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A NEW TECHNIQUE SINGLE CELL MASS SPECTROMETRY-REVIEW

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ABSTRACT

Recent advancements in Mass spectrometry (MS), which is enable the detection of dozens to hundreds of lipids, tiny chemicals, and protein molecules in a single cell. The ability to describe the cellular heterogeneity in the individual cells is crucial for defining the whole spectrum of cell subtypes and determining their function. In this paper, we address single-cell mass spectroscopy, a methodology that allow for thorough proteome & metabolomic profiling of individual cells using both antibody-free approaches as well as high-throughput, focused mass cytometry-based methodologies. The benefits and drawbacks of various approaches are examined, along with the difficulties and chances for additional advancements in single-cell Mass Spectrometry.

Keywords: Cellular Heterogeneity, Lipidomics, Mass Cytometry, Mass Spectrometry, Metabolomics, Proteomics, Single cell analysis

INTRODUCTION [1, 2]:-

Mass spectroscopy (MS) is a method of analysis for figuring out the masses of particles and the chemical make-up of a sample or substance. Mass is measured by

mass spectrometers. In proteomics, mass provides details regarding the structure, chemical changes, and identification of the protein.

The multiplexed investigation of peptides, lipids, proteins and metabolites in individual cells is possible using the label-free approach of MS. Thanks to recent developments in the science of mass spectrometry (MS), tens to hundreds of proteins, lipids, and minute molecules may now be found in a single cell. To define the complete range of cell subtypes and determine their function, it is essential to be able to characterise the molecular heterogeneity in individual cells.

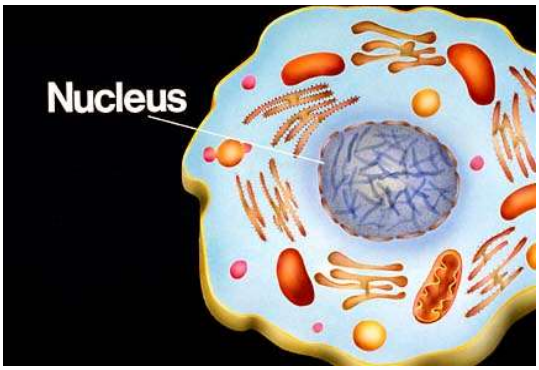

Figure 1

The purpose of single-cell mass spectrometry [3]

Small metabolic bio-molecules created by cells include amino acids, lipids, carbohydrates, and others. These biomolecules have the capacity to provide crucial information regarding the ongoing biological processes in cells.

To understand the structural & functional roles in cells, it is therefore required to investigate and analyse the metabolites & components in each cell. MS is a good method for single-cell investigation combining qualitative, quantitative, & spatially resolved analysis to the molecular level because of its minimal sample consumption and many detections.

History [4]:-

The examination of cell-cell reactions, genomics, transcriptomics and proteomics, and metabolomics at the level of single cell is referred to as single-cell analysis. The idea of single-cell analysis first emerged in 1970. Prior to discovering the heterogeneity, single-cell analysis often referred to the observation or modification of a single cell among an enormous number of cells utilizing an optical or even an electronic microscope. Scientists can observe mechanisms that are not visible when examining a large population of cells

because of the variances present from both eukaryotes and prokaryotic cell populations. High-throughput individual cell partitioning technologies enable the simultaneous molecular study of hundreds of thousands unsorted individual cells. Single-cell analysis may offer useful information for research projects in the field of biological science, medical research, pharmacological studies, pathology, toxicology, etc. Single-cell mass spectrometry is commonly called the tiniest scale in the world, not due to its size but due to the small size of the things it weighs. The MS methods high detection potential & low sample consumption make it appropriate for single-cell investigation at the cellular level. However, single-cell analysis may be quite difficult due to the tiny size of every cell as well as the wide diversity and incredibly low amounts of chemicals found in individual cells. With its excellent sensitivity and selectivity, mass spectrometry has a lot of potential as an effective single-cell analysis tool.

Approaches To Mass Spectrometry

1. ESI-MS, or Electrospray ionization with mass spectrometry
2. SIMS, or secondary ion mass spectrometry
3. LDI-MS, or Laser Deposition/Ionization Mass Spectrometry
4. ICP-MS, or Inductively coupled plasma mass spectrometry

Esi-Ms Or Electrospray Ionization With Mass Spectrometry [5, 6, 7]:-

Electrical energy is used by ESI to help ions move through the solution towards the gaseous phase prior to mass spectrometric measurement.

Large, polar molecules including proteins, nucleic acids, and peptides can be analysed using this frequently used ionization approach in mass spectrometry.

When a high voltage is supplied to a liquid that is flowing through a capillary, it dissolves the liquid meniscus, which results in a minute spraying of charged droplets. As the droplets evaporate, polar molecules that dissolved in the liquid are ionized and transferred into the gas phase. The term “electrospray ionization” (ESI) is used to describe this event. Mass spectrometric analysis (MS), which is a method that can be used to identify the molecular weights & chemical structures that compose gaseous ions. With the aid of ESI, polar analytes such as viruses, macromolecules like synthetic and natural polymers, and low molecular-weight compounds like peptides, amino-acids, and nucleotides can all be ionisable.

ESI-MS has developed over the last three decades into a potent instrumental technique that (bio) chemists can use for a variety of applications.

Ionic species are removed by ESI through the solution to gas state in three steps:

- A light mist of charges droplets disperses.
- In the event that the solvent goes exhausted,
- Highly charged droplets release ions.

A spray of highly charged particles will be produced using identical polarity that defines the capillary voltage. Using a nebulizing gas that shears around the drained sample solution can increase sample flow rate. After being produced from the electrospray tips output, the charged droplets move along a potential gradient and a pressure gradient towards the mass spectrometer's analyser region.

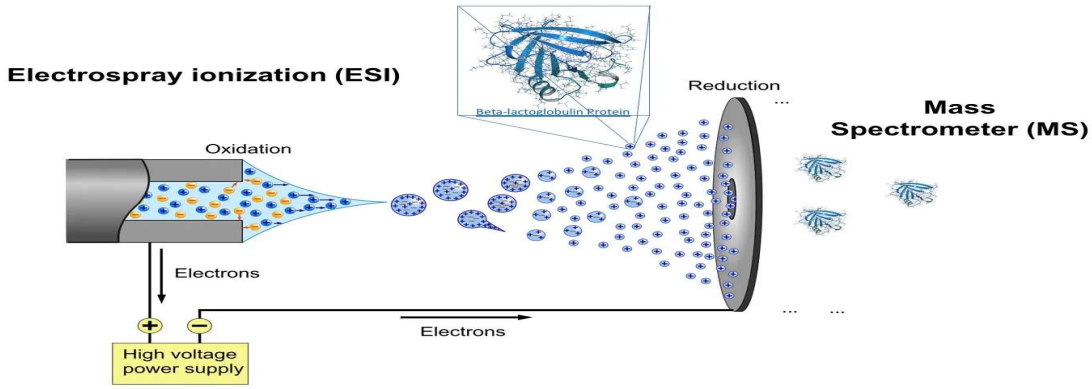

Figure 2

Components Of Esi-Ms:-

1. Ion source
2. Desolvation system
3. Mass analyser
4. Detector
5. Data analysis software.

Ion Source- It is composed of high-voltage power source, a metallic capillary, and a gas source for the nebulizer. Once the sample has been injected using a syringe into a metallic capillary, high voltage is applied. Using nebulizer gas, liquid samples are divided into tiny droplets.

Desolvation System- It is responsible for removing the solvents which are present in the droplets and producing gas phase ions. This can be achieved by combining of heating, vacuum and gas flow.

Mass Analyser- It separates the ions based on their mass-charge ratios. TOF, Quadrapole, and ion trap are mass analysers.

Detectors- commonly used detectors are Electron multiplier tube, Faraday cups, Micro channel plate detector.

Sims Or Secondary Ion Mass Spectrometry [8, 9]:-

In SIMS process, primary gallium ions move secondary ions and molecules from the atomic layers on the surface. The quadrupole mass spectrometer detects up these secondary ions as a mass-to-charge ratio. A large number of electrons are

generated this sputtering process in addition to the secondary ions, and these electrons can be employed to create a physical image of the sample surface similar to what is seen in a SEM.

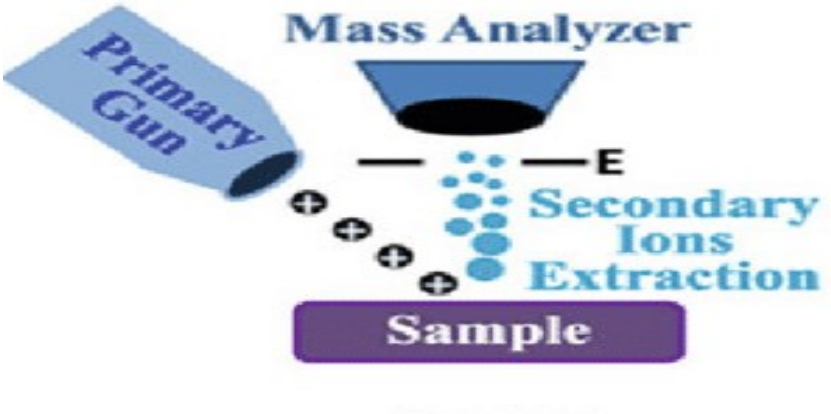

Figure 3

High vacuum with pressures below 104 Pa is necessary for SIMS. This is necessary to prevent background gas collisions between secondary ions and the detector as well as to prevent measurement surface contamination from background gas particle adsorption.

A secondary ion mass spectrometer is made up of the following components: A high-vacuum samples chamber that holds a sample and the secondary ions extraction lens a primary ion column which accelerates as well as concentrates the ion beam onto the sample, a mass analyzer that separates the ions according to their mass-to-charge ratio, as well as a detector.

Ldi-Ms, Or Laser Deposition/Ionization Mass Spectrometry [10]:-

The analyte is ionised by a laser during laser desorption mass spectrometry. In rare circumstances, the sample can be directly exposed to the laser. It will ionise the molecules after removing them from the material's surface. Because there is very little parent ion breakdown, molecular ions are constantly dominant. In some cases, the laser cannot directly desorb compounds, in these situations a matrix is utilised. The procedure is then referred to as MALDI.

The LDI-MS method was also used to asses low molecular weight samples such as lipids, amino-acids, micro-molecule metabolites, and other tiny compounds. LDI techniques can be applied in matrix-free and matrix-assisted laser desorption/ionization procedures. No extra matrix is required for the plant cell analysis because there are greater number of pigmented cells there which are capable of absorbing light and make it simpler to ionize chemical compounds within the cells. Animal cells,

however, require the extra matrix for their examination.

Frequently, the target molecules cannot simply absorb the irradiation of a laser at a particular wavelength. In order to address this problem, a second drug is included in addition to the target organism in order to hasten ionization. This method is known as MALDI-MS.

Icp-Ms, Or Inductively Coupled Plasma Mass Spectrometry [11, 12]:-

The six fundamental divisions of the single quadrupole ICP-MS are the sample administration, the Inductively-Coupled Plasma, the interface, ion optics, the mass analyser, and detector. Liquid samples are first nebulized by the sample administration mechanism to create a fine aerosol, which is then given to an argon plasma. These ions are then collected via the interface region and placed through a series of electrostatic lenses termed as the ion optics.

Figure 4

The important information on cell-to-cell variance between each cell is made possible by the ICP-MS technology. The substance is atomized, leading to the creation of atomic and smaller polyatomic ions that are later identified. Then, the ionized sample is divided according to its charge in relation to a reference and indicated readings. A mass spectrometer is used to assess these ions after that. An inductively linked plasma is used in the mass spectroscopy technique known as ICMS to ionize the sample. The material is atomized, resulting in the production of atomic and minute polyatomic ions that are later discovered.

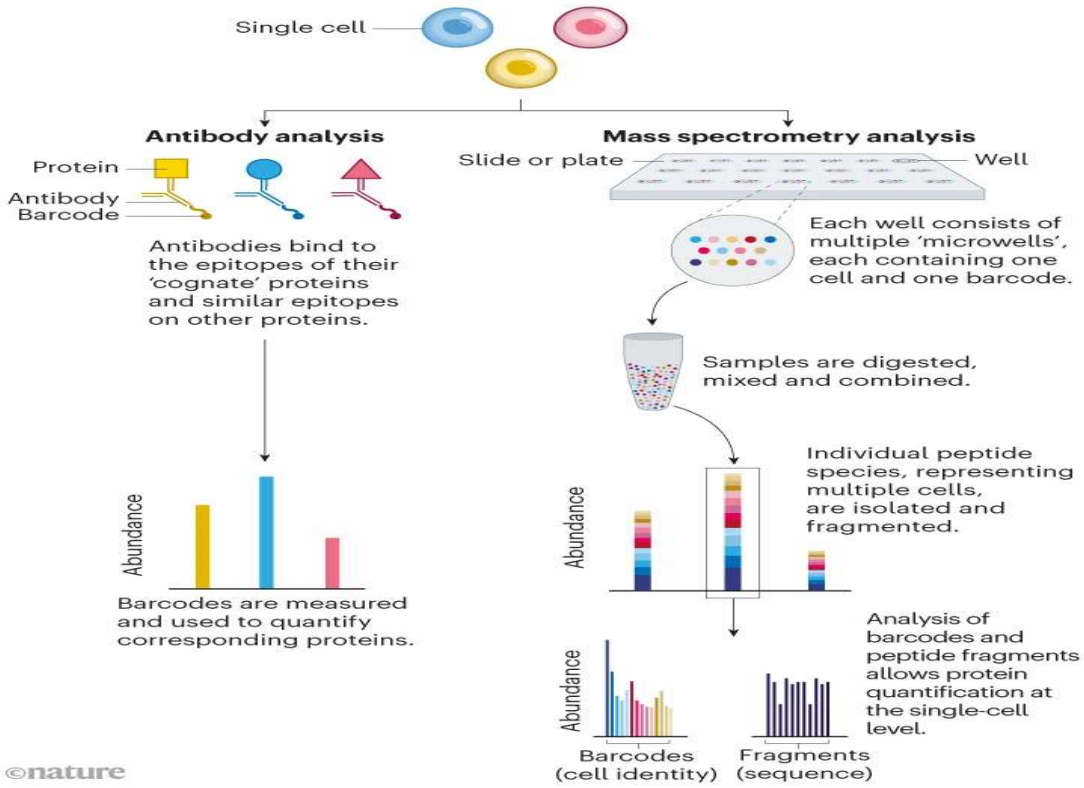

Figure 5 [13]

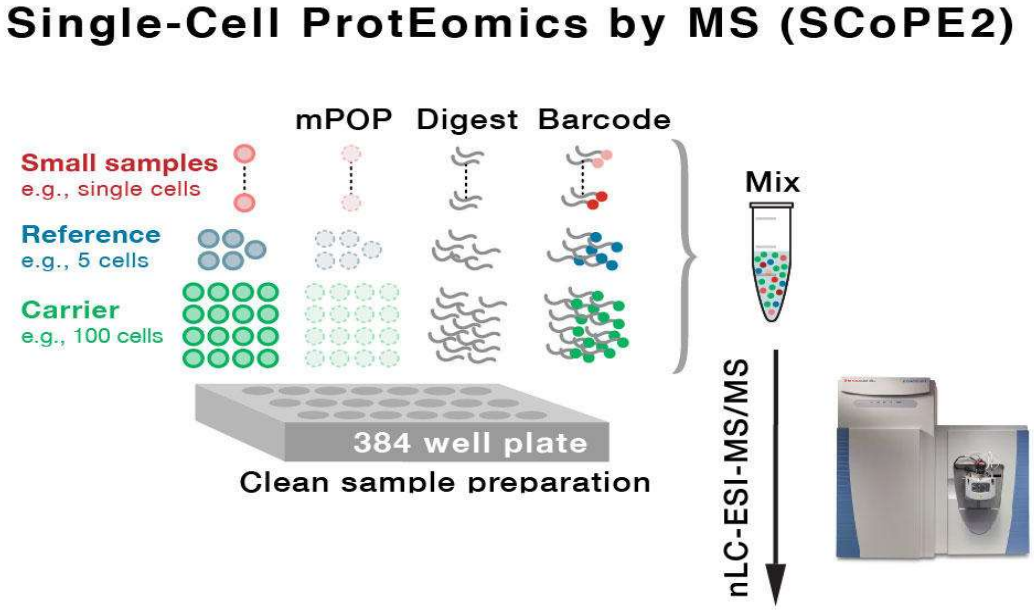

Figure 6

In the single cell proteomics, the process involves: collect the different sample cells like single cell, 5-10 reference cells and 100 of carrier cells. Digest all sample cells separately and give the barcode to each single cell with different colours then mix all the samples. Then pour the mixed sample cells into the 384 well plate. Keep well plate in the mass spectrometer to analyse the samples.

Sample preparation, peptide/protein separation, ionization, and tandem MS analysis are typically steps in the protein analysis by MS process **[14]**.

Sample Preparation- It includes sample collection, Sample extraction, Sample clean up and sample introduction. The sample may be taken from a variety of places, including tissues, bodily fluids, or environmental samples. It could be necessary to extract after the sample has been collected. Liquid-liquid extraction, solid-phases extraction, and protein precipitation are examples of common extraction techniques. Following sample extraction, cleaning may be done to get rid of contaminants. The sample is then placed inside the mass spectrometer for examination. Direct injection, liquid chromatography are all methods of sample introduction.

Peptide/Protein Separation- The separation can be done by two ways: 1. Liquid Chromatography-Depending on the chemical characteristics, divided complex mixtures into distinct components. Prior to doing a mass spectrometry analysis, it is utilized to separate the proteins. 2. Capillary electric filed. It is used to separate the peptide based on their charge and size.

Ionization- EI, MALDI, SIMS, and laser desorption are examples of commonly used ionization techniques.

Tandem Ms Analysis- Used for the identification and quantification of molecules.

Single Cell Mass Spectrometer

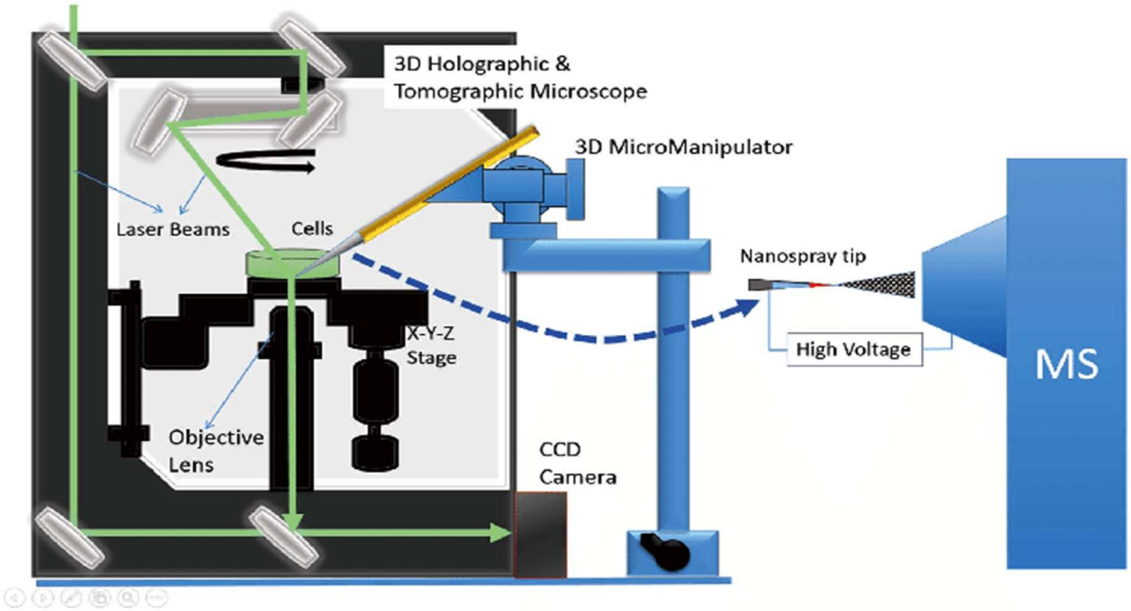

Figure 7: Schematic diagram of Single-cell Mass spectrometer

RESULTS AND DISCUSSION

Analysis Challenges With Single-Cell Mass Spectrometry [15,17,19]:-

Figure 8

Delivering Proteins:-

Delivering the MS detectors receive an adequate number of copies of ions allow for precise quantitation.

Sequence Identification:-

Reliable ion quantification by amino acid sequencing.

Increasing Throughput:-

Economical expansion of the inquiry to thousands of individuals of individual cells.

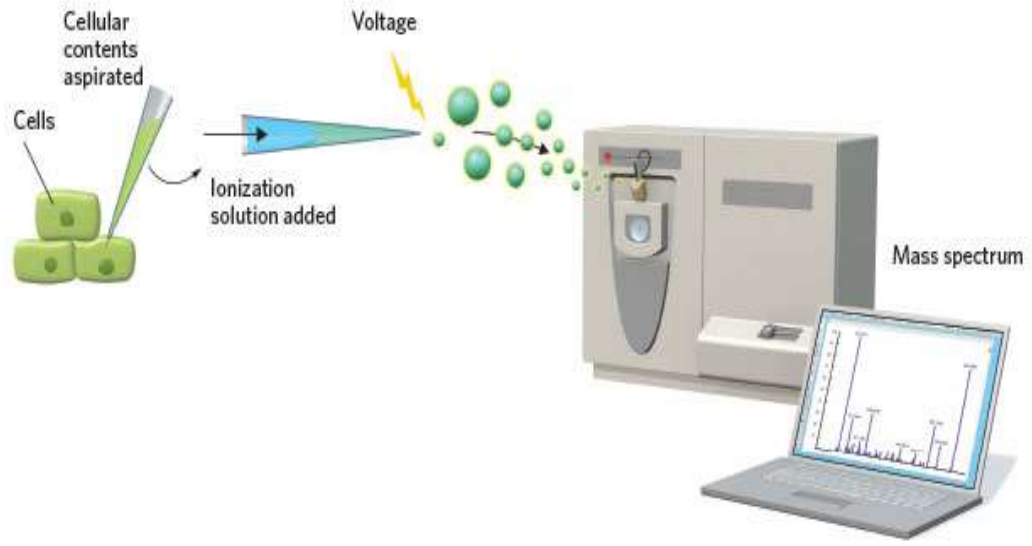

Figure 9

Case Studies

Lsc-Ms, Or Live Single-Cell Mass Spectrometry [20]

The LSC-MS, or live single-cell mass spectroscopy technique is shown schematically below. Patients with colorectal and stomach cancer had their blood samples taken. Circulating tumour cells (CTCs) were enriched using a microfluidics method. The LSC-MS method was used to sample and analyse individual CTCs. Red blood cell; EDTA, ethylenediaminetetra acetic acid; PCA-DA, principal component analysis-discriminant analysis. The CTC samples were enriched before being centrifuged at 500g about 10 minutes, aspirating the supernatant, and resuspending in 300 L PBS. Following reconstitution, the CTC solution sample was moved to a Cells Imaging Dish. Under the microscope, a single CTC was picked out and sucked onto a cellomics tip. Utilizing a piston syringe and a micromanipulator. A single lymphocyte was also drawn into a tip similarly as a control. Morphology was used to choose individual CTCs and lymphocytes. Later, the samples were stored in a refrigerator at 80^{o}c for a subsequent MS analysis. Before an organic solvent was introduced through the cellomics tip's end, the collected samples were thawed. Mass spectrometry measurements were performed.

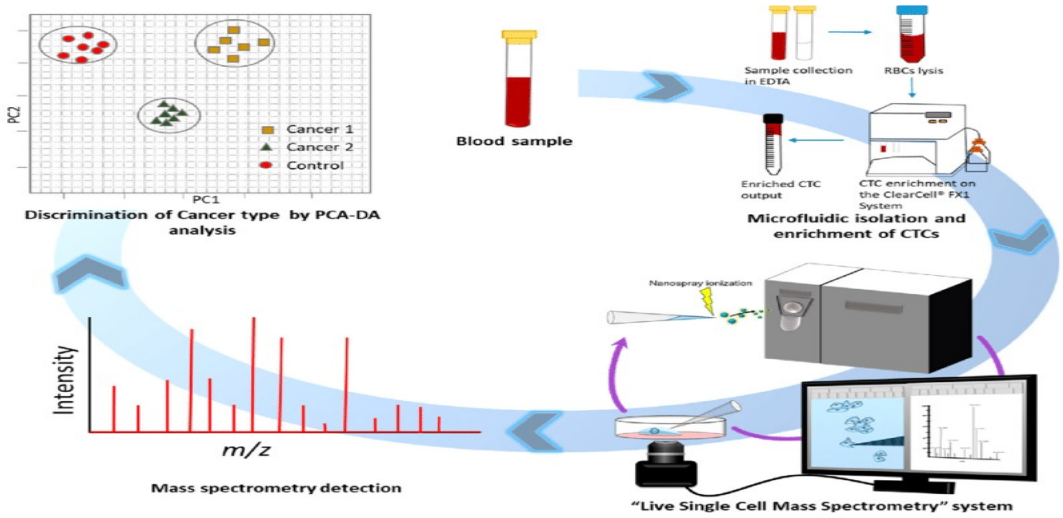

Figure 10

Plant Single-Cell Mass Spectrometry Analysis [27, 28]:

The components of a single target cell are directly suctioned up using the metal-coated glass capillary called as a "Nano spray tip" under a stereo microscope, and the contents are then directly fed into the input channel of a mass spectrometer. The mass spectrometer can identify hundreds or thousands of molecular peaks in a matter of minutes. These peaks may then be compared to databases to identify whether metabolites were found in the plant cell under the particular circumstances it was subjected to at the time the contents were extracted.

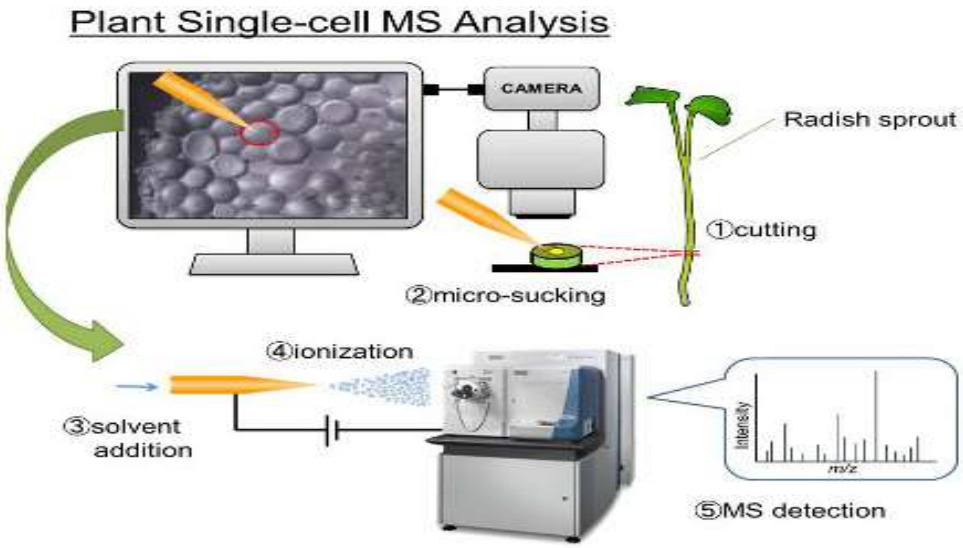

Figure 11

Scms For The Expression Of Lipid And Metabolites [30]:-

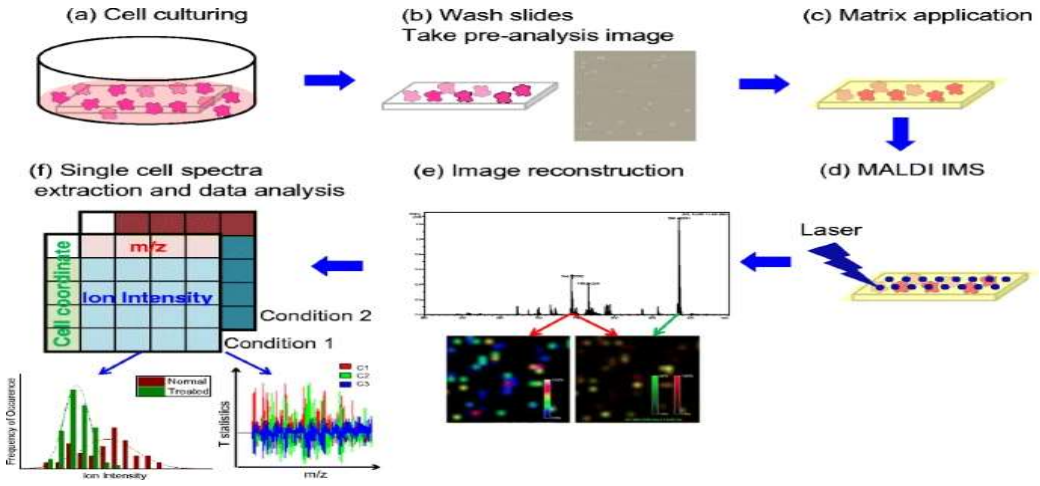

Figure 12

Using imaging mass spectrometry, single-cell analysis was carried out. On optical slides, cells are grown under various growth circumstances to see how they impact the cellular expression of one single cell. After removing the glass slide from the culture media, the subsequent cells are washed to remove any residual culture medium. The slide scanner is utilized to take pictures of the cells in order to record their relative location on the plate. The sample target is subjected to MALDI matrix. MALDI-IMS captures mass spectrum at each distinct location by rastering a laser across the entire cell population. The optical picture is registered to the MALDI ion image. Extractions are made using mass spectra which have been obtained from single cells. To make additional analysis and visualization easier, each of the ion abundances are removed.

Success Factors For Single-Cell Mass Spectrometry:

- It uses a tiny sample size,
- Is quicker, and
- Can distinguish between isotopes,
- It is the ideal technique for determining if a chemical is present or absent in a particular sample because it is exceedingly sensitive.

CONCLUSION:-

With its great adaptability, good sensitivity, quick processing speed, and wide dynamic range, and capacity to detect thousands of chemicals at once, mass spectrometry is an excellent test for single-cell investigation. Mass spectrometry-based single-cell analysis techniques are ubiquitous and don't need fluorescent labelling or derivatization because it determines the molecular weight

(MW) of every component. As a result, single cell metabolomics is widely used.

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