kunghadkar

Dipali Kumbhare

Himayu Pawar

Kirti Nagpure

Pranali Dupare

Shraddha Dhole

Digital Camera

Construction

Basically digital camera is constructed by the following components:

1. Lenses
2. Diaphragm
3. Shutter
4. CCD (charge coupled device)

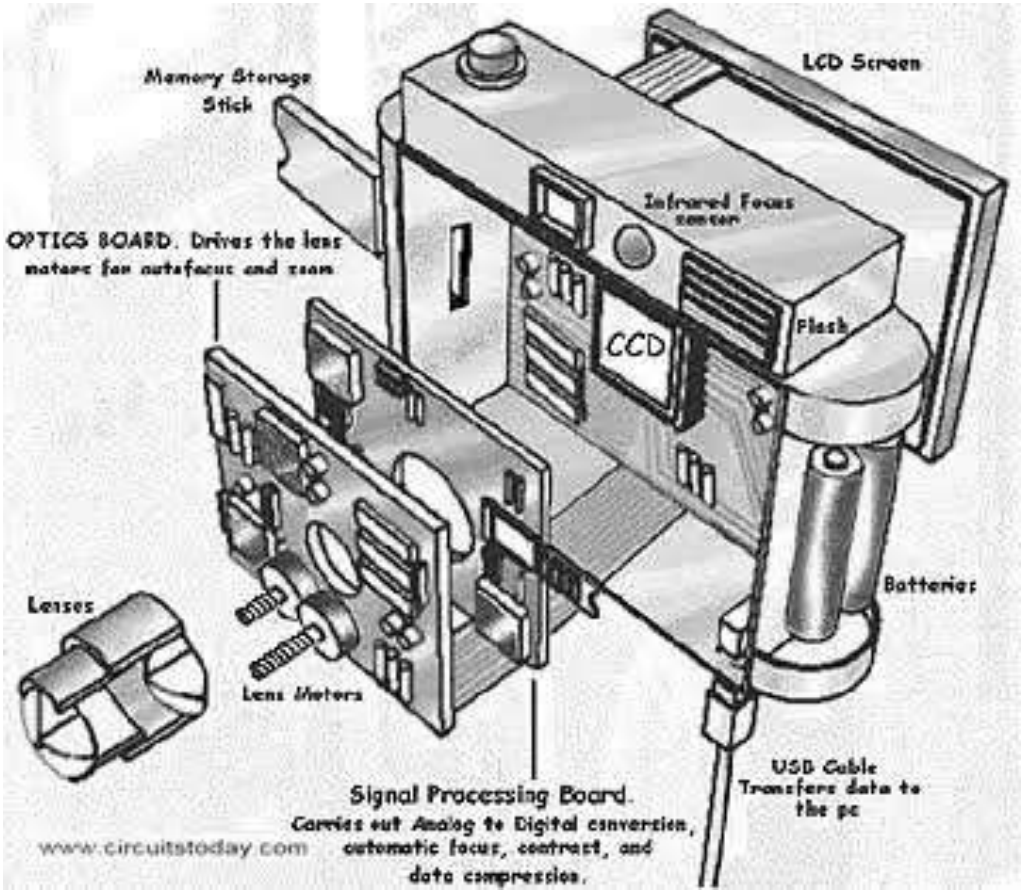


Fig. D1: Components of Digital Camera

Lenses:

It collects the light coming from the object and focuses it on the sensor. Most digital cameras use automatic focussing technology. Different cameras may have different types of lenses like fixed focus, fixed zoom lenses, optical zoom lens with automatic focus, digital zoom lenses, replaceable lens system etc.

Diaphragm:

In a camera, diaphragm determines the amount of light that enters through the lens, it is measured as f-number. The greater the f-number, smaller is the opening of diaphragm.

Shutters:

It determines the amount of time of exposure of camera sensors. It is measured in fraction of seconds. The faster the shutter, smaller is the exposure of the camera sensor.

CCD (Charge Coupled Device):

These are the group of sensors that converts light into corresponding electric charge. This sensor is made of light sensitive diodes called photosites that convert photons into electrons. CCD also includes filters that generate the colour images. A series of filter must unpack the image into discrete value of red, green and blue (RGB). Sometimes CMOS sensor is also used in place of CCD.

Beside these basic components, the digital camera have other important parts like batteries, flash capacitor, flash lamp, small LED, USB connector, SD card slot and processor chip which control all the camera's function.

Working Principle of Digital Camera

1. **Capturing Image:**
   Lenses focus the light rays coming from the object and make it into coherent image. Diaphragm determines the amount of light that enters and shutter speed determine the time of exposure. Then light is made to fall onto CCD sensor to get corresponding electric charge.
2. **Binary System Processor:**
   To convert analog signal (electric charge from photosites) to digital signal in binary form, an ADC converter is used. Each of the charge stored in photosites is assigned to a binary value storing them as pixels.
   A pixel is a point sample of an image which contains three basic colour (RGB) components. Pixel per square inches determines the resolution of camera which indicate the size and quality of image.

3. **Compression and Storage:**
   Digital camera have internal memory chip that is used to store images. Once the image is digitalized, it is compressed by microprocessor and stored as image file (JPEG,TTF, etc).

Applications (in general)

1. Keeping record of friends and family, important events in day to day life.
2. **Creating Insurance Record:** Digital cameras are an excellent way to keep a visual inventory for insurance purpose.
3. **Creating Graphics for Websites:** As digital photos are electronic, it enables to create ones photos and graphics for web site.
4. **Textures and Objects for Presentation:** Digital cameras are great for recording textures for websites and presentation. We can shoot objects as per our requirement.
5. **Record an Event and Meeting:** Digital camera is easy to use to record an historic event or an important meeting.

Applications (in Forensic Laboratories)

1. Digital camera is most often used in crime scene investigation to keep record of crime scene.
2. Digital photos are used to reconstruct the crime scene.
3. It is most important tool in documentation of crime scene.
4. In laboratories, it can be used to keep record of findings of evidence analysis.

Other Relevant Information

1. A digital camera stores image digitally rather than recording them on film. Once a picture has been taken, it can be downloaded to a computer system, and then manipulated with a graphics program and printed.
2. Digital cameras comes in wide range of sizes and capabilities e.g. compacts, action cameras, 360-degree cameras, bridge camera, DSLR (Digital Single-Lens Reflex Cameras), DSLT(Digital Single Lens Translucent), SLR (Single Lens Reflex) camera.
3. Digital cameras are incorporated into many devices ranging from PDAs and mobile phones (mobile camera) to vehicles.

E

Double Beam Absorption Spectrometer

Apurva Shinde

Bharti Verma

Govind Patel

Krunal Mahatpure

Manjusha Bawankar

Double Beam Absorption Spectrometer

Construction

The schematic of the Double beam absorption spectrometer is given below,

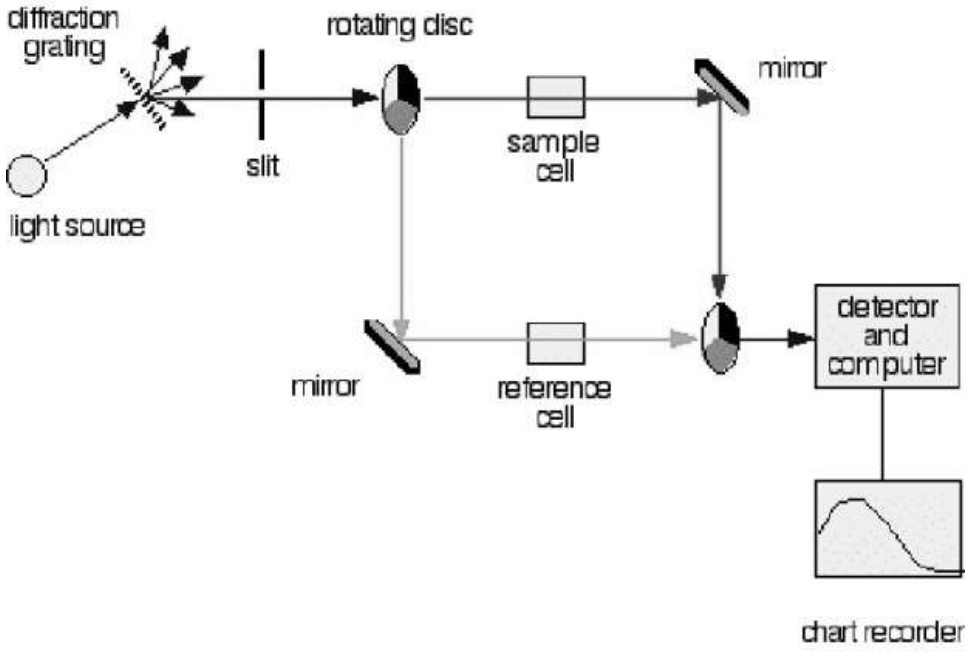


Fig. E1: Schematic of Double Beam Absorption Spectrometer

The Double beam absorption spectrometer consists of the following components:

1. A light source
2. Diffraction grating and the slit
3. The rotating disk
4. The sample cell and reference cell
5. The detector and computer
6. Chart recorder

1. Light Source:

The Double beam absorption spectrometer operates in visible as well as ultraviolet region of the electromagnetic spectrum. Hence, the light source covering the visible as well as ultra-violet region must be used i.e. the range from about 200nm to about 800nm (slightly into the near infrared). Thus to satisfy this condition a

deuterium Lamp for UV region and a tungsten/ halogen lamp for the visible region is used.

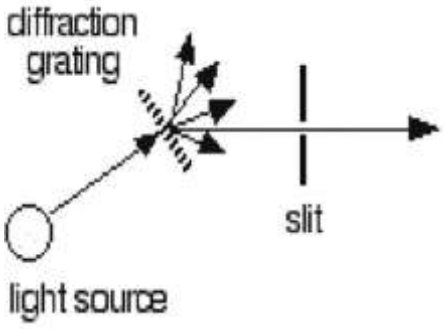


Fig. E2: Light Source

2. **The Diffraction Grating and the Slit:**

The diffraction grating is present in front of the light source so that the light falls on it. The slit is next to the diffraction grating. The diffraction grating does the same work as the 'prism' i.e. to split the light into its components.

The slit is made in such a way that it allows only a narrow range of wavelength to pass through it.

3. **The Rotating Disk:**

The rotating disk consists of the three sections which are as follows,

1. Transparent Section
2. Mirrored section
3. Black section

It keeps on rotating during the operation of the instrument. There are 2 rotating disks; the first rotating disk is present before the sample cell while the second rotating disk is present before the detector.

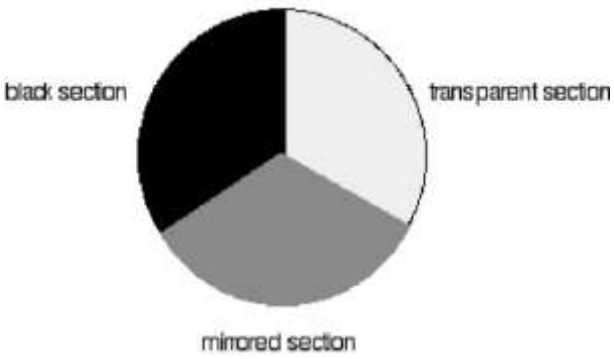


Fig. E3: Rotating Disk

4. **The Sample Cell and the Reference Cell:**

   The sample cell and reference cell are rectangular containers made up of glass or quartz. They are designed in such a way that the light incident onto the sample cell passes through it for only 1cm.

   The sample cell contains the sample which is to be identified mixed with a solvent (which absorbs very less light or negligible light). The reference cell contains the same solvent which is used in sample cell but in pure form.

5. **The Detector and Computer:**

   The detector converts light into electrical current. More the intensity of light, more is the current. This current is fed to the computer that calculates the absorbance.

6. **Chart Recorder:**

   The chart recorder plots a graph between wavelength on X-axis and absorbance on Y-axis.

Principle

When the white light passes through the coloured substances, the wavelengths that are absorbed are not shown, but the wavelength that is emitted is seen as the colour of that substance. For example, colour of the leaf is green because it reflects only green colour and absorbs all the other colours. In case of colourless substances, the absorption occurs in the UV region which can't be seen by us.

In the double beam absorption spectrometer, the light of single wavelength passes through the sample cell at a time. If the sample absorbs the particular wavelength, the intensity of that wavelength decreases when it comes out of the sample cell. This intensity is given by 'I'. The same wavelength of light is passed through the reference cell which contains only the solution (that absorbs very less or negligible light). This intensity is denoted as 'I_0'. If the intensity of light through sample cell is less than that from the reference cell i.e. $I < I_0$, then it can be said that the sample has absorbed some amount of light. By using these intensities and the absorbance can be calculated as;

$$A = \log_{10}\left({}^{I_0}/_{I}\right)$$

If the sample does not absorb any light, then the intensity (I) and intensity (I_0) will be same. Thus, we get absorbance as;

$$A = \log_{10}(1) = 0$$

Thus, if no amount of light is absorbed the absorbance will be zero.

Depending upon the components present in the sample, different wavelength of light will be absorbed. On this basis the components present in the sample can be identified. Also the amount of light absorbed by the sample depends upon the concentration of the components absorbing light in the solution.

Thus, the double beam absorption spectrometer measure the intensity of light i.e. the amount of light absorbed by a compound and then the emitted light after absorption, hence absorbance across the UV and visible spectrum.

Working of the Instrument

The light source (a deuterium lamp for UV and tungsten/ halogen lamp for the visible part of spectrum) emits the light that covers the visible and UV spectrum i.e. the wavelength range of 200nm to about 800nm is given by the light source.

This emitted light falls upon the diffraction grating as shown in the Fig. 1. The diffraction grating then breaks the light into its components. Thus, from diffraction grating we get components of different wavelengths in different directions. But the slit present next to the diffraction grating allows only a narrow range of wavelength of light to pass through it (as shown in Fig. 2). To get different wavelengths of light to pass through slit the diffraction grating can be rotated.

The light from the slit falls on the first rotating disc. As the disc has three sections, transparent section, mirrored section and black section, there are three possibilities,

1. If the light falls on the transparent section, it passes as it is onto the sample cell. The sample may or may not absorb the light, but the light further falls on the mirror next to it in such a way that the mirror sends the light to the second rotating disc. The second rotating disc

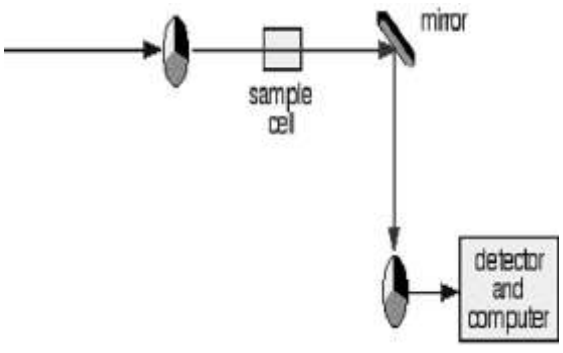


Fig. E4: Light Falling on Transparent Section

rotates such that the mirrored section facing the fallen light on it. Thus the light further reflects from this mirrored section and falls on the detector. Then the detector converts this light into current.

2. If the light falls on the mirrored section, it gets reflected to the mirror present at its other side (towards the reference cell). From this mirror the light passes through the reference cell and then to the second rotating disc. This light passes through the second rotating disc as it is and then goes to the detector where it is converted into current.

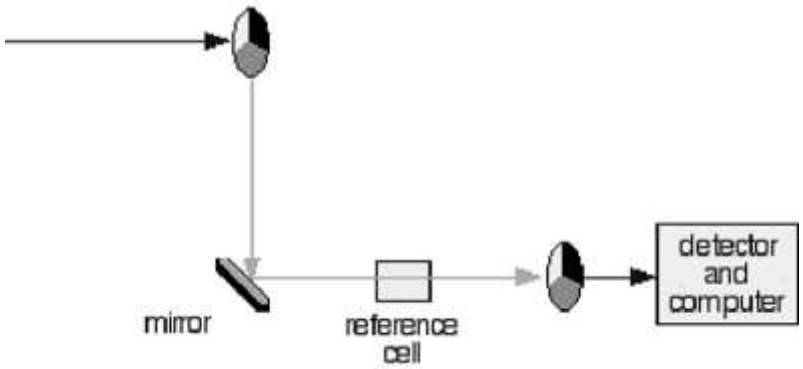


Fig. E5: Light Falling on Mirror Section

3. If the light falls on the black section of the first rotating disc, it is blocked and does not go any further. Thus there is no current in the detector due to light. If there is still some current in the detector, it flows through to the computer and this by which is not due to the light will not interfere with the current generated by the light.

When the light reaches detector, it is converted into electric current. More the intensity of light, more is the current produced. This current is fed to the computer on the basis of which the computer performs the calculation in the following manner,

i. When the light passes through the sample cell, some amount of light may be absorbed. Thus the intensity of light may decrease thus decreasing the current. This intensity is given by 'I'.

ii. When the light passes through the reference cell, a very small or negligible amount of light is absorbed, as the solvent in the reference cell is so selected that it will absorb very small amount of light. Thus the intensity of this light is given by 'I_0'. This intensity is also converted into current.

Then both the intensities (I_0 and I) are used to calculate the absorbance which is done in the computer by using the formula of absorbance given as;

$$A = \log_{10}\left(I_0/I\right)$$

Finally, the chart recorder which is attached to the computer plots the graph between wavelength (nm) on X-axis and absorbance on Y-axis.

General Application of Double Beam Absorption Spectrometer

1. It helps to determine lead in various cosmetic products such as lipsticks, aerosols, etc.
2. It also helps for the determination of metal such as copper, calcium in inorganic fertilizers.
3. It helps for the determination of iron in haemoglobin.
4. It is used to measure certain ingredients in a drug to make sure that it is effective and safe for consumers.
5. It is used to measure bacterial growth or diagnose a patient based on how much uric acid is present in their urine.
6. Scientists also use this spectrometer to see how a reaction has progressed.
7. In many industries such as semiconductors, laser and optical manufacturing uses this spectrometer for the study of chemical substances.
8. It is used as detector in HPLC.

Forensic Application of Double Beam Absorption Spectrometer

1. It is used for the determination of calcium, magnesium in blood serum as well as in plasma.
2. It is also used for the determination of lead in gunshot residues for identification of the shooter in gunshot cases.
3. It is used for the determination of haemoglobin and its degradation products in bruises in human subjects for the determination of age of bruise.
4. It is used for toxicological examinations in heavy metal poisoning cases
5. It is also used to detect multi-metal traces in injured skin for investigation of fatalities caused by electrocution.
6. It is also used for the determination of chemical composition of ball-point pen ink analysis which helps in forensic questioned document examination.

Advantages of Double Beam Spectrometer over the Single Beam Spectrometer

1. Measuring the reference and the sample beam simultaneously or alternately, depending on the instrument configuration increases the speed with which measurements can be taken
2. The measurements and readings are more efficient as compare to the single beam spectrometers. It decreases the chance for human error during setup and calibrations.
3. It is more flexible in use of diffraction grating as compared to the single beam absorption spectrometer.
4. The double beam absorption spectrometer is simple to use than the single beam absorption spectrometer.

F

Colorimeter

Kalptaru Dhakate

Nikita Borkar

Priya Sharma

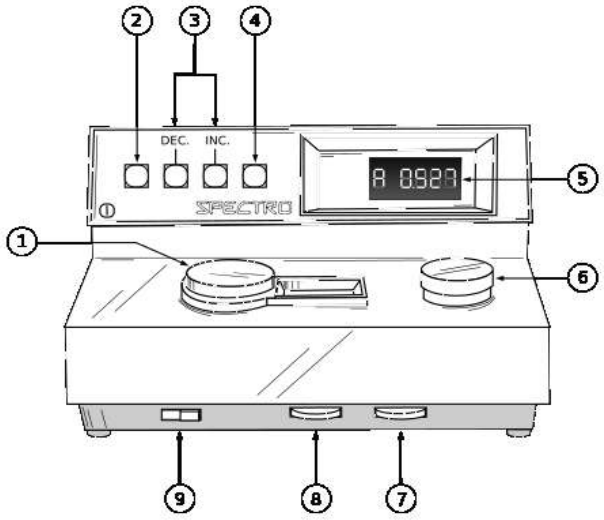
Sudeshna Bag

Vishakha Lanjewar

Colorimeter

Construction

The schematic diagram of a colorimeter is as shown in the figure,



1. Wavelength Selection
2. Printer Button
3. Concentration Factor Adjustment
4. UV Mode Selector
5. Readout
6. Sample Compartment
7. Zero Control
8. Sensitivity Switch
9. ON/OFF Switch

Fig. F1: Schematic of a Colorimeter

The essential parts of a colorimeter are,

1. Light source
2. Adjustable aperture
3. Set of filters
4. Cuvette
5. Detector
6. Ammeter to display the output from the detector

In addition there may be a voltage regulator and second light path.

Filters: Changeable optics filters are used to select the wavelength of light which the sample absorbs the most. Wavelength of light ranges used usually is from 400-700 nm.

Cuvettes: Cuvettes are inserted and removed by hand. Generally it holds the solution.

Output: The output may be displayed by analog or digital meter.

Detector: Generally photoresistor is used to measure the transmitted light.

Principle

Colorimetry is a technique, frequently used in biochemical investigation, which involves the quantitative estimation of colours. It helps in measuring the concentration of any unknown substance. If the substance is colourless, then it can be chemically converted to a coloured substance.

In colorimeter, when monochromatic light passes through a coloured solution, some specific wavelengths of light are absorbed which is related to colour intensity. The amount of absorption or transmission by a colour solution is according to Beer's and Lambert's law.

Colour can be produced by any substance when it binds with colour forming chromogens. The difference in colour intensity results in the difference in the absorption of light. And intensity of colour is directly proportional to the concentration of the compound to be measured. The colorimeter can also be used to measure the turbidity of solution.

General Applications

1. Colorimeters are used to monitor the growth of bacterial or yeast culture. They are also used to measure and monitor the colour in various foods and beverages, including vegetable products and sugar.
2. Diamond merchants use colorimeters to measure the optical properties of precious stones. In cosmetology, the device is used to measure the sun protection factor of products applied to the skin.
3. Colorimeters analyze skin tones and tooth colour to help diagnose certain diseases, and to test the concentration of haemoglobin in blood.

Forensic Applications

1. This technique is used for testing water quality by screening chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc and hydrazine.
2. This technique is also used to determine the concentrations of plant nutrients such as ammonia, nitrate, and phosphorus in soil. It is also used to measure change of pH of the soil.
3. Hypostasis was measured in 93 cadavers using a tristimulus colorimeter in order to investigate its relationship with the time of death.

4. It is also used to determine the age of blood stain. If the blood is fresh, the range of visual spectrum is between 400 and 600 nm.
5. It also helps in analysis of dyed fibre by using different range of spectra.
6. The colorimetry is used for assessing the age of a bruise by measuring yellowness of a bruise upto 32% of the age of a bruise.
7. It is also used for the screening of alcohol in vitreous humor and urine samples in autopsy cases and in saliva from drunken drivers. The method gives instant and semi-quantitative results on presence of alcohol.
8. It also detects concentration or amount of chemicals used in drugs which helps to determine genuineness of drugs. It is also used to detect whether the substance or suspected sample contain a prohibited drug or not.
9. It is also used in identification of cocaine, cocaine of salt, phencyclidene (angel dust, also a major drug of abuse) comprising cobalt thiocyanate, a polyol, an aliphatic solvent, a non-ionic emulsifier and a silicon antifrom agent. These things give positive results because these are effectively dissolved in solvent and give good result but drugs like heroine, methadone, quinine, etc. generally give false positive results as they are not dissolved in aqueous reagent of polyhydroxy compound.

Mass Spectrometer

Lily Harle

Poonam Moon

Prachi Kathane

Pragati Gupta

Pranay Charpe

Mass Spectrometer

Mass spectrometry uses an instrument called a **Mass Spectrometer**. Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (M/Z) and relative abundances.

This technique basically studies the effect of ionizing energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species.

Principle

A mass spectrometer generates multiple ions from the sample under investigation. It then separates them according to their specific mass-to-charge ratio (M/Z), and then records the relative abundance of each ion type.

The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio and are detected in proportion to their abundance.

A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of M/Z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.

Construction

The main components of a mass spectrometer are:

1. Inlet system (LC, GC, Direct probe etc...)
2. Ion source (EI, CI, ESI, APCI, MALDI, etc...)
3. Mass analyzer (Quadrupole, TOF, Ion Trap, Magnetic Sector)

4. Detector (Electron Multiplier, Micro Channel Plates MCPs)

All mass spectrometers have an ion source, a mass analyzer and an ion detector. The nature of these components varies based on the type of mass spectrometer, the type of data required and the physical properties of the sample. Samples are loaded into the mass spectrometer in liquid, gas or dried form and then vaporized and ionized by the ion source.

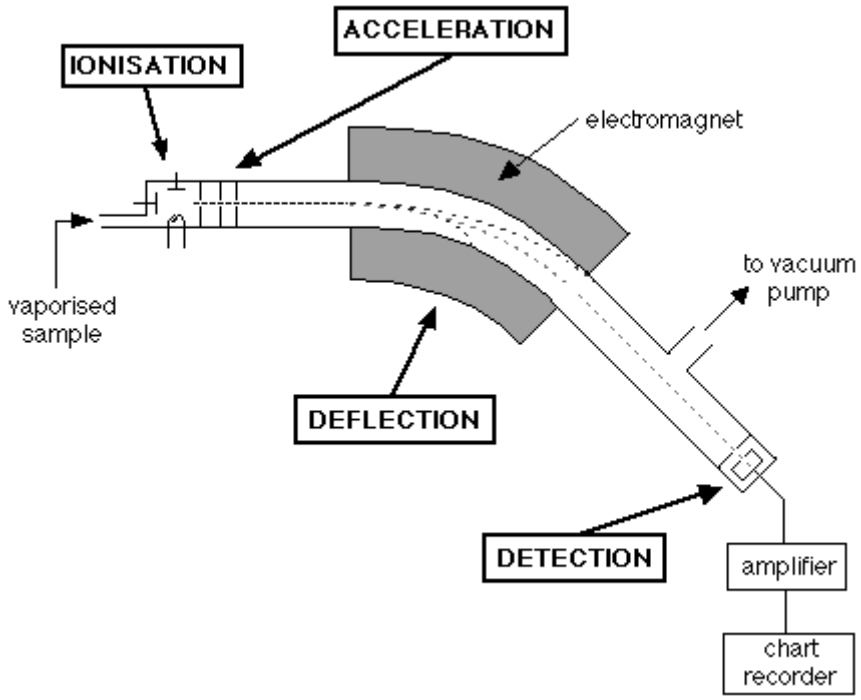


Fig. G1. Schematic of a Mass Spectrometer

Working

Mass spectrometer works on four basic processes,

1. Ionisation
2. Acceleration
3. Deflection
4. Detection

Stage 1: Ionisation

The atom or molecule is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Most mass spectrometers work with positive ions. The vaporized sample passes into the ionisation

chamber. The electrically heated metal coil gives off electrons which are attracted to the electron trap which is a positively charged plate.

The particles in the sample (atoms or molecules) are therefore bombarded with a stream of electrons, and some of the collisions are energetic enough to knock one or more electrons out of the sample particles to make positive ions.

Most of the positive ions formed will carry a charge of $+1$ because it is much more difficult to remove further electrons from an already positive ion.

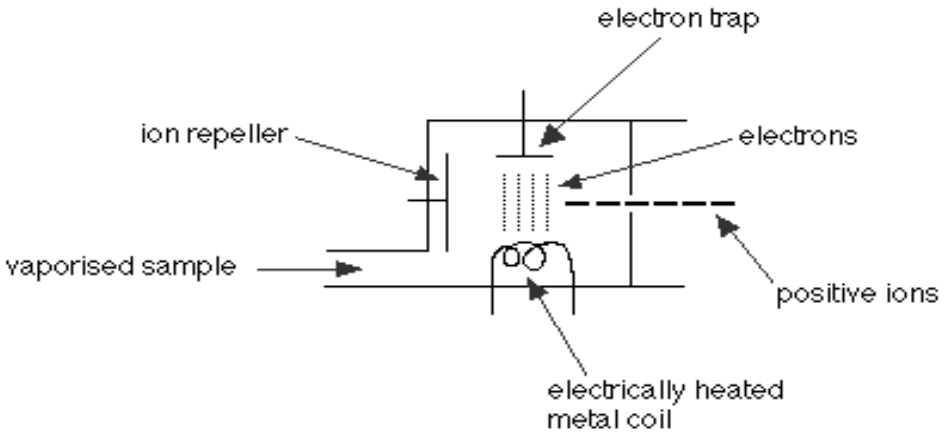


Fig. G2: Ionization Chamber

These positive ions are persuaded out into the rest of the machine by the ion repeller which is another metal plate carrying a slight positive charge.

Stage 2: Acceleration

The ions are accelerated so that they all have the same kinetic energy. The positive ions are repelled away from the very positive ionisation chamber and pass through three slits, the final one of which is at 0 Volts. The middle slit carries some intermediate voltage. All the ions are accelerated into a finely focussed beam.

Stage 3: Deflection

The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion. In other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.

Different ions are deflected by the magnetic field by different amounts. The amount of deflection depends on,

- The mass of the ion. Lighter ions are deflected more than heavier ones.
- The charge on the ion. Ions with 2 (or more) positive charges are deflected more than ones with only 1 positive charge.

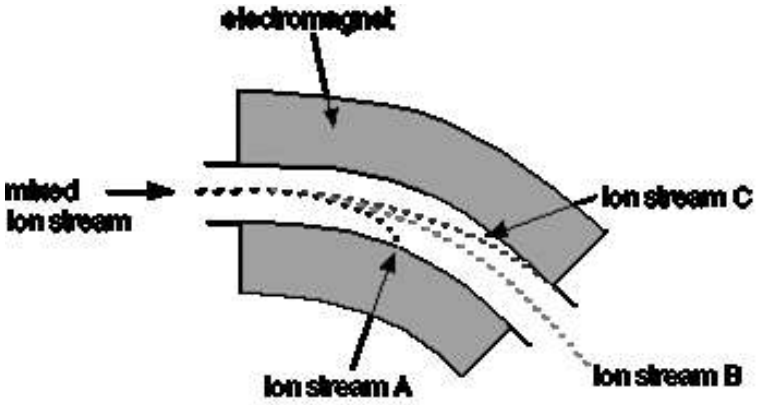


Fig. G3: Deflection of Ions by the Magnetic Field.

These two factors are combined into the ***mass/charge ratio.*** Mass/charge ratio is given the symbol M/Z (or sometimes m/e).

From the figure it can be seen that ion stream A is most deflected - it will contain ions with the smallest mass/charge ratio. Ion stream C is the least deflected - it contains ions with the greatest mass/charge ratio.

If we assume that the charge on all the ions is $+1$ Most of the ions passing through the mass spectrometer will have a charge of $+1$, so that the mass/charge ratio will be the same as the mass of the ion.

Assuming $+1$ ions, stream A has the lightest ions, stream B the next lightest and stream C the heaviest. Lighter ions are going to be more deflected than heavy ones.

Stage 4: Detection

The beam of ions passing through the machine is detected electrically. Only ion stream B makes it right through the machine to the ion detector. The other ions collide with the walls where they will pick up electrons and be neutralised. Eventually, they get removed from the mass spectrometer by the vacuum pump.

When an ion hits the metal box, its charge is neutralised by an electron jumping from the metal on to the ion. That leaves a space amongst the electrons in the metal, and the electrons in the wire shuffle along to fill it.

A flow of electrons in the wire is detected as an electric current which can be amplified and recorded. As the ions start to build up, the current also increases.

Detecting the other Ions (Streams A and C)

The stream A was most deflected - it has the smallest value of M/Z (the lightest ions if the charge is $+1$). To bring them on to the detector, they should be deflected less by using a smaller magnetic field (a smaller sideways force).

To bring Stream C with a larger M/Z value (the heavier ions if the charge is $+1$) on to the detector they should be deflected more by using a larger magnetic field.

If the magnetic field is varied, each ion stream in turn can be brought on to the detector to produce a current which is proportional to the number of ions arriving. The mass of each ion being detected is related to the size of the magnetic field used to bring it on to the detector. The machine can be calibrated to record current (which is a measure of the number of ions) against M/Z directly. The mass is measured on the ^{12}C scale.

Note: The ^{12}C scale is a scale on which the ^{12}C isotope weighs exactly 12 units.

Applications of Mass Spectrometer

1. Astronomers use mass spectrometry to determine the elements and isotopes found in the solar wind. For example, the mass spectrum of solar wind reveals that the following elements are common, carbon (12 amu), oxygen (16 amu), neon (20 amu), magnesium (24 amu), silicon (28 amu) and iron (56 amu).
2. Environmental scientists use mass spectrometry to detect toxins in contaminated fish. They can also use the technique to measure the amount and nature of airborne particles in the atmosphere, data that can be used to monitor climate change.
3. Biologists use mass spectrometry to identify the structures of complex biological molecules, such as carbohydrates, proteins and nucleic acids.

4. Anaesthesiologists use mass spectrometry during surgery to measure the metabolic gas exchange of their patients. The technique enables them to determine the **respiratory quotient**, the volume of carbon dioxide produced divided by the volume of oxygen consumed, which indicates that the patient's cells are getting enough oxygen and eliminating enough carbon dioxide to remain healthy.
5. Palaeontologists rely on spectrometers for carbon dating, which requires the measurement of carbon-12 and carbon-14 isotopes in a sample to determine the sample's age.

Forensic Applications of Mass Spectrometer

1. **Toxicology Analysis**

   One area where a mass spectrometer is handy is in cases involving poisons or toxins. Forensic analysts can take samples of a subject's tissues or bodily fluids and determine if any toxic substances are present, and if so, in what concentration. This can give vital clues to investigators as to how a victim died as well as help identify the time and dosage of any poison or medication ingested. Investigators can also determine if a victim was a regular user of any substances that might have contributed to his or her death.
2. **Trace Evidence**

   Mass spectrometry is also useful in analyzing trace evidence. Investigators at a crime scene may find microscopic materials like carpet fibres, glass splinters or paint flakes.

   Ordinarily, these substances might be extremely difficult to use as a starting point to identify a suspect. However, a mass spectrometer can determine the precise mix of dyes used in carpet fibres, the makeup of materials that went into any particular glass fragment and the precise set of polymers present in any paint sample.

   This information can lead investigators to a particular manufacturer, who may be able to narrow down where a given sample came from, helping detectives to identify suspects and build a case.
3. **Arson Investigations**

   Arson investigations can also benefit from the use of mass spectrometry. While an arson investigator might be able to identify the use of an accelerant through burn patterns or lingering odours, a mass spectrometer can break down any residue

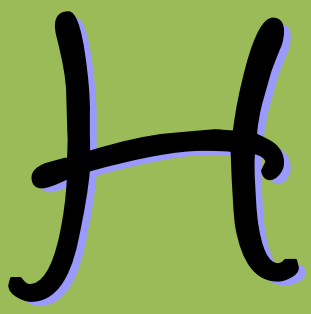
and provide an accurate report of its molecular makeup. This can help identify any unique or exotic compounds that may be present. Discovering a similar mix used at multiple crime scenes may be useful for identifying the work of a serial arsonist.

4. **Explosive Residue**

Another area where spectrographic analysis is extremely useful is in analyzing explosive residue. When a bomb detonates, it may not leave behind much in the way of physical evidence - perhaps only small fragments and chemical residues.

However, commercial explosive manufacturers utilize their own unique mix of chemicals, and a spectrometer can analyze this residue to identify the particular composition of the explosive involved. Even in cases where a bomber used a homemade mix, the analysis may identify the type of materials used and give investigators a push in the right direction to identify the source.

Forensic analysis of explosive residues is difficult because explosives are almost completely destroyed during the explosion. Specialized MS techniques allow the detection of tiny amounts of explosives.



Electron Microscope

Mansi Singare

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Riya Ganer

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Electron Microscope

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. Electron beam also follows the properties like light radiation for example wavelength, amplitude, intensity, frequency, absorption, transition. Hence, the electron beam can be used in microscopy in place of light. This examination can yield information about the surface features of an object, shape and size of the particles making up the object, composition and structure of various samples.

Construction of Electron Microscope

The different components involved in the magnification of an electron microscope are:

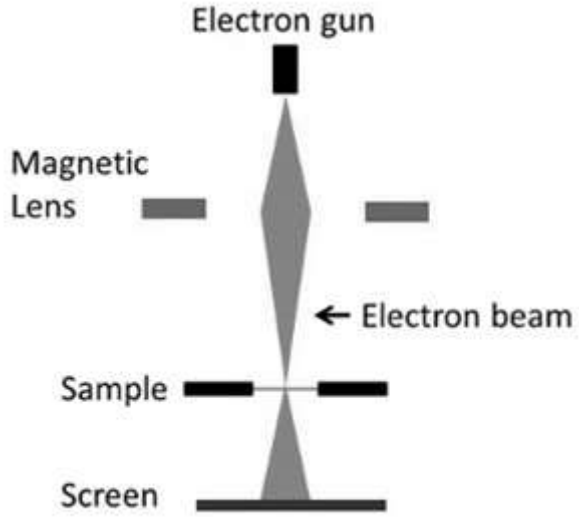

Fig. H1: Schematic of an Electron Microscope

1. **Electron Source**

   It is also known as an electron gun. It produces a sharp, narrow and thin collimated beam of electrons that has precise kinetic energy. A metallic cylinder is placed around the source to control the spreading of electrons.

2. **Electrostatic lens**

   When the electron beam passes from one electrostatic field to another, the path is charged. It guides the electrons emitted from the source to the electron analyzer. The cylinders of different potential produce electric lines differently, leading to the acceleration of electrons. The electrostatic lenses are designed in the same way as

optical lenses so that they can easily magnify or converge the electron trajectories. An electron lens can be used to focus an ion beam.

3. **Magnetic lens**

   Magnetic lens helps to converge the electron beam by the use of magnetic Lorentz force (combination of electric as well as magnetic force on a point charge due to electromagnetic fields). The magnetic coils applied with different current causes the convergence of electrons.

4. **Intermediate Screen (S1)**

   The specimen is focused on the intermediate screen and passes through it.

5. **Fluorescent Screen (S2)**

   The final image is focussed on the fluorescent screen or photographic plate. Due to the absorption of the specimen examined by the X-rays it is not always possible to obtain images directly visible to the human eye, hence the fluorescent screen enhances the intensity of the image.

6. **Vacuum Chamber**

   The entire chamber is kept inside a high vacuum chamber where no air and gases are present. These gases are removed with the help of a vacuum pump.

Scientific Principle and Theory of Operation

Electron Microscopes (EMs) function exactly as their optical counterparts except that they use a focused beam of electrons instead of light to "image" the specimen and gain information as to its structure and composition.

The basic steps involved are,

i. A stream of electrons is formed in high vacuum (by electron guns).
ii. This stream is accelerated towards the specimen and also confined and focussed using metal apertures and magnetic lenses into a focussed, monochromatic beam.
iii. The sample is irradiated by the beam and interactions occur inside the irradiated sample, affecting the electron beam.
iv. These interactions and effects are detected and transformed into an image.

When a beam of electrons fall on the surface of specimen, it shows different types of electron rays which consist of X-rays, visible rays, back-scattered electrons, elastically

scattered electrons, inelastically scattered electrons and transmission electrons. Transmitted beam of electrons passes straight through the specimen on the other side and fall on the fluorescent screen and can be observed.

Scanning Electron Microscope (SEM)

Operation

In Scanning Electron Microscope (SEM), a source of electrons is focused in vacuum into a fine probe that is rastered over the surface of the specimen. As the electrons penetrate the surface, a number of interactions occur that can result in the emission of electrons or photons from or through the surface.

A reasonable fraction of the electrons emitted can be collected by appropriate detectors. In this way an image is produced on the CRT. The principle images produced in SEM are of two types: secondary electron images and backscattered electron images. When the energy of the emitted electron is less than about $50\,eV$, it is referred as a secondary electron and backscattered electrons are considered to be the electrons that exit the specimen with an energy greater than $50\,eV$. Detectors of each type of electrons are placed in the microscope in proper positions to collect them.

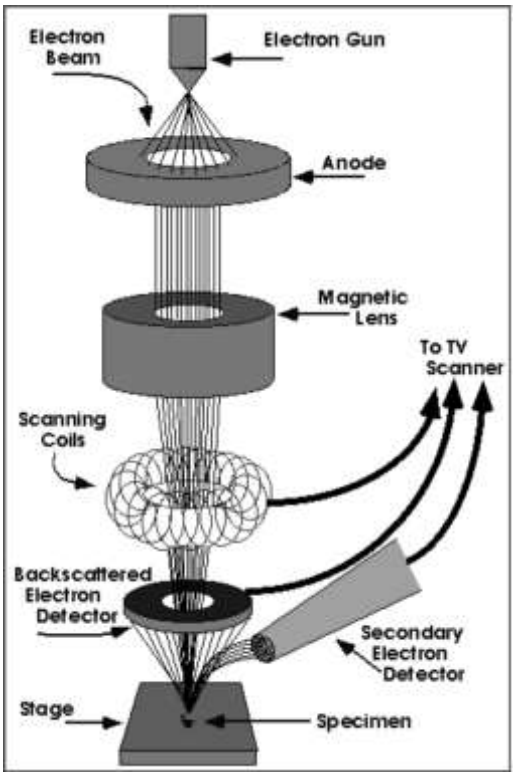

Fig. H2: Geometry of SEM

Transmission Electron Microscope (TEM)

Operation

Transmission Electron Microscopy (TEM) is a technique where an electron beam interacts and passes through a specimen. The electrons are emitted by a source and are focussed and magnified by a system of magnetic lenses. The electron beam is confined by the two condenser lenses which also control the brightness of the beam, passes the condenser aperture and "hits" the sample surface.

The electrons that are elastically scattered consists the transmitted beam, which pass through the objective lens. The objective lens forms the image display and the following apertures, the objective and selected area aperture are used to choose of the elastically scattered electrons that will form the image of the microscope. Finally, the beam goes to the magnifying system that is consisted of three lenses, the first and second intermediate lenses which control the magnification of the image and the projector lens. The formed image is shown either on a fluorescent screen or in monitor or both and is printed on a photographic film.

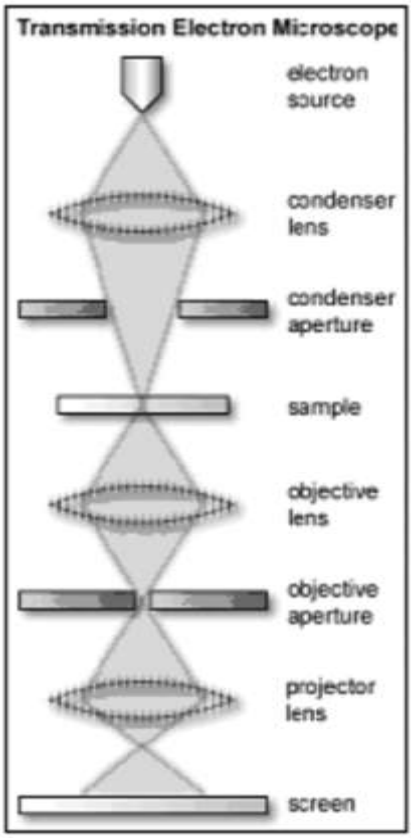

Fig. H3: Geometry of TEM

Applications of Electron Microscopy (in General)

Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, metals and crystals. Industrially, the electron microscope is often used for quality control and failure analysis.

1. Diagnostic electron microscopy
2. Virology
3. Electron tomography
4. Nanometrology
5. Fractography
6. Failure analysis

Applications of Electron Microscopy (in Forensics)

Due to its superior performance, Electron Microscopy is used in a lot of Forensic laboratories for,

1. Gunshot residue analysis
2. Firearms identification (bullet markings comparison)
3. Investigation of gemstones and jewellery
4. Examination of paint particles and fibres
5. Filament bulb investigations at traffic accidents
6. Handwriting and print examination / forgery
7. Counterfeit bank notes
8. Trace comparison
9. Examination of non-conducting materials
10. High resolution surface imaging

Electrophoresis Apparatus

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Electrophoresis Apparatus

Electrophoresis is the migration of charged particles or molecules under the influence of an applied electric field using semisolid or porous medium. The usual purposes for carrying out electrophoresis are,

1. To determine the amount, and mobility of components in a given sample or to separate them.
2. To identify and detect molecules which form electrical double layers about their surroundings.

In modern times, electrophoresis is used as a tool for diverse purposes such as,

i. Determination of molecular weight of proteins,
ii. DNA sequencing
iii. Protein –Protein interaction
iv. Cellular interaction
v. Enzyme interaction

Electrophoresis technique was fully devised by Tiselius in 1937. It is carried out on semisolid or porous supporting medium such as Agar-Agar, Agarose and polyacrylamide gel etc.

Construction

The electrophoresis equipment consists of two components,

1. Buffer reservoir
2. D.C power supply

The buffer reservoir system has an upper and a lower buffer reservoir connected by the gel. Except the gel, there is no other electrical connection between the two reservoirs. Electric current is passed through platinum electrodes to terminals extending from the top of the system. The sample, prepared in a high density component such as glycerol or ficoll to prevent its mixing with the upper reservoir buffer is loaded on top of the gel.

A 'tracking dye' (usually bromophenol blue) is often mixed with the sample. The extent of migration of the dye gives an index of electrophoretic process. The dye migrates

faster than all macromolecules. Thus if the electrophoresis is stopped before or just as the dye comes out of the bottom of the gel, it indicates that all macromolecules are still within the gel.

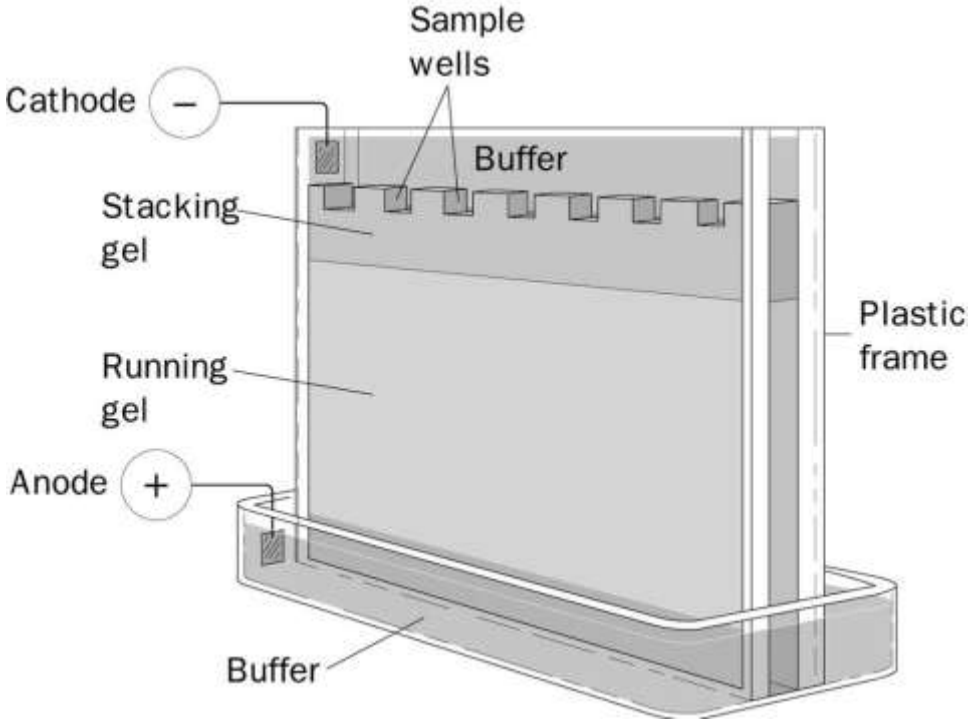

Fig. I1: Schematic of Electrophoresis Apparatus

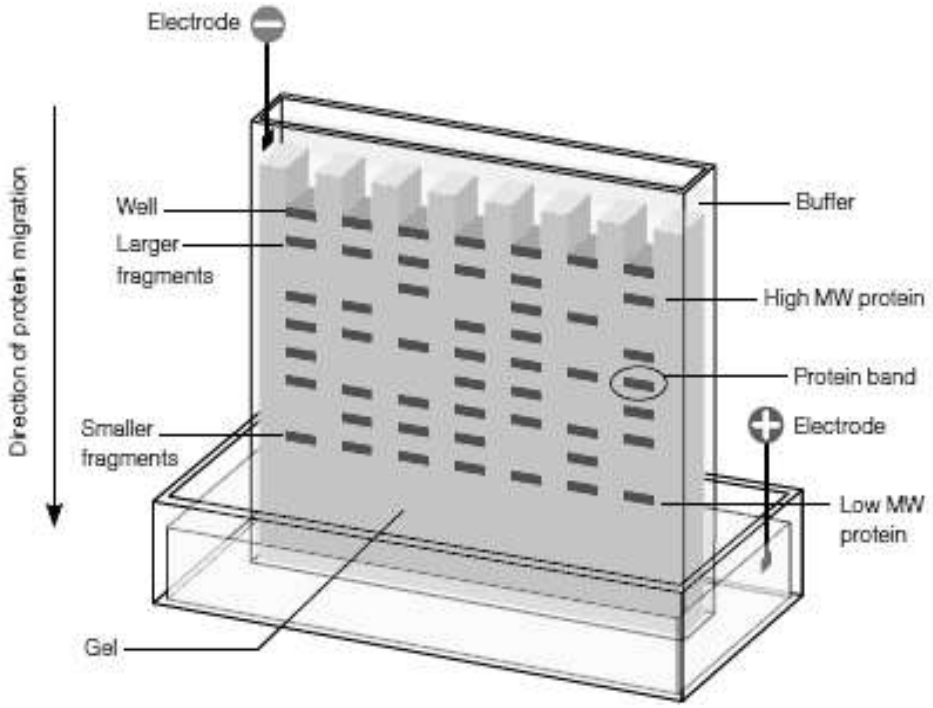

Fig. I2: Protein Migration in Electrophoresis Apparatus

For the separation of macromolecules the pH of the system is fixed at 9, which gives a negative charge to most macromolecules. The anode, to which these negatively charged macromolecules would migrate under an electric field, is therefore placed in the lower buffer reservoir.

When the power is switched on, we can see that the amount of bubbles generated in the reservoir containing the anode is much less than that in the reservoir containing the cathode. The reason for this are the reactions that permit the flow of current from cathode to anode. They are essentially the electrolysis of water producing hydrogen at the cathode and oxygen at the anode.

Principle (Disc Gel Electrophoresis)

In all types of electrophoreses (free electrophoresis, paper and cellulose acetate electrophoresis) charge on the molecule is the major determinant for its electrophoretic mobility and separation from the rest of the molecules. But in gel electrophoresis, the gels are porous and the size of the pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. The separation on gel, thus not only depends on the charge on the molecule but also on its size. And thus resolution of a sample is sharper and better in a gel than in any other type of medium.

Disc gel electrophoresis (so called because of the discontinuous buffer employed and discoid appearance of the macromolecular zones) is a modification of conventional zone electrophoresis. This allows the sample to enter the gel as a sharp band, thereby helping further resolution. Here the macromolecule mixture to be analysed is subjected to an electric field in a retarding gel support that is separated into two sections differing in porosity and having buffers at different pHs.

The macromolecular mixture migrates from the more porous gel into the less porous gel, which is accompanied by a change in pH. As a result, each macromolecule type becomes concentrated into a very thin, sharp band, producing much higher resolution than can be achieved in a continuous buffer.

Working

Glycine is present in the upper buffer reservoir in two forms; as a zwitterion which does not have a net charge, and as a glycinate anion with a charge of -1. When the power is switched on, chloride, protein, and glycinate anions begin to migrate towards the anode. On entering the stacking gel, the glycinate ions encounter a condition of low pH (pH of the stacking gel buffer is about 2 pH units lower than that of the buffer in the upper reservoir) which shifts the equilibrium towards formation of zwitterions.

As zwitterions do not possess a net charge, they are immobile. This immobility of glycine zwitterions to migrate into the stacking gel along with high mobility of the chloride ions creates a very high localised voltage gradient between the leading chloride and the trailing glycinate ions. Since the mobility of proteins is intermediate between the trailing and the leading ions, they carry the current in this region and migrate rapidly in this strong local electric field.

The proteins, however, cannot overtake the chloride ions as the strong local field exists only between the chloride and the glycinate ions. As a result, the proteins migrate quickly until they reach the region having more chloride ions and then slow down. These two different speed movements of the proteins results in piling up of the protein sample in a tight, sharp disc between the glycinate and the chloride ions.

It is in this sharp band form that the macromolecules enter the running gel. The smaller pores of the running gel retard the movement of the sharp band of the macromolecules for a time long enough for the glycinate anions to catch up. Since the running gel pH is higher than that of the stacking gel the glycine ions become fully charged again and the localized high voltage gradient disappears. From this point on, the separation of proteins takes place as in zone electrophoresis. But since the macromolecules enter the running gel as a sharp band the further resolution in disc electrophoresis is sharper than conventional zone electrophoresis.

Applications of Electrophoresis

Apart from separation of protein and other macromolecules, electrophoresis is utilised for a larger number of analytical applications.

1. **Determination of DNA Sequences:** The methods of DNA sequencing are dependent on high resolution polyacrylamide gel electrophoresis. These techniques depend on generating specific sets of radio labelled fragments, each terminating at a particular base. The use of high resolution polyacrylamide gels allows fragments differing by only a single nucleotide to be resolved as a distinct band and the sequence can be deduced.
2. **Southern and Northern Blotting:** The complementary nature of the two strands of DNA provides a unique method to find whether the DNA or the mutation in DNA is present in the sample or not. If the sequence of a portion of the desired DNA is known, one can synthetically prepare a complimentary oligonucleotide, radiolabel it, and make it react with the sample which has been separated by agarose gel electrophoresis and transferred onto a nitrocellulose paper. The paper can then be auto-radiographed. Retention of radioactivity onto the paper shows us whether or not the sequence we are interested in is present. It is also used for northern blotting which is concerned with RNA rather than DNA.
3. **Restriction Mapping of DNA:** During the study of genomic or cloned DNA, it is essential to have some sort of map to differentiate one area from another. One of the techniques of mapping is known as restriction mapping which uses electrophoresis. The technique consists of
    i. digesting the DNA with different restriction endonucleases.
    ii. separating the resulting nucleotide fragments by agarose or sometimes polyacrylamide gel electrophoresis.
    iii. visualizing these fragments while-they are in the gel, by ethidium bromide staining.
    iv. estimating their size in relation to the standards run on the gel simultaneously.
4. **DNA Footprining:** It is used to find out the regions of DNA which interact with proteins and restriction fragment length polymorphism (RFLP), used to detect mutations in various genes in carcinogenesis and other diseases.
5. **Detection of Precursor Molecules:** These molecules are processed (degraded) to give rise to the product mature species. The precursors differ very little in molecular

weight from their products and the resolving power of the gel is the decisive factor in the experiment. Precursors of t-RNA, r-RNA, and m-RNA have all been observed by their different mobilities on high-resolution gels.

Forensic Applications

1. In Forensic science, suspects can be eliminated if their DNA pattern does not match the pattern of DNA molecules found at the crime scene. Other people may become suspects become if their DNA pattern matches the pattern of the person who committed the crime. This helps in following,
    i. **DNA Profile Interpretation:** The primary purpose of forensic DNA profiling is to obtain a DNA 'fingerprint' from a biological sample and compare this to profiles obtained from DNA from a crime scene, an individual or profiles stored on a database.
    ii. **Analysis of Y Chromosome:** One branch of DNA analysis focuses on the amelogenin marker, the only marker on the sex chromosome, useful in the analysis of the Y chromosome. The Y chromosome is generally found only in males. Y chromosome analysis discrimination is comparatively low. Y chromosome analysis is particularly useful in cases of sexual assault and rape in which mixed DNA profiles may be encountered.
2. **Illicit Drug Analysis and Forensic Toxicology:** Electrophoresis is also applied to the analysis of drugs and pharmaceuticals. It has become a tool for illicit drug analysis in both clandestine preparations and biological samples.
3. Electrophoresis is used in the field of forensic and clinical toxicology with a method based on a phosphate-borate buffer, pH 9.1, with $75\ mM$ SDS for the qualitative determination in urine of drugs of abuse.
4. Electrophoresis method used with a buffer of $2.5\ mM$ borate, $25\ mM$ SDS; helps to resolve as many as 11 components of gunshot residues and 15 high-explosive constituents. Application to forensic cases included the investigation of spent ammunition casings, reloading powders and plastic explosives.
5. In the case of home-made explosives (the so called low-explosives), anions and cations left behind from the blast represent useful pieces of evidence to determine the type and source of the explosive mixture used. Moreover, traces of the explosive mixture can be looked for in the environment where the device was assembled and/or on the perpetrator.

6. **Analysis of Pen Inks:** In forensic laboratories, gel electrophoresis is used to investigate ink composition, and classify almost all the components of 17 blue and black ink samples from different manufacturers.

Other Relevant Information

1. When preparing Agarose for electrophoresis, it is best to sprinkle Agarose into room temperature buffer, swirl, and allow it to settle for 1 minute. This allows the Agarose to hydrate, preventing foaming during heating.
2. Loading DNA in the smallest volume possible will result in the sharpest bands.
3. Electrophoresis buffer can affect the resolution if DNA.
4. Migration of DNA is retarded and band distortion occurs when too much buffer covers the gel.
5. Slower migration results from a reduced voltage gradient across the gel.
6. DNA can be preserved in Agarose gel for long term storage in 70% ethanol.
7. Using a hot gel can cause the DNA to denature in the gel. It can also cause the Agarose gel to deform. Cool the gel using a fan during electrophoresis.