Introduction to Proteomics
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Lecture 25
Mass Spectrometry: Mass analyzers

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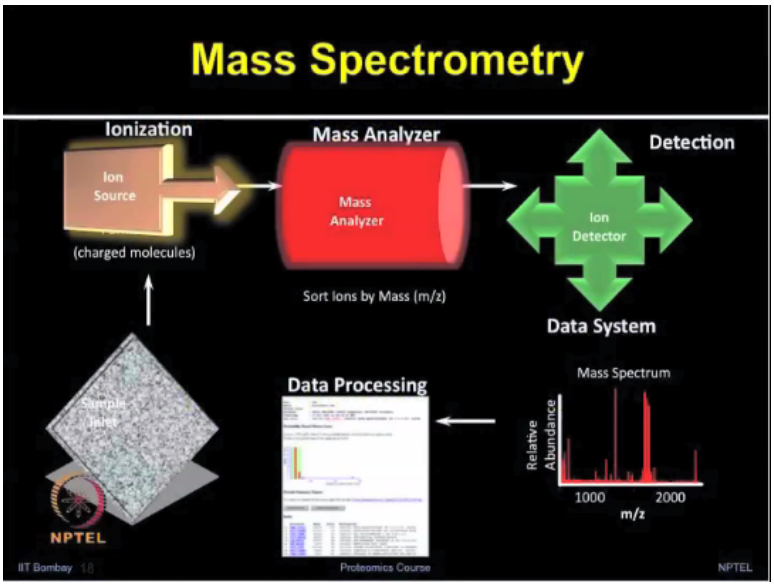

Mass Spectrometry is a technique for protein identification and analysis by production of charged molecular species in vacuum and its separation by magnetic and electric fields based on mass to charged issue. Mass spectrometry has become the method of choice for analysis of complex protein samples in proteome study due to its ability to identify thousands of proteins.

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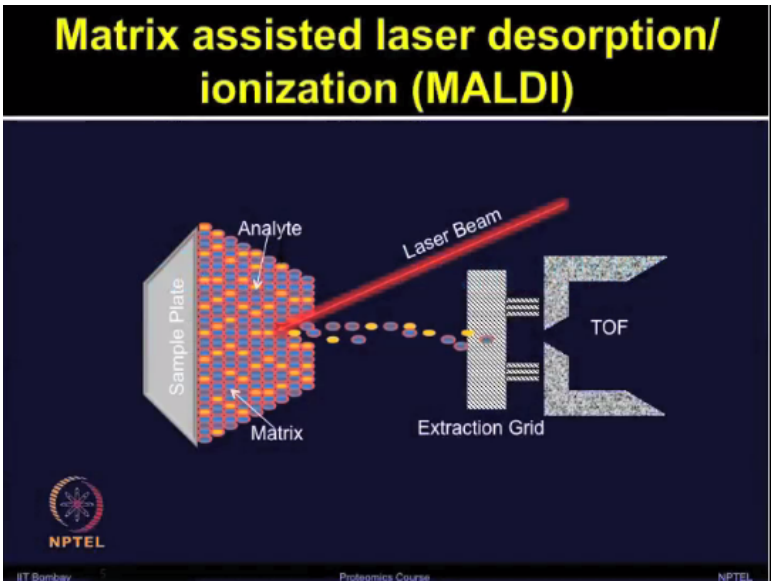

The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum.

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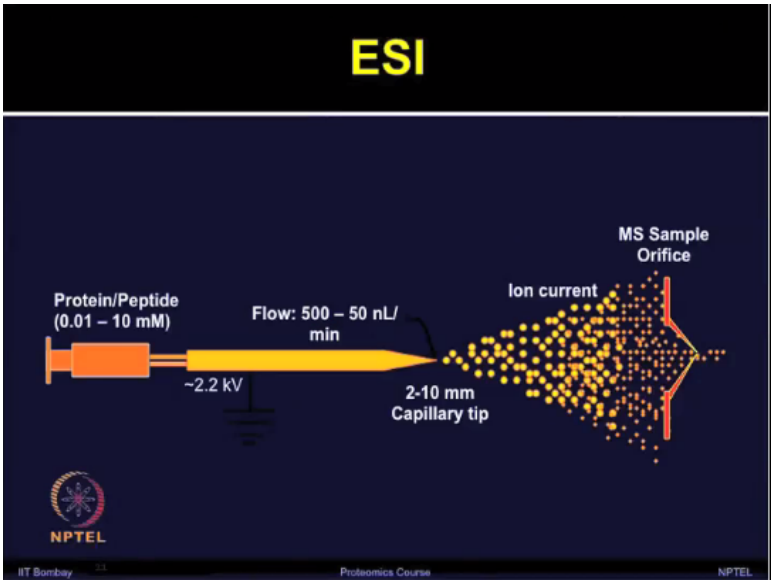

This has been made possible by the development of Soft ionization techniques which ensures that the non-volatile protein sample is ionized without completely fragmenting it. Most commonly used ionization sources are MALDI and ESI.

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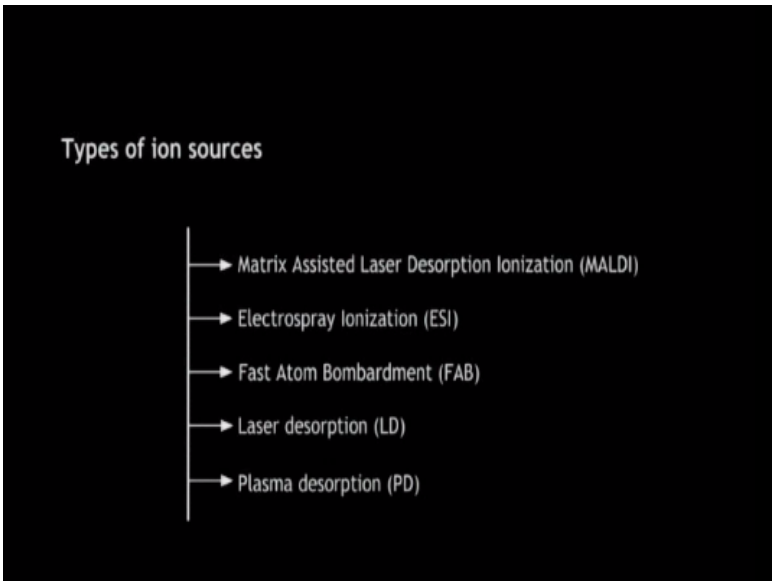

Additionally, there are other ionization sources such as Fast Atom Bombardment FAB, Laser desorption LD, Plasma desorption PD. Let discuss the two most commonly used Soft ionization technique MALDI and ESI.

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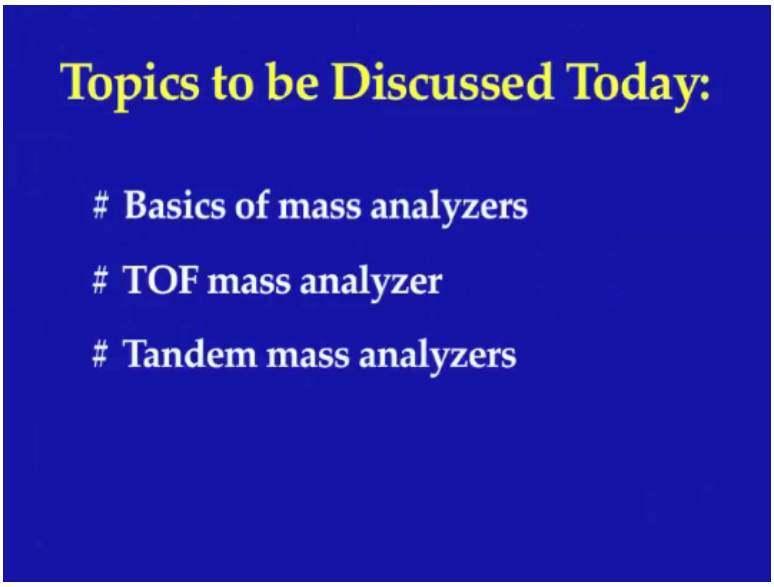

The Mass Analyzers disperses all the ions based on their mass to charge ratio and focuses all the mass resolved ions at a single focal point and maximizes their transmission.

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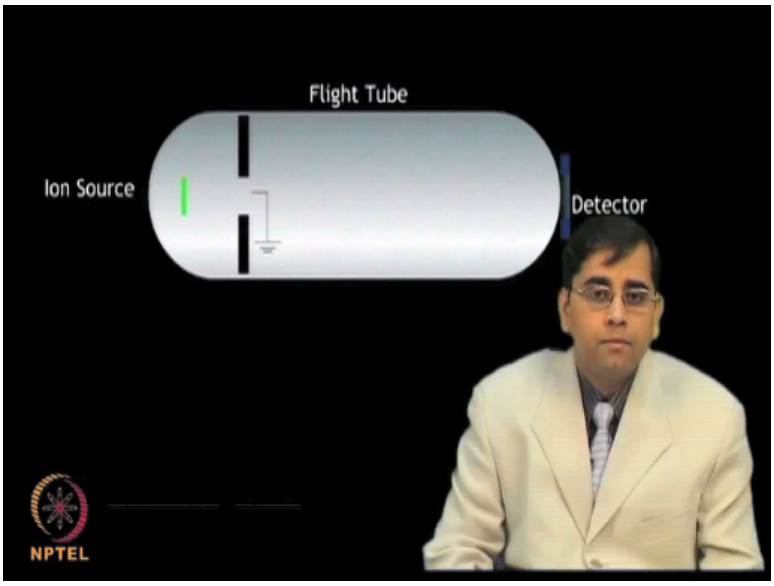

The Time-of-Flight measured M/Z ratio of ions based on the time it takes for ions to fly in the analyzer and strike to the detector.

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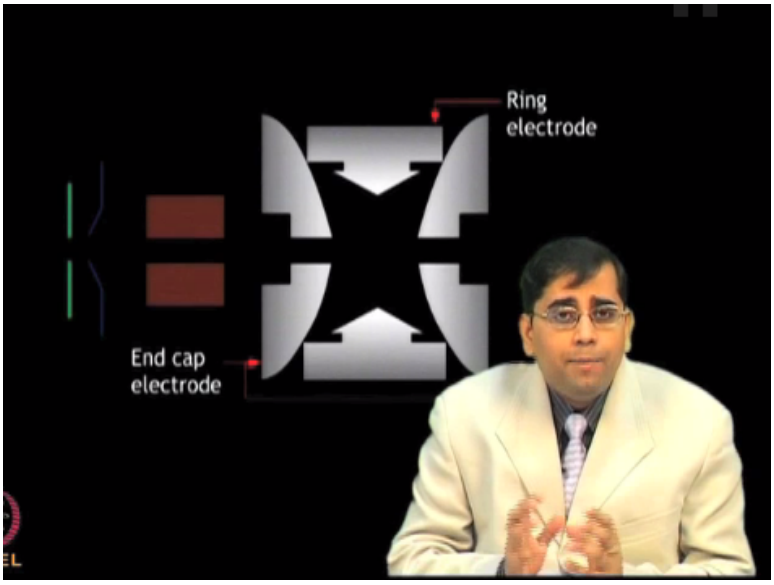

The Ion Trap, it traps ions using electrical fields and measures mass by selectively ejecting them to rejecter.

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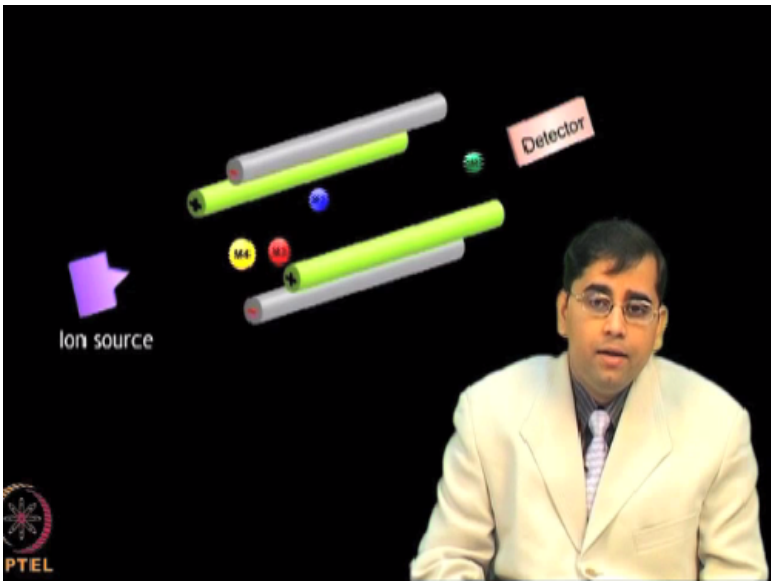

Quadrupole, it consists of four parallel metal rods and mass separation is accomplished by the stable vibratory motion of ions in a high frequency oscillating electric field that is created by applying direct current and radio frequency potentials to these electrodes.

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The different type of mass spectrometers currently available but for proteomics the two configurations which are most commonly or most oftenly used. The Quadrupole time-of-flight or Q-drup based configurations and hybrid linear ion Orbittrap instruments. The TOF configuration separate peptides in time as they reach on the detector so the time-of-flight is measured whereas the Orbitrap mass analyzers they measure frequency of peptide ions which are oscillating in the ion trap.

Now different type of resolution and sensitivity can be obtained from each of these configurations.

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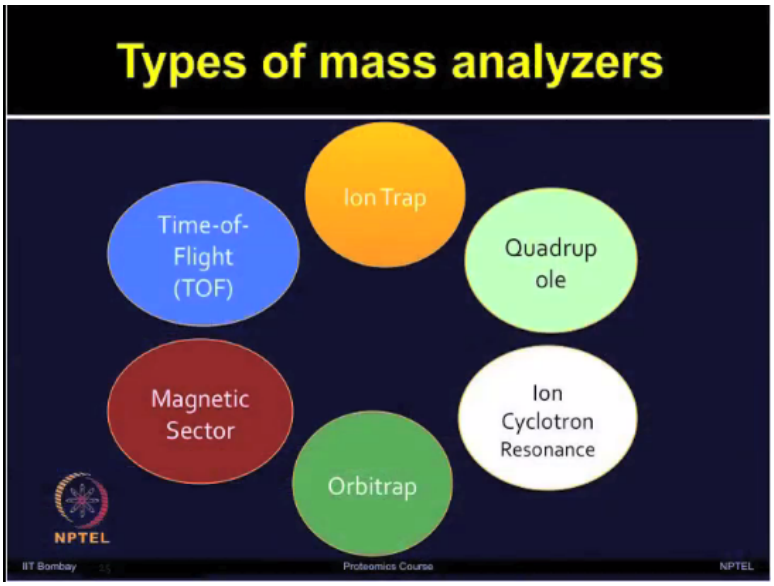

In the previous lecture, I gave you an overview of different type of mass analyzers currently available. Each of those have, its own unique properties in mass range, analysis speed, resolution, sensitivity, the ion transmission, and dynamic range. The time-of-flight analyzers use Time-Flight, Ion Trap, Orbitrap and Ion Cyclotron resonances this separate ions based on their mass to charged resonance frequency whereas Quadrupole or cube they use oscillating electrical field for selective stabilization of ions.

This just gives you an overview of various type of mass analysis and briefly we discussed about their principles.

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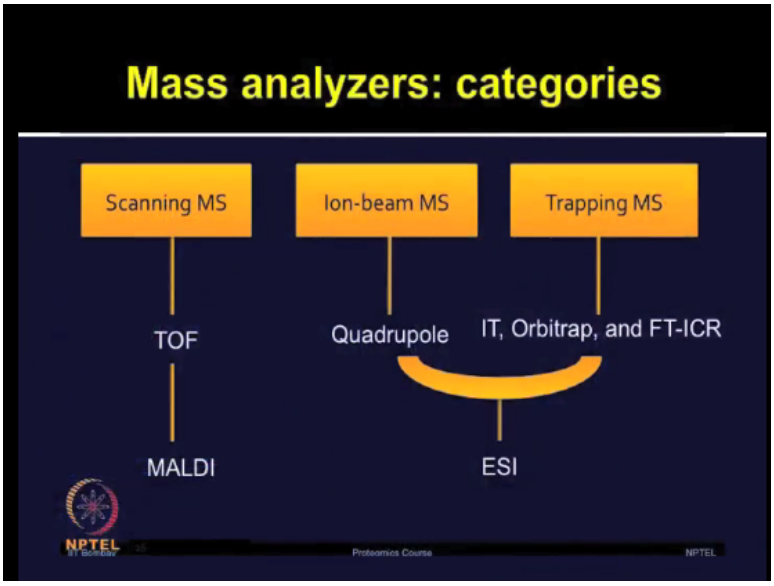

Now mass analyzers can be categorized broadly into the scanning MS, Ion-beam MS and Trapping MS. Scanning MS is more commonly use with the TOF which further coupled with the MALDI ionization sources. The Ion-beam MS is commonly used for the Quadrupole whereas trapping MS for Ion traps, Orbitraps and FT-ICR, all these can be coupled with the Electrospray ionization ESI.

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First of all, the important mass analyzers let discuss a little more detail. First talk about Time-of-Flight which is one of the simplest mass analyzer currently used in combination with the MALDI. The TOF has immersed as one of the main stream technique for the analysis of bio-molecules and it is widely used for various applications.

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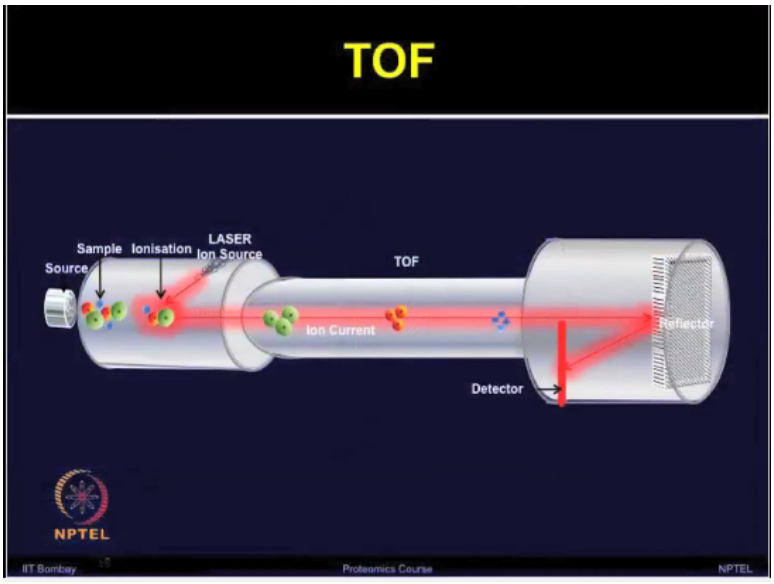

In TOF, the ions are accelerated to high kinetic energy and due to their different velocities they are separated in a flight tube. One can also use the reflectron mirror so that ions can turnaround into a reflected and it can compensate for minor differences in the kinetic energy and provide long separation. Another commonly used mass analyzer is Quadrupole. The instruments are one of the most widely used type of mass analyzers currently used in proteomic.

It consists of 4 mesh parallel mesh metal rods and mass separation is accomplished by the stable vibratory motion of ions in a high frequency oscillating electric field that is created by applying direct current and radio frequency potentials to these electrodes.

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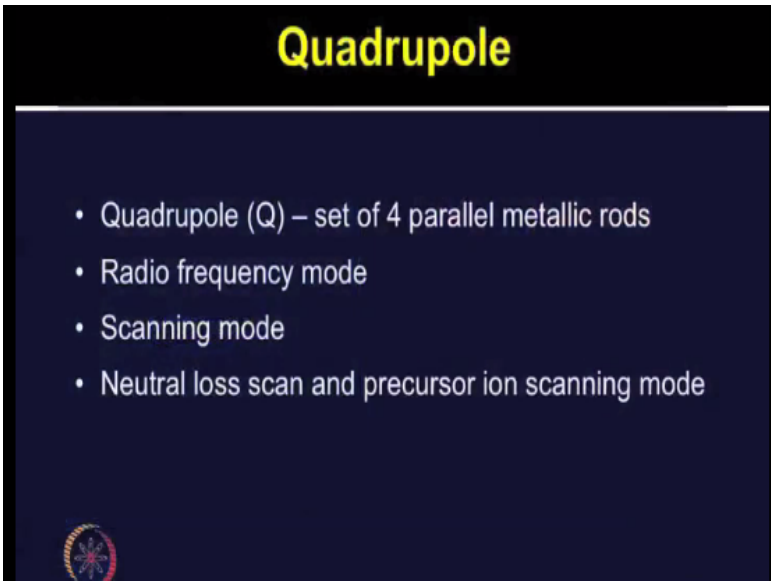

So as talked Quadrupole is set of 4 parallel metallic rods with opposite players electrically connected. The different modes one can use of this analysis RF or radio frequency mode which allows ions of any M/Z ratio to pass through scanning mode, ions of selected mass by charge can be allowed by the detector the potential difference applied and instrument can be used as a mass filter.

The Neutral loss scan and precursor ion scanning method they are used for the phosphor relation to distinguish the phosphorylated and non- phosphorylated peptides.

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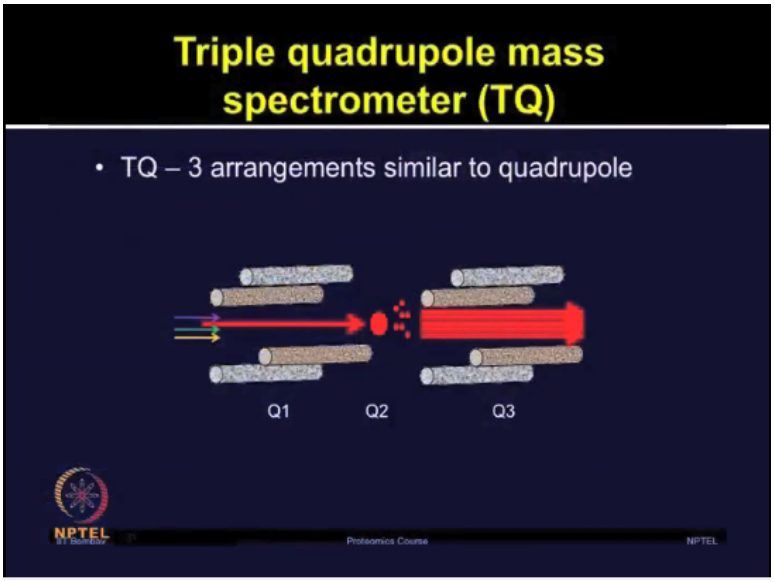

Now Triple quads, which is arrangement of Quadrupoles is widely use for the proteomics. In triple quad the Q1 scans ions it streams, it direct ions of a selected M/Z ratio into the second quadrupole, Q2 which is the collision cell. As you can see in this slide the collision cell operates in the radio frequency mode, the fragmentation of intact peptide ions can be induced by colliding with inured gases and then selected ions are further moved into the Q3.

Q3 scans the stream of ion fragments which are emerging from the collision cell to generate a collision induced dissociation spectrum. The mass spectrum of fragments drives from one peptide after one analysis complete. Then Q1 direct a different intact peptide into the collision cell. So in this sequential manner it can process various peptides.

Now let us talk about another important mass analyzer Ion Trap.

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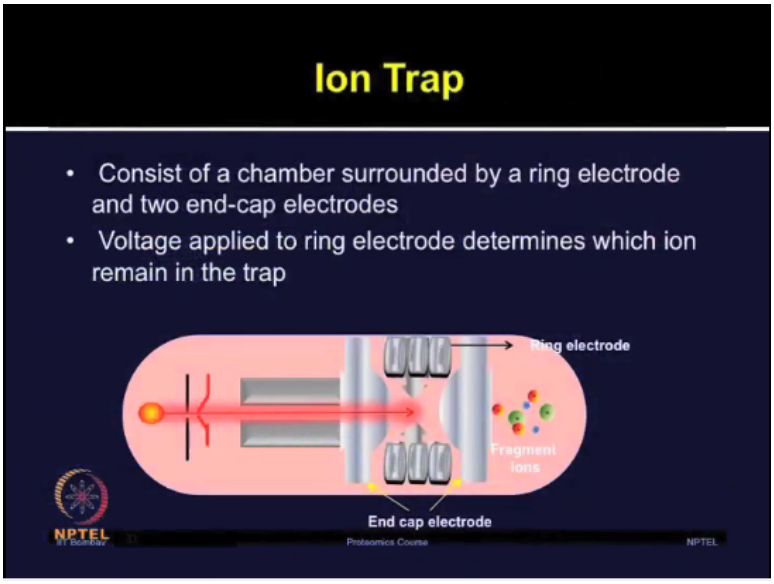

The Ion Trap, traps ion using electrical fields and it measures by selectively ejecting them to a detector. It consists of a chamber which is surrounded by a ring electrode and two end-cap electrodes as you can see in this figure here. The voltage applied to the ring electrode determines which ion remain inside the trap. So ions above a threshold of M/Z ratio they remain inside the trap and others can be ejected through the small hole.

Theoretically, ion trap can provide MS an analysis and it can also provide a mass filter.

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One important mass analyzer is Fourier transform ion cyclotron resonance or FT-ICR. Due to its high resolution and MS/MS capabilities application of FT-ICR MS in combination with electrospray ionization has been employed for the large bio-molecules and now it is also used in the proteomics.

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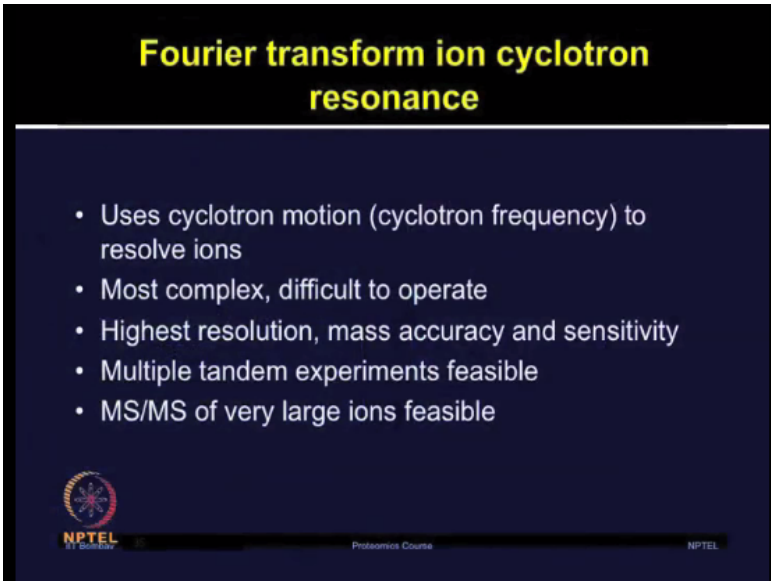

And FT-ICR MS can be considered as an ion trap system where ions are trapped in the magnetic field. It uses cyclotron motion or cyclotron frequency to resolve the ions, although operationally it is complex and not really to operate but it provides highest resolution mass accuracy and

sensitivity. It also provides a capability of multiple tandem experiments and MS/MS of very large ions are possible by using FT-ICR MS.

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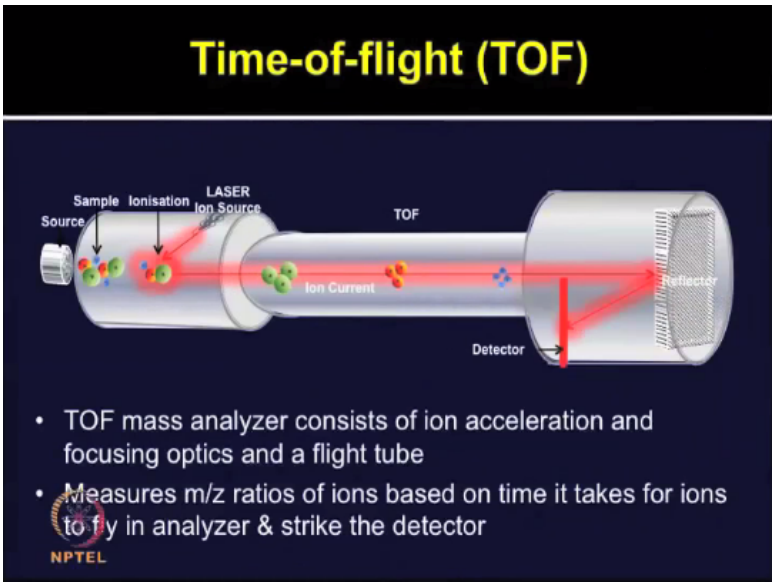

So the TOF mass analyzers, they consist of ion acceleration and focusing optics and a flight tube. As shown in the slide, you have source, the sample ionization is occurring due to the laser beam bombardment then ions are moving in the time-of-flight tube and reaching towards the detector. Now often we can also add the refractor and ion mirror which can increase the path length.

So this time-of-flight tube it measures the mass to charge ratio of ions based on time it takes for ions to fly in the analyzer and strike to the detector. To the mass is exponentially proportional to the flight time, how much time it takes to travel in the time-of-flight tube. So ions of the lower masses are accelerated to the higher velocity. The Time-of-flight tubes often outperform scanning mass analyzers in its sensitivity and scan speed.

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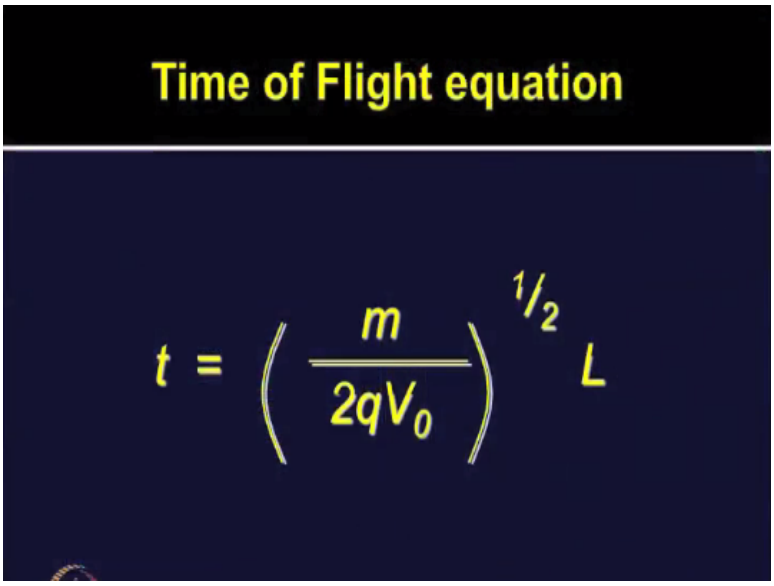

The time of flight of a charged ion can be calculated by using the equation shown in this slide. The flight time is directly proportional to the square root of mass of the ion. Now this equation represents T represents the time-of-flight, m is mass of the ions, q charged on ions, V0 is accelerating potential and L is the length of flight tube.

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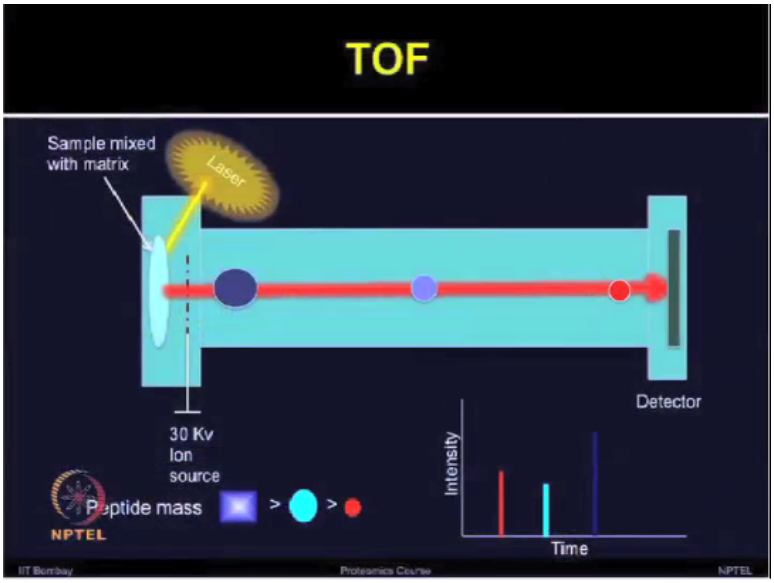

In Time-of-flight tube, the ions are accelerated to high kinetic energy and due to the different velocities they are separated in a flight tube. As I mentioned earlier, by adding the reflect form or a reflected the ions can turnaround in the reflector that can compensate for minor different in the kinetic energy. Now if you take an example where you have three ions as shown in the dark blue, light blue and the red color in the slide.

Now you will expect that the small ion which is the red one will show the first peak followed by the blue ion and then the dark blue one.

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After discussing some of the basic concepts of using MALDI and TOF now let me give you an overview of entire MALDI-TOF experiment by showing you the following animation.

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Fundamentals of MALDI-TOF MS.

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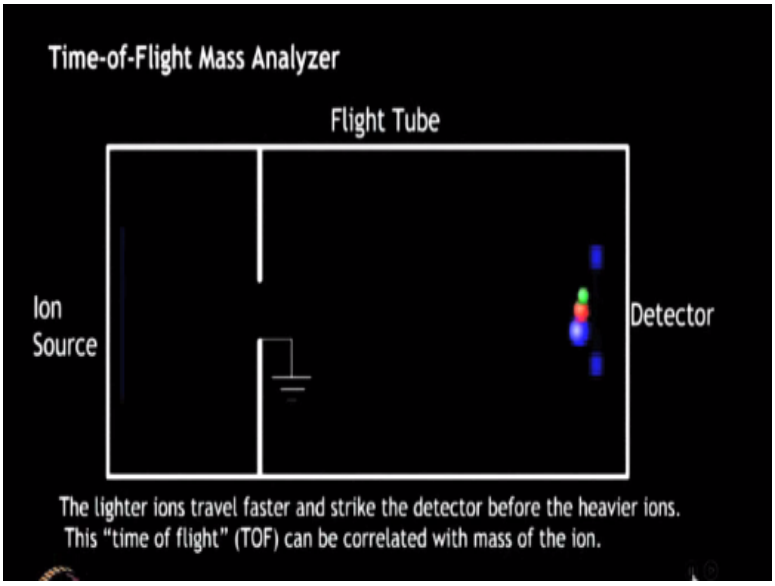

The Time-of-Flight Mass Analyzer resolves ions produced by the ionization source on the basis of their mass to charge ratio. The Time-of-Flight tube can be operated in the linear mode or the reflectron mode which depends on the sample to be analyzed. In case of a small molecules this mode usually provides sufficient resolution. The generated ions are accelerated towards the detector with the lighter ion travelling through the TOF tube faster than the heavier ions.

So the lighter ions travel faster and it strike the detector before heavier ion reaches to the detector. This time of light or the TOF tube can be correlated with the mass of the ion. So the time of flight of the ions can be correlated with the mass to charge issue. As we talked earlier, the TOF analyzer can also be operated in the Reflectron mode.

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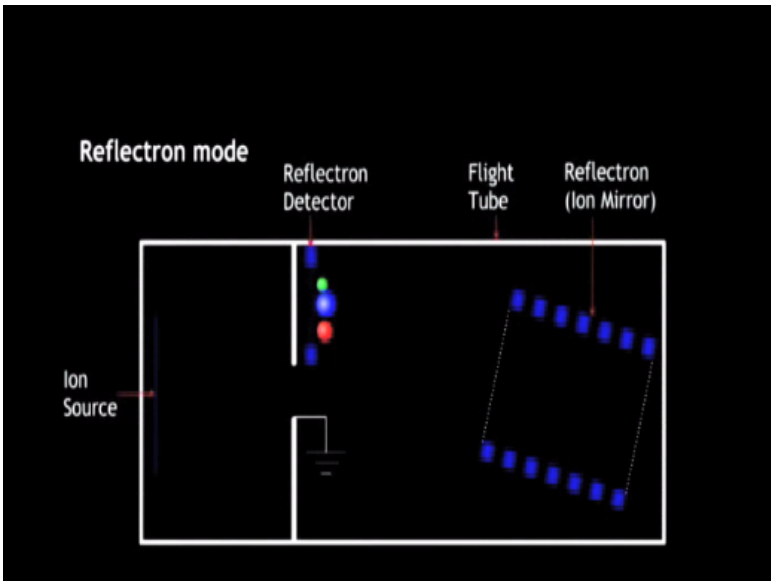

So this is more commonly use for the proteomics studies. A reflectron which acts as a ion mirror it is incorporated at one end of the Time-of-Flight tube. This helps in extending the path length and in turn the flight time of the ion without having to increase the actual size of the instrument. So rather than using very long Time-of-Flight tube by including the reflectron ion mirrors, we can increase the path length.

This helps to even out any kinetic energy differences between ions having the same mass and thereby improving the resolution.

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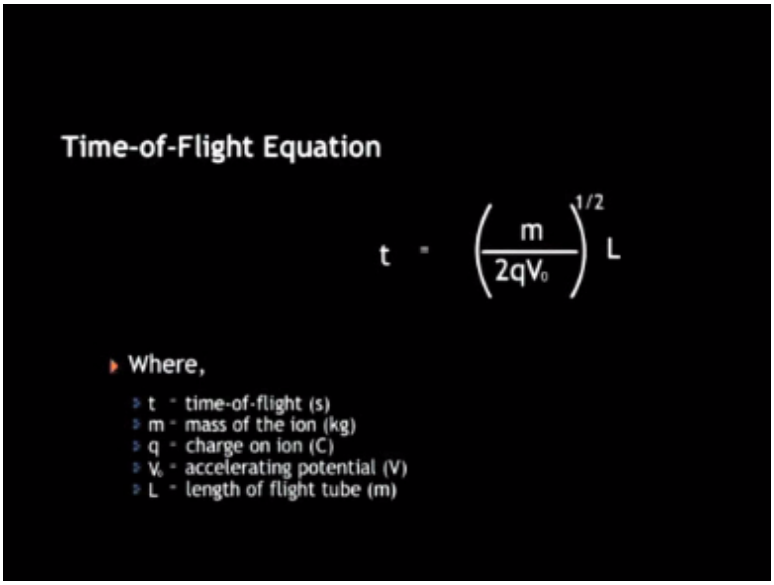

The Time-of-Flight of a charged ion can be calculated by means of a equation shown here. The flight time is directly propositional to the square root of mass of the ion.

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So we have discussed all the important components of liquid chromatography, mass spectrometry. Now, one can apply these configurations in tandem, one can select different type of mass analyzers and use it based on their applications. So now we will look at some of the popular hybrid and MS/MS configurations.

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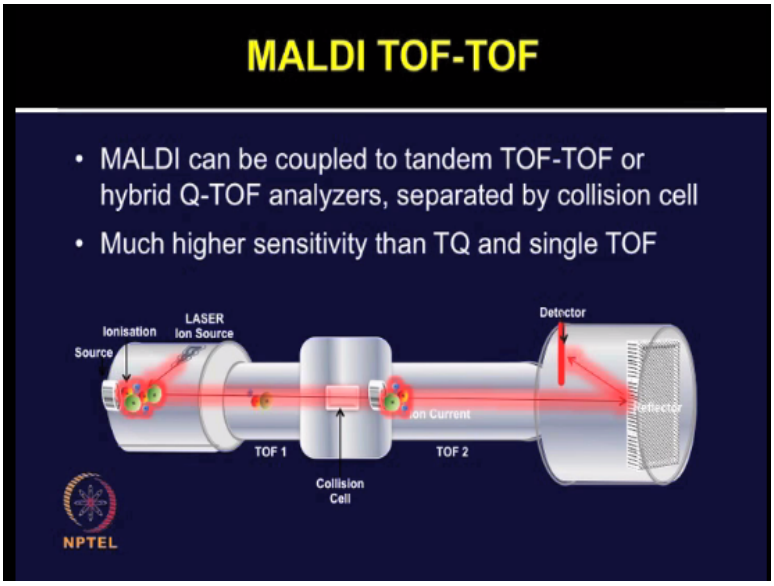

MALDI TOF-TOF that is one of the widely used tandem MS configuration. In this one the TOF-TOF or two time-of-flight tubes as well as hybrid Quadrupole time of flight analyzers can be

used. We have discussed the MALDI TOF-TOF system in some more detail in the previous lecture. Shall move onto some other configurations which is Q-TOF.

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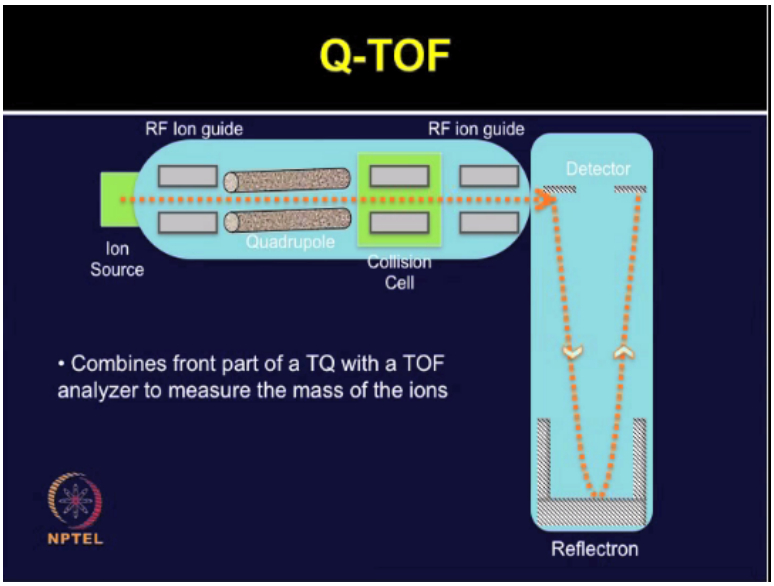

The Q-TOF it combines front part with Quadrupole or it can be triple quad TQ along with the TOF analyzer to measure the mass of ion.

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Some of the important concepts involved ionization, mass analyzers and Tandem MS. I will describe those in following animation.

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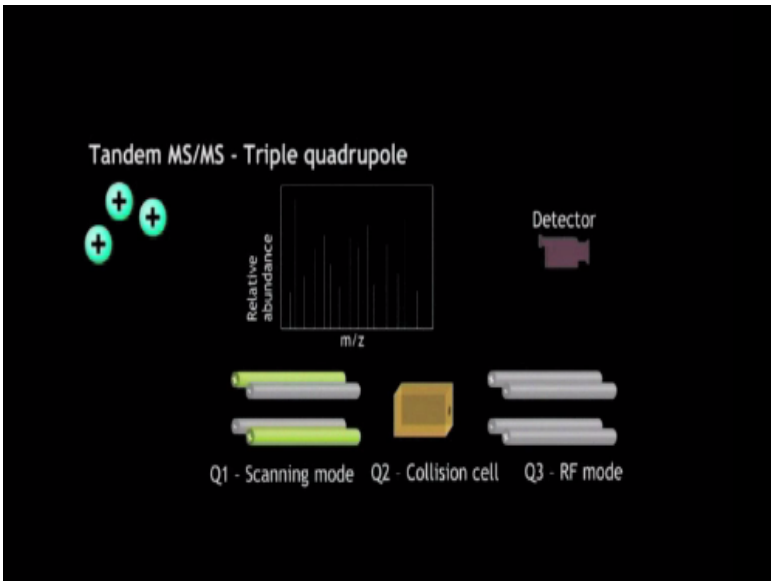

The Triple quadrupole consists of two sets of parallel metallic rods intersperse by a collision cell. The first quadrupole scans the ions coming from the ionization source and allow only ions of a particular M/Z ratio to pass through. Once ions are selected these ions enter the collision cell where they are fragmented by collision against an inner gas like argon.

The smaller fragments then enter the third quadrupole which it can all the ions in radio frequency or RF mode to generate spectrum based on the varying behavior of ions in an oscillating electrical field.

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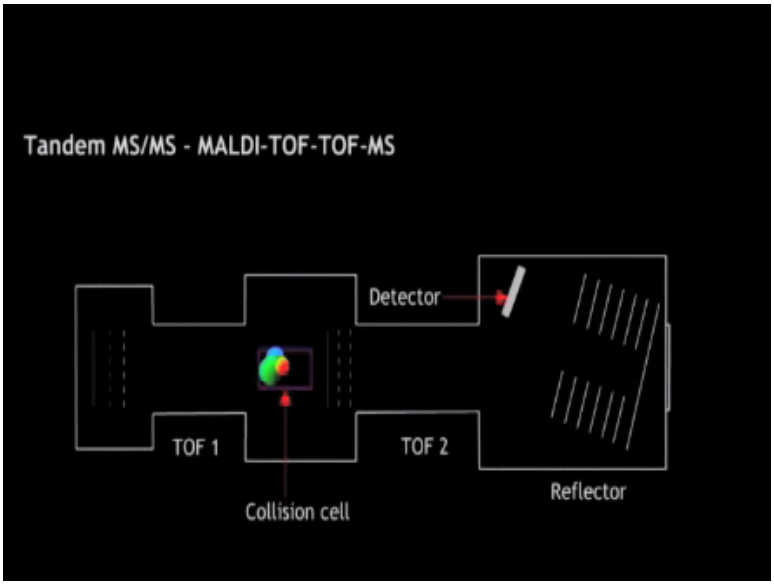

The different type of tandem MS/MS configuration such as MALDI TOF-TOF. The MALDI TOF-TOF MS is as common tandem MS configuration in which the ions are first resolved on the basis of the time of flight in the first TOF analyzer. The selected ions enter the collision cells where they are further fragmented. The fragmented ions are accelerated and further resolved on the basis of their M/Z values in the second time of flight tube after which they can be detected.

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ESI-Q-TOF is another common tandem MS configuration that first selects ions in radio frequency mode. Selected peptides are fragmented in collision cells and resulting ions are accelerated and resolved on the basis of the time of flight.

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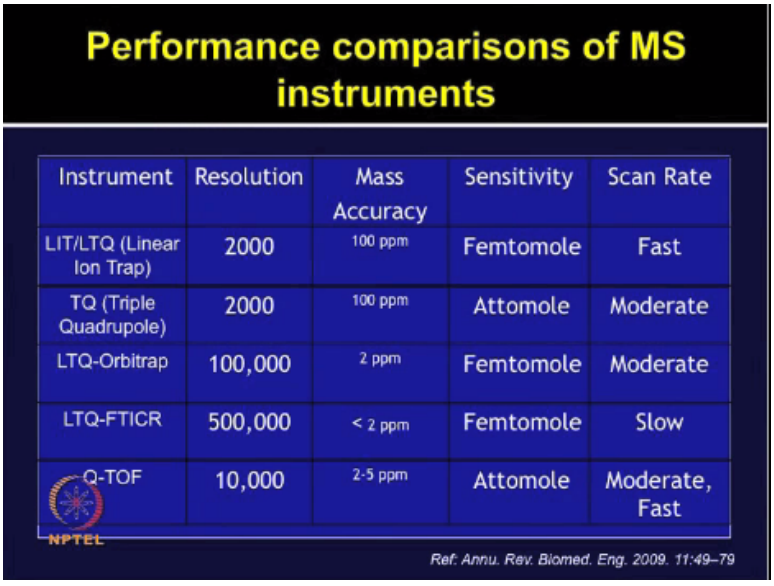

So finally, there are so many mass spectrometers currently available commercially. So now depending on individuals' application one can select different type of configuration. Based on excellent review from (()) (21:52) colleagues I have provided this performance comparison of MS instruments in following slide.

Here you can see the linear ions traps are LID or LTQ they have a resolution of 2000, mass accuracy 100 ppm, Sensitivity Femtomole and scan rate is very fast. The Triple Quadrupoles or TQ with the resolution of 2000; mass accuracy 100ppm; the sensitivity is Attomole and scan rate is moderate. The LTQ-Orbitrap they can provide high resolution 100,000, mass accuracy 2ppm; sensitivity in Femtomole and scan rate is moderate to low.

LTQ-FTICR, they can provide very high resolution of 500.000; mass accuracy < 2 ppm, sensitivity in Femtomole range, slow scan rate. The Quadrupole Time-of-Flight they provide resolution more than 100,000; mass accuracy 2-5ppm; sensitivity in Attomole range and scan rate is moderate to fast.

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