Mass Spectrometry Based Proteomics
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Mod 02 Lecture Number 09

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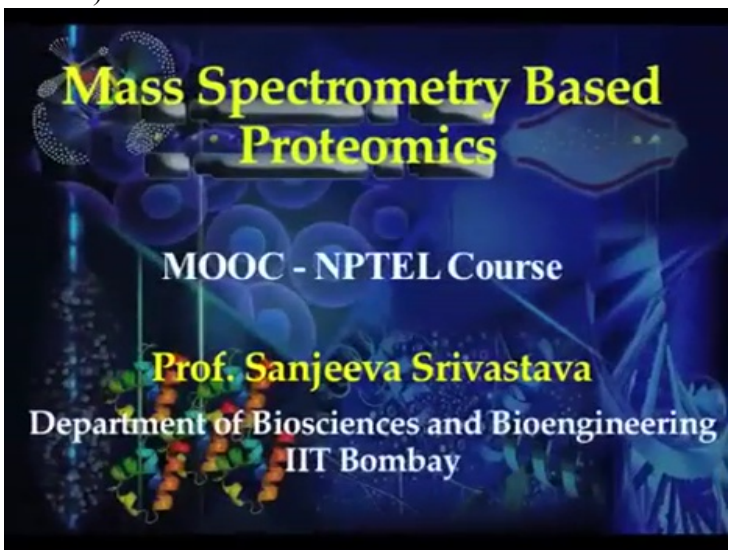

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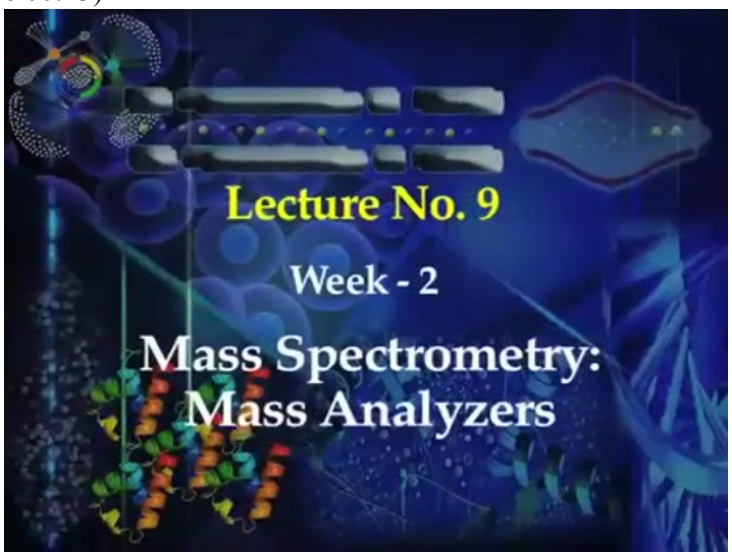

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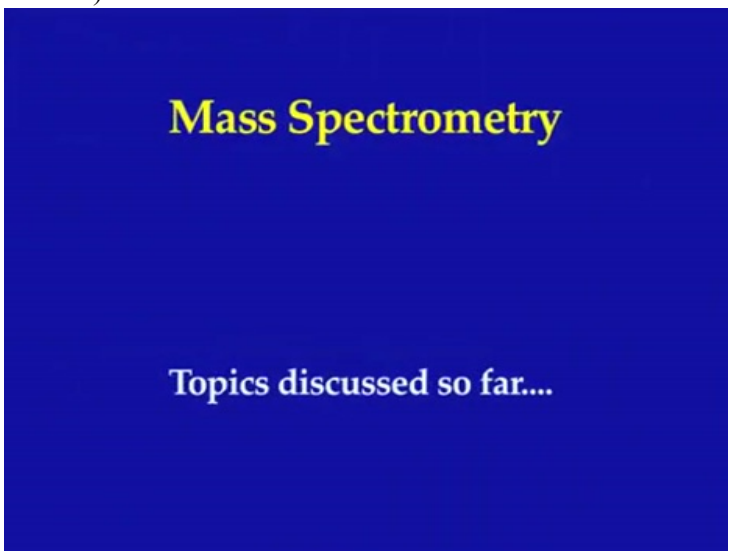

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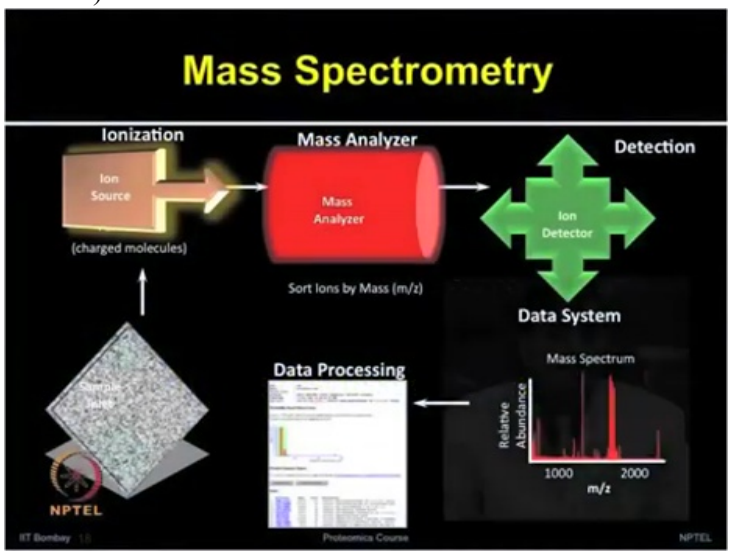

Mass spectrometry is technique for protein identification and analysis by production of charged molecular species in vacuum and its separation by magnetic and electric fields based on mass to charge ratio.

Mass spectrometry has become the method of choice for analysis of complex protein samples in proteomics studies due to its ability to identify thousands of proteins.

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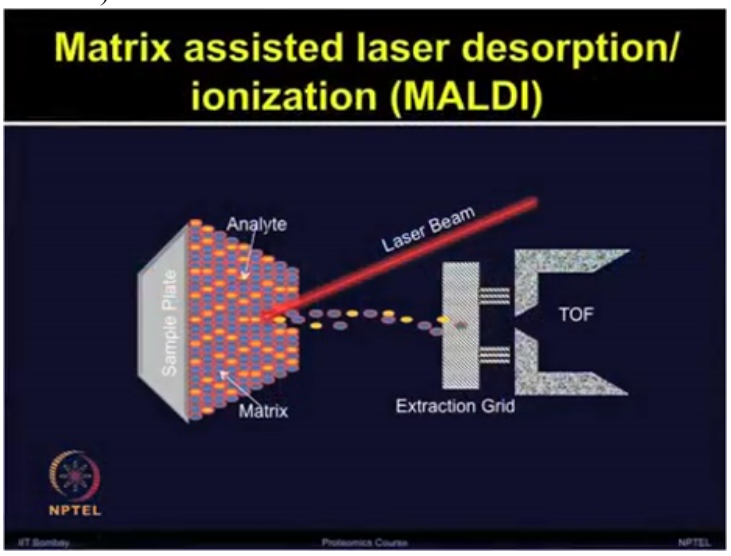

The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum. This has been made possible by the development of soft ionization techniques…

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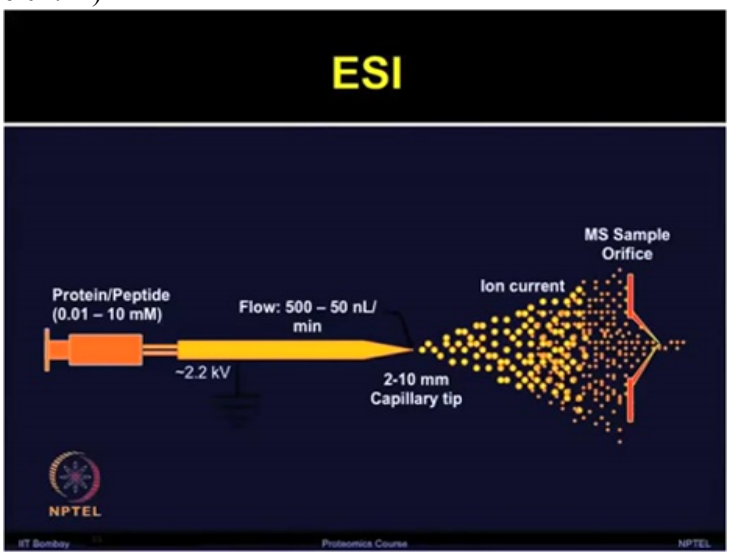

… which ensures that the non-volatile protein sample is ionized without completely fragmenting it Most commonly used ionization sources are MALDI and ESI.

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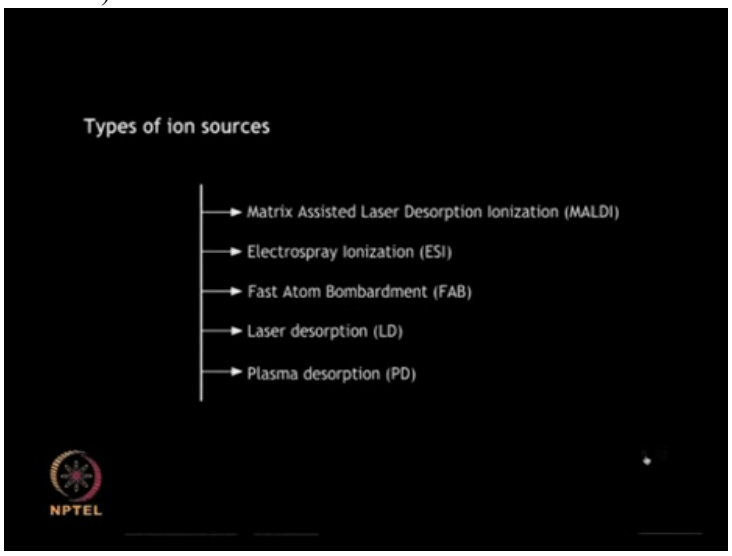

Additionally there are other ionization sources such as Fast Atom Bombardment FAB, Laser Desorption LD, Plasma Desorption PD. We discussed the two most commonly used soft ionization techniques MALDI and ESI.

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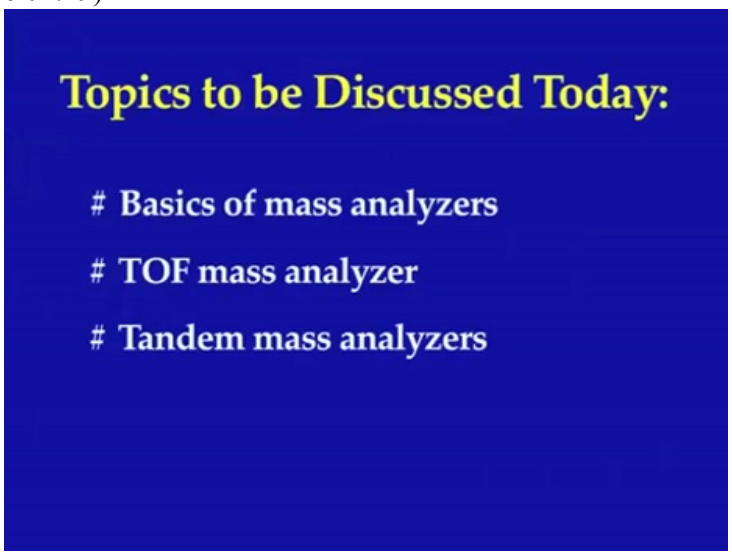

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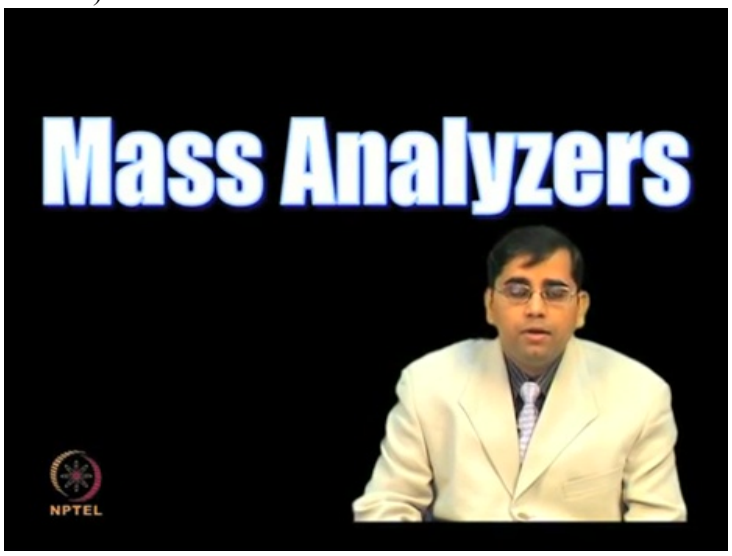

The mass analyzer disperses all the ions based on their mass to charge ratio and focuses all the mass-resolved ions at a single focal point and maximizes their transmission.

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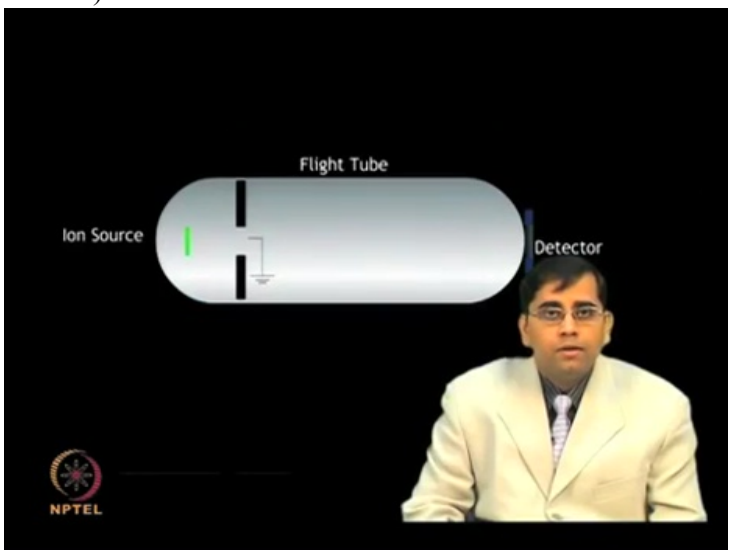

The Time of flight measures the m by z ratio

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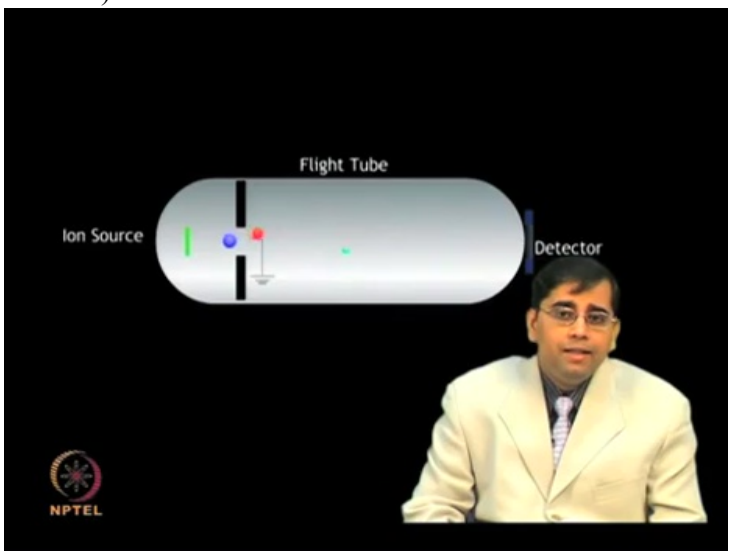

… of ions based on …

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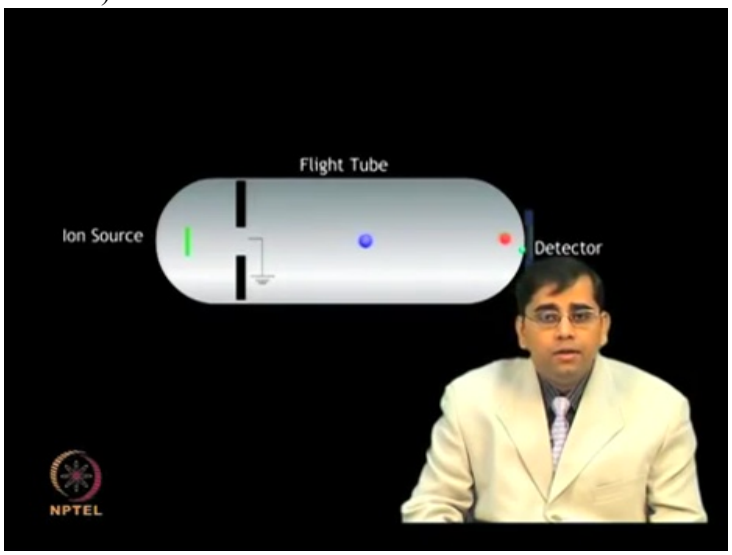

…the time it takes for ions to fly in the analyzer and strike to the detector.

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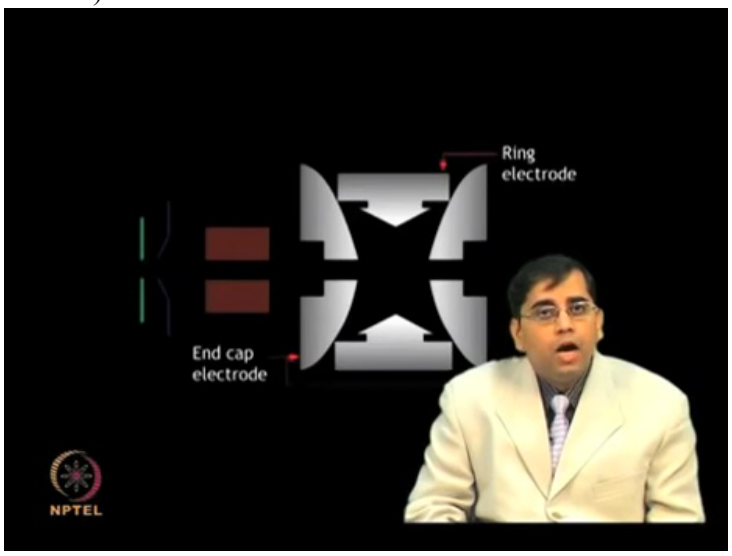

The Ion Trap, it traps ions using electrical fields …

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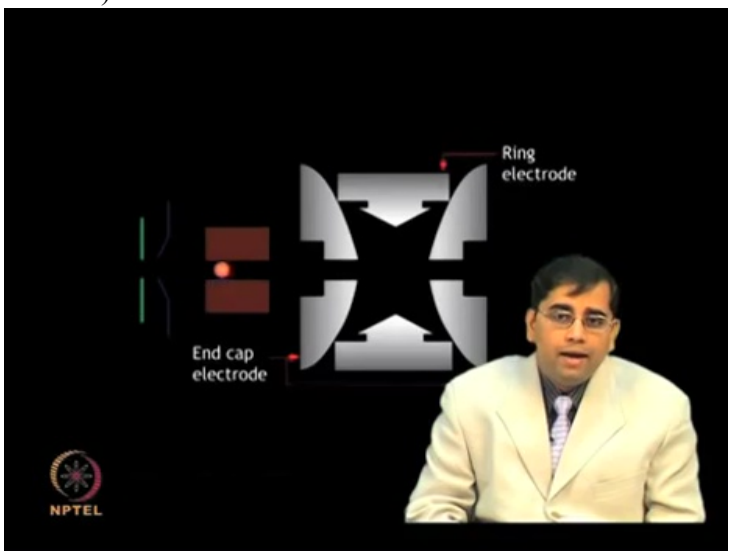

… and

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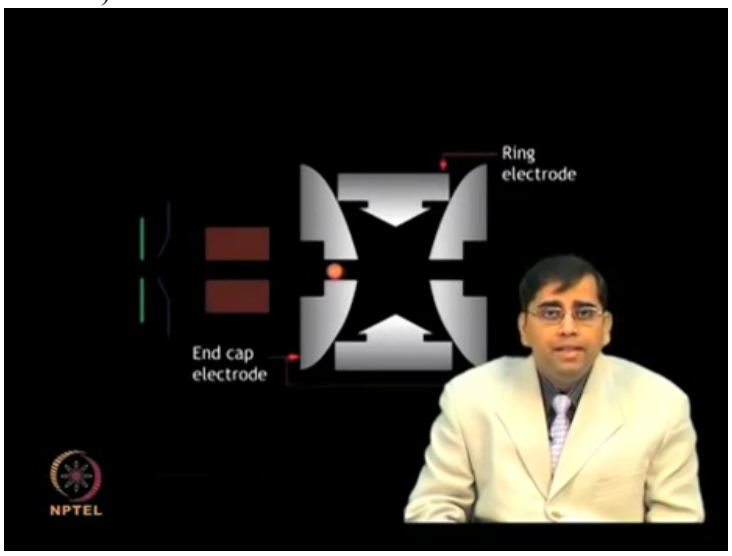

measures mass

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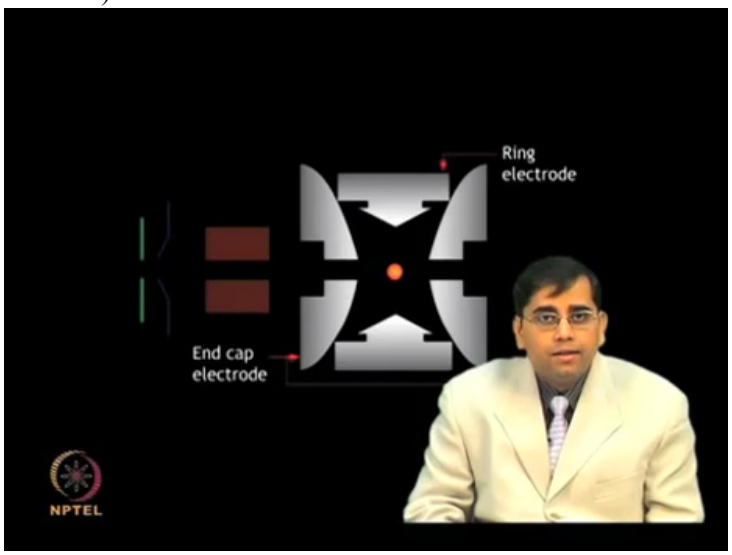

…by selectively ejecting them…

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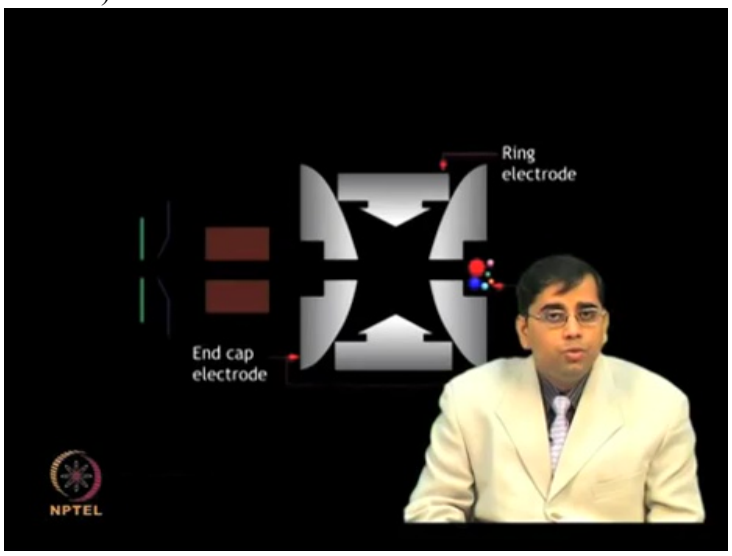

… to the detector.

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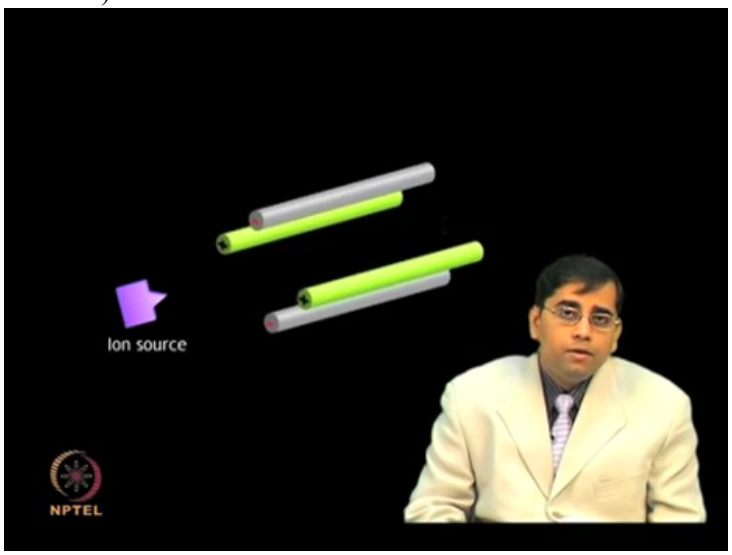

Quadrapole, it consists of

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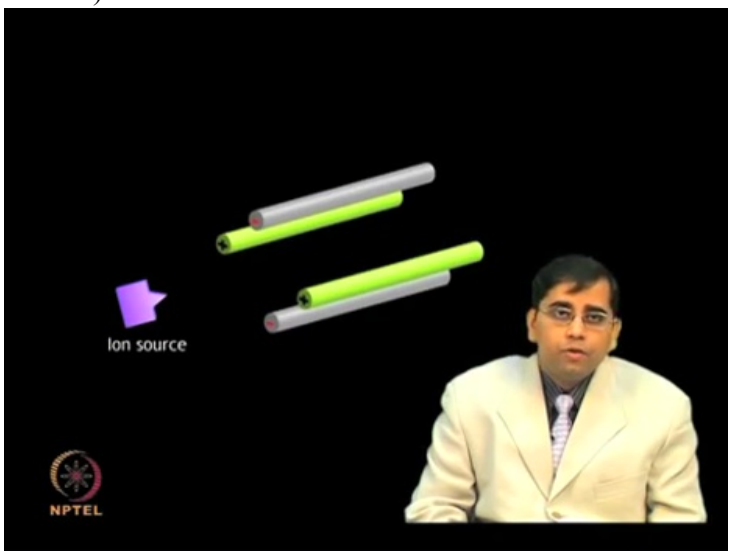

4 parallel

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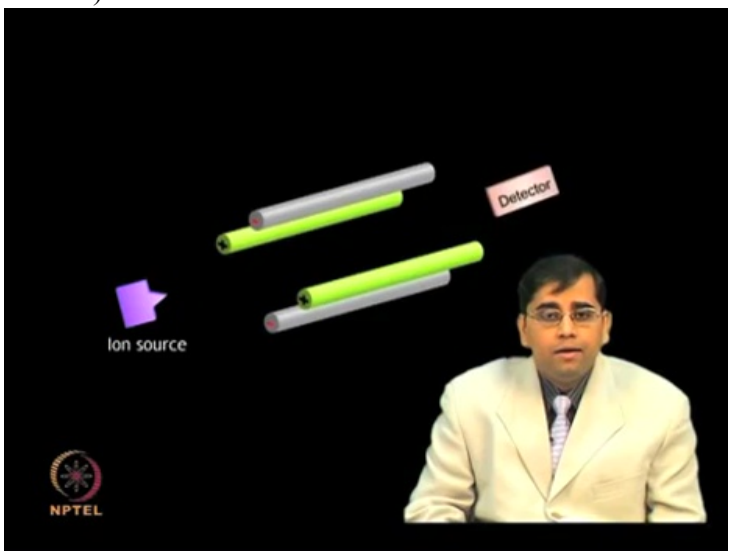

metal rods

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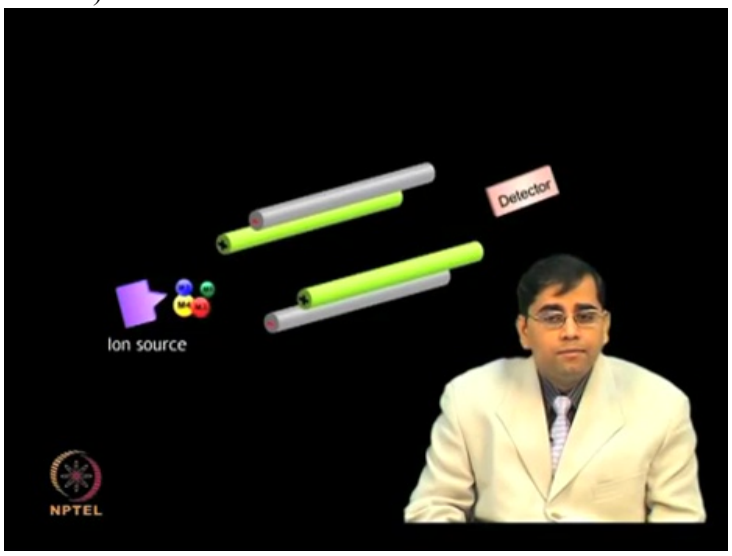

and mass separation is

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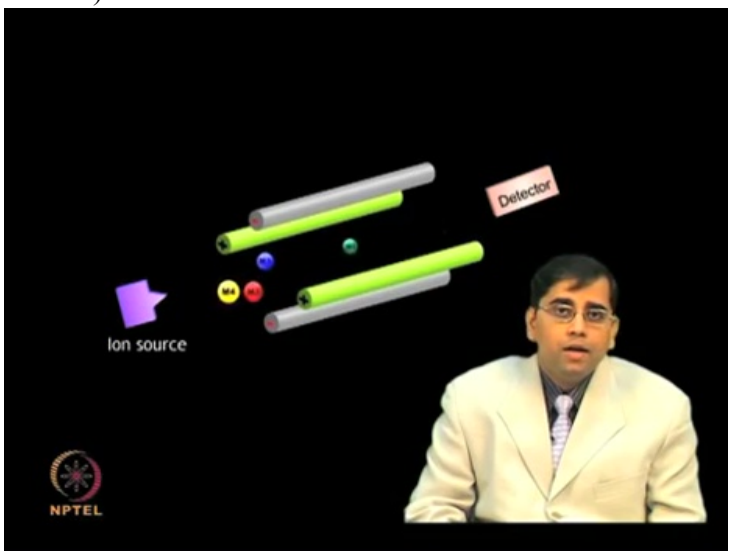

accomplished by the stable vibratory motion

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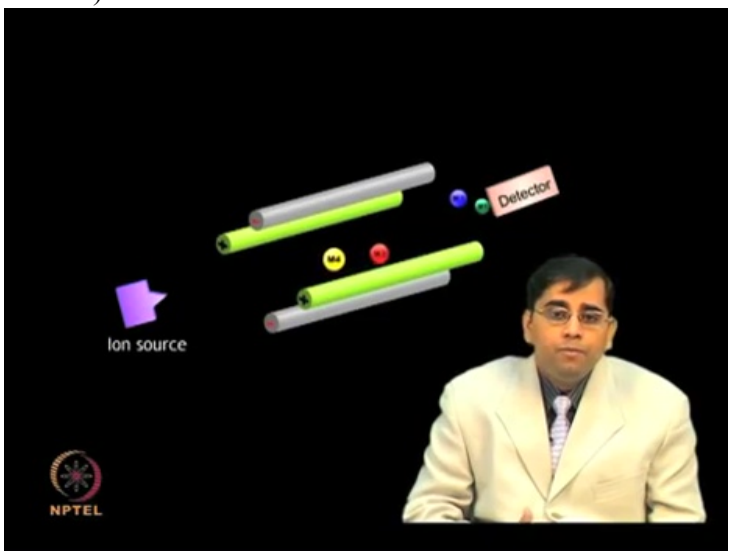

of ions in high frequency

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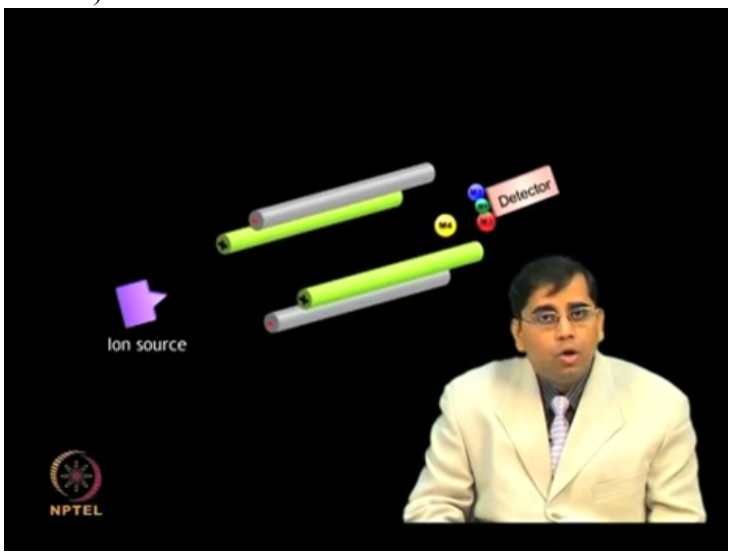

oscillating electric field that is created by applying direct current and radio frequency potentials to these electrodes

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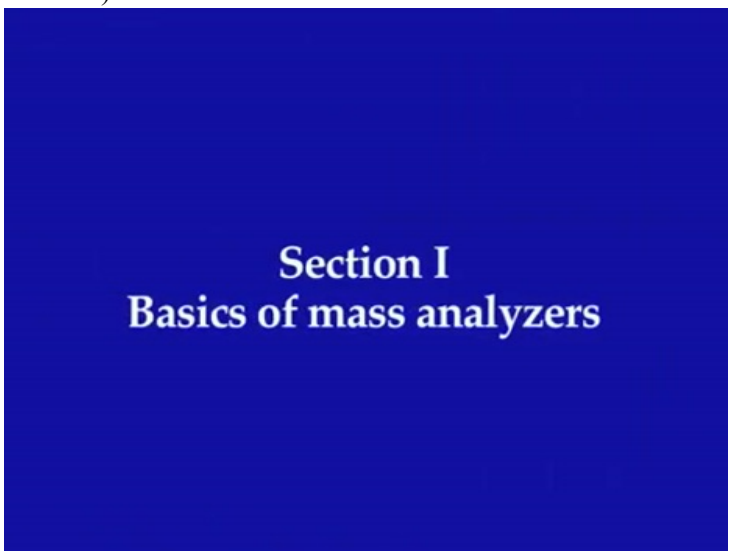

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There are different types of mass spectrometers currently available but for proteomics there are 2 configurations which are most commonly or most oftenly used; the Quadrupole-Time Of Flight or Q-TOF-based configurations and hybrid linear Ion Orbitrap instruments.

The TOF configurations separate peptides in time as they reach on the detector, so the time of flight is measured, whereas the Orbitrap mass analyzers, they measure the frequency of peptide ions which are oscillating in the ion trap. Now different types of resolution and sensitivity can be obtained from each of these configurations.

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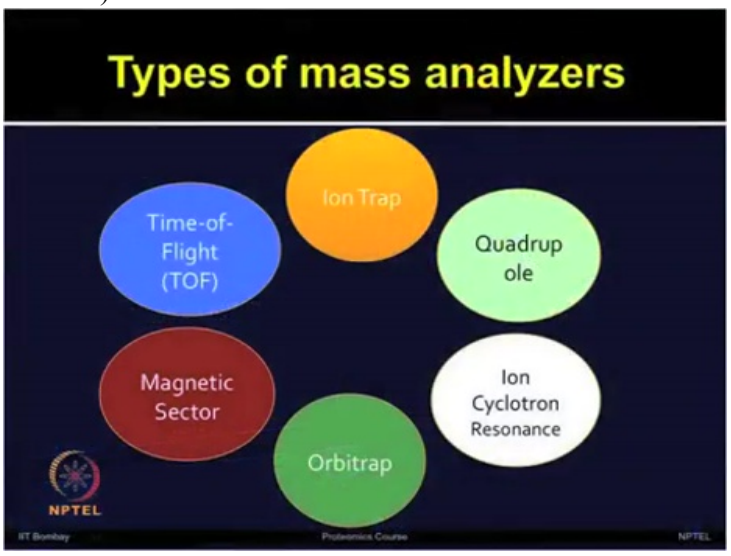

In the previous lecture I gave you an overview of different types of mass analyzers currently available. Each of those has its own unique properties in mass range, analysis speed, resolution, sensitivity, the ion transmission and dynamic range.

The Time Of Flight analyzers use time flight. Ion Trap, Orbitrap and Ion Cyclotron Resonance, they separate ions based on their mass to charge resonance frequency, whereas the quadrupoles or Q, they use an oscillating electrical field for selective stabilization of ions.

This just gives you an overview of various types of mass analyzers and briefly we discussed about their principle.

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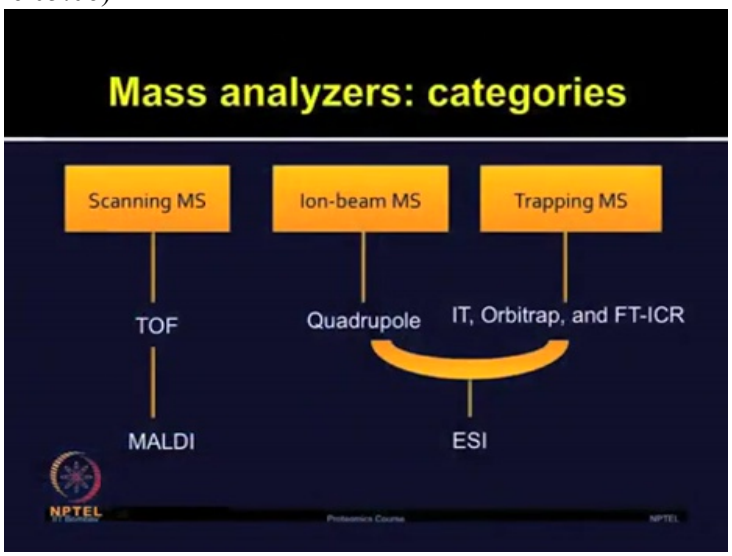

Now mass analyzers can be categorized broadly into the scanning MS, ion-beam MS and trapping MS. Scanning MS is more commonly used with TOF which is further coupled with the MALDI ionization sources.

The ion-beam MS is commonly used for the quadrupoles, whereas, trapping MS for the ion traps, Orbitrap and FT-ICR. All these can be coupled with the Electrospray Ionization ESI.

Some of the important mass analyzers, lets discuss a little more detail.

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First talk about Time Of Flight which is one of the simplest mass analyzers currently used in combination with the MALDI.

The TOF has emerged as one of the mainstream techniques for the analysis of the biomolecules and it is widely used for various applications.

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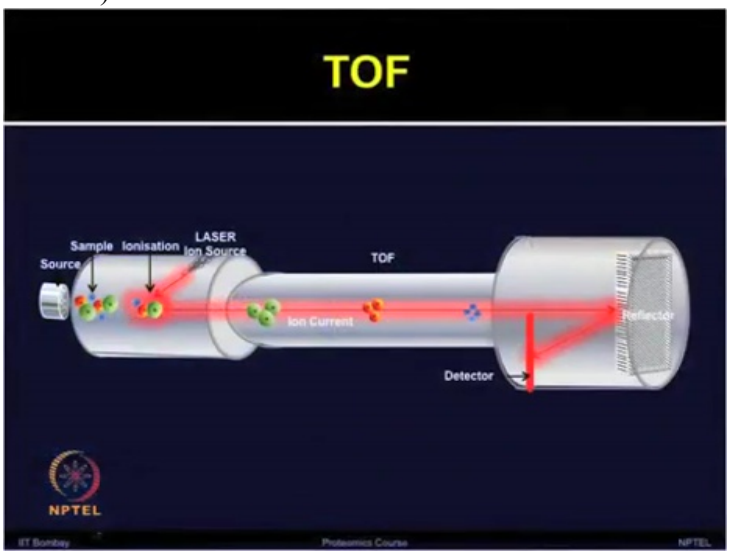

In TOF, the ions are accelerated to high kinetic energy and due to their different velocities; they are separated in a flight tube. One can also use the reflectron mirror so that ions can turn around into a reflector and it compensate for minor differences in the kinetic energy and provide long separation.

Another commonly used mass analyzer is Quadrupole.

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The Q instruments are one of the most widely used type of mass analyzers currently used in proteomics.

It consists of four matched 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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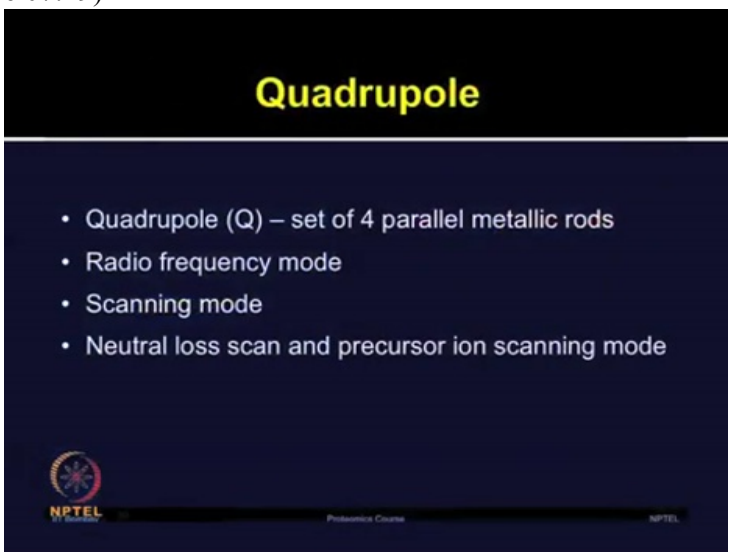

So, as we talked, quadrapole is set of 4 parallel metallic rods with opposite pairs are electrically connected.

There are different modes one can use for analysis

RF or radio frequency mode which allows ions of any m by z ratio to pass though;

Scanning mode, ions of selected mass and charge can be allowed by the detector. The potential difference applied and the instrument can be used as the mass filter

The neutral loss scan and precursor ion scanning method, they are used for the phosphorylation to distinguish between phosphorylated and non-phosphorylated peptides.

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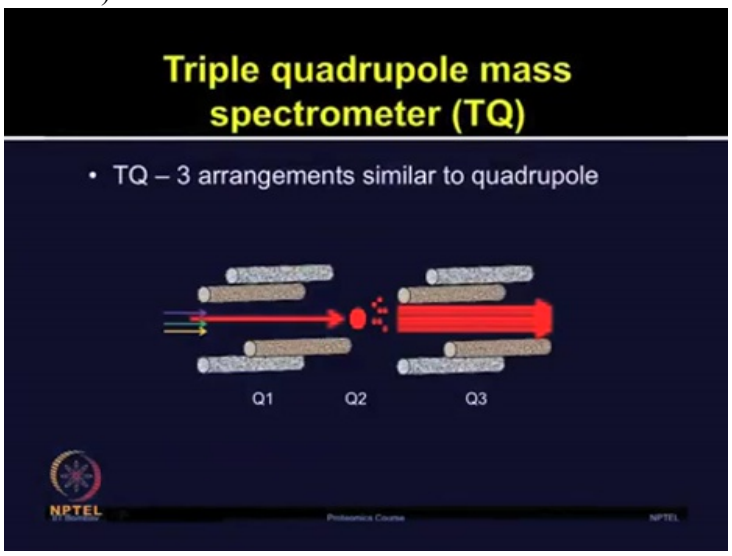

Now triple quads, which is arrangement of quadrupoles is widely used for the proteomics. In triple quad, Q1 it casts the ionic streams. It directs ions of a selected m by z ratio into the second quadrupole, Q2 which is a collision cell.

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 inert gases and then selected ions are further moved into the Q3.

Q3 scans the streams of ions fragments which are emerging from the collision cell to generate a collision induced disassociation spectrum.

The mass spectrum of fragments derived from one peptide after one analysis is complete.

Then Q1 directs a different intact peptide into the collision cell. So in this sequential manner, it can process various peptides.

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Now let's talk about another important mass analyzer, the Ion Trap.

The Ion Trap traps ions using electrical fields and it measures by selectively ejecting them to a detector

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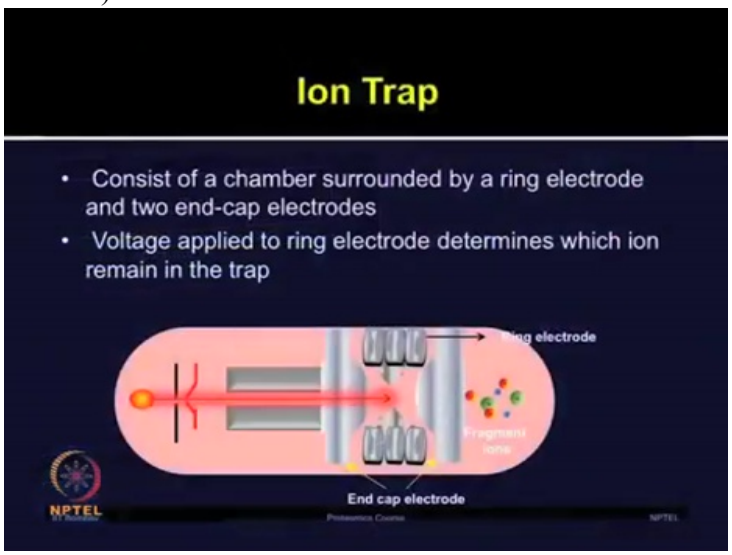

It consists of a chamber which is surrounded by a ring electrode and two end-cap electrodes, as you can see in the figure here. The voltage applied to the ring electrode determines which ions remain inside the trap. So ions above a threshold of m by z ratio, they remain inside the trap and others can be ejected through a small hole.

Theoretically ion trap can provide MS 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 biomolecules and now it is also used in the proteomics.

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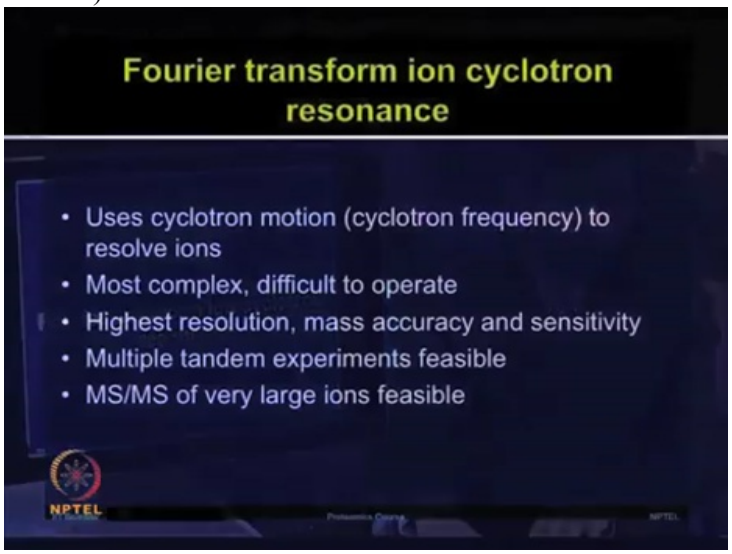

An FT-ICR MS can be considered as ion trap system where ions are trapped in the magnetic field. It uses cyclotron motion or cyclotron frequency to resolve the ions.

Although operationally it is very complex and not very easy to operate; but it provides highest resolution, mass accuracy and sensitivity. It also provides the capability of multiple tandem experiments and MS/MS of very large ions are possible by using FT-ICR MS.

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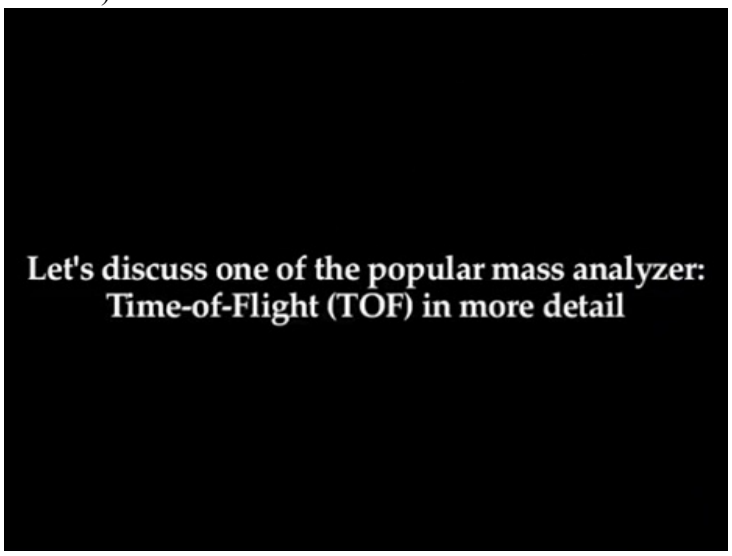

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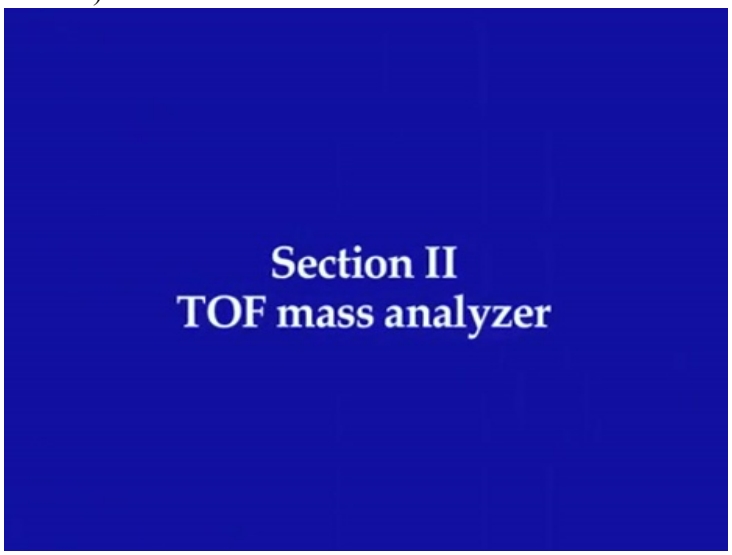

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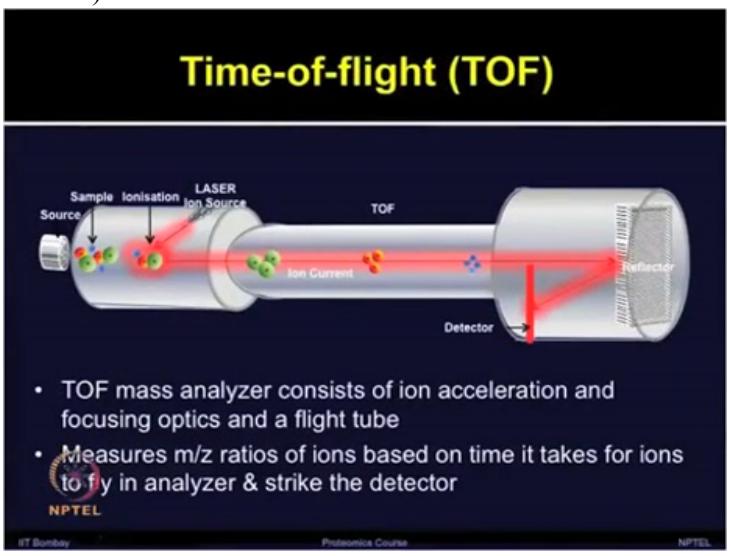

So the TOF mass analyzers, they consist of ion acceleration and focusing optics and a flight tube.

As shown in the slide, you have a source, 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 reflector, an ion mirror which can increase the path length.

So the 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.

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 velocities.

Now Time of Flight tubes often out-performs the scanning mass analyzers in its sensitivity and scan speed.

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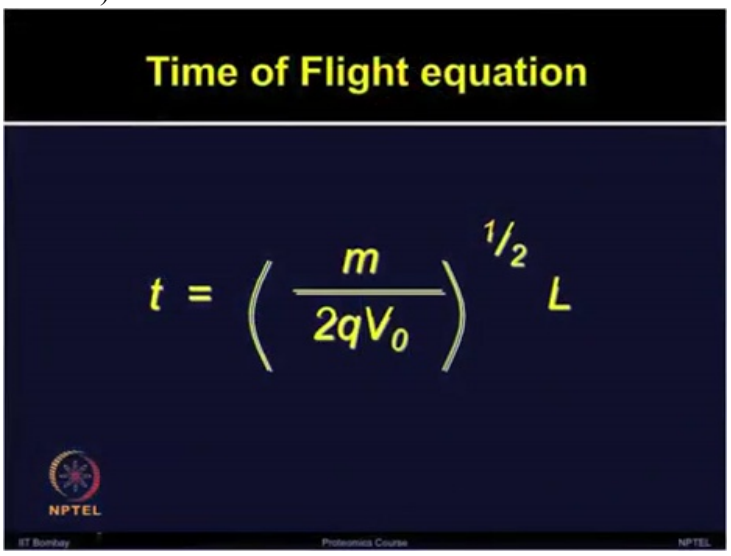

The Time of Flight of a charged ion can be calculated by using the equation shown in this slide, the flight time is directly proportional to the square root of mass of the ion.

Now in this equation, "t" represents the time of flight, "m" is mass of the ion, 'q" charge on the ion, "V zero" is accelerating potential and "l" is the length of flight tube.

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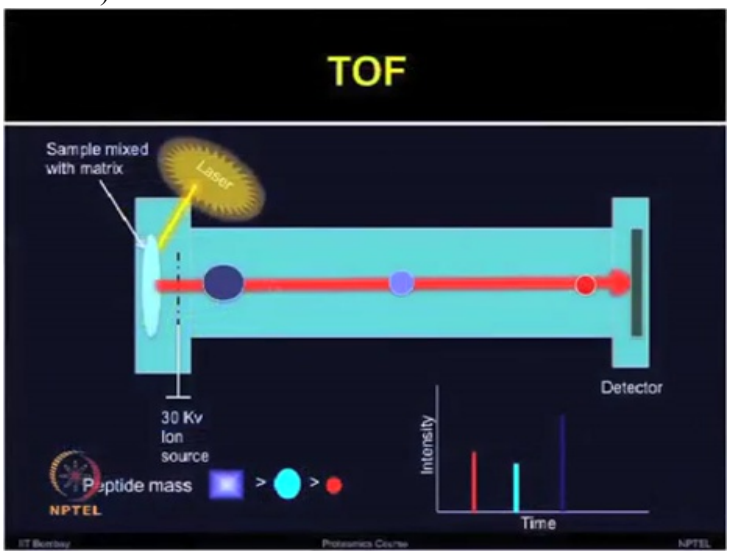

In time of flight tube, the ions are accelerated to high kinetic energy, and due to different velocities, they are separated in a flight tube. As I mentioned earlier, by adding the reflectron or a reflector, the ions can turn around in the reflector that can compensate for minor differences in the kinetic energy.

Now if you take an example where you have 3 ions as shown in the dark blue, light blue and the red color in the slide, now you will expect the small ion which is the red one will show the first peak, followed by the blue ion followed by the dark blue ion.

After discussing some of these basic concepts of using MALDI and TOF…

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…now let me give you an overview of entire MALDI-TOF experiment by showing you the following animation.

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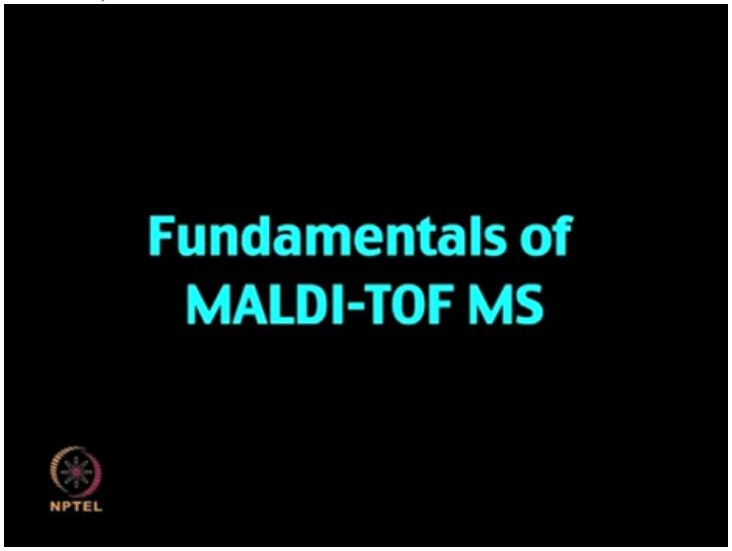

Fundamentals of MALDI-TOF MS,

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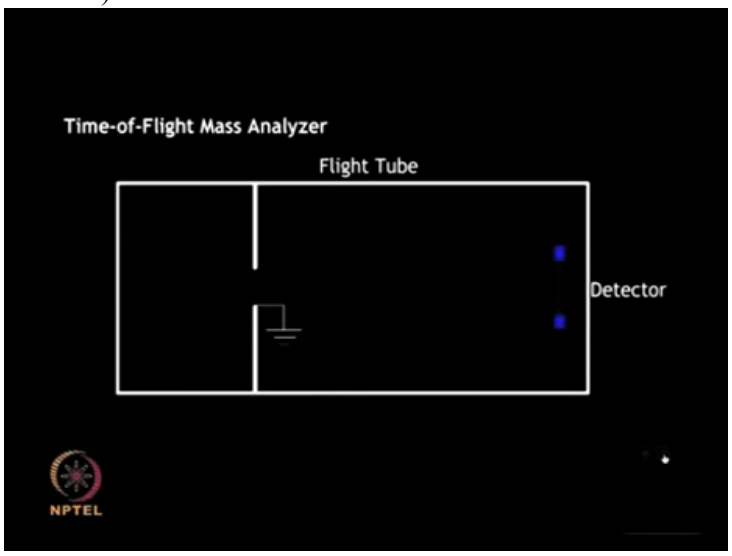

the Time of Flight analyzer resolves ions, produced by

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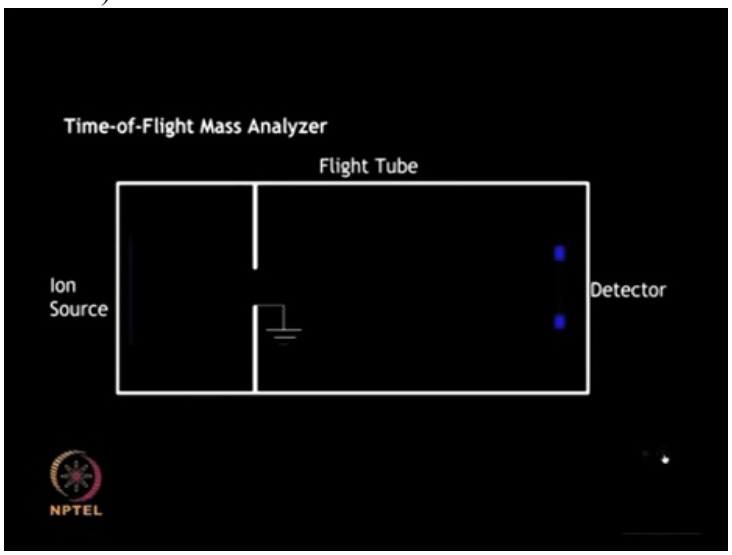

the ionization source, on the basis of their mass to charge ratio. The time of flight tube can be operated in the linear mode ….

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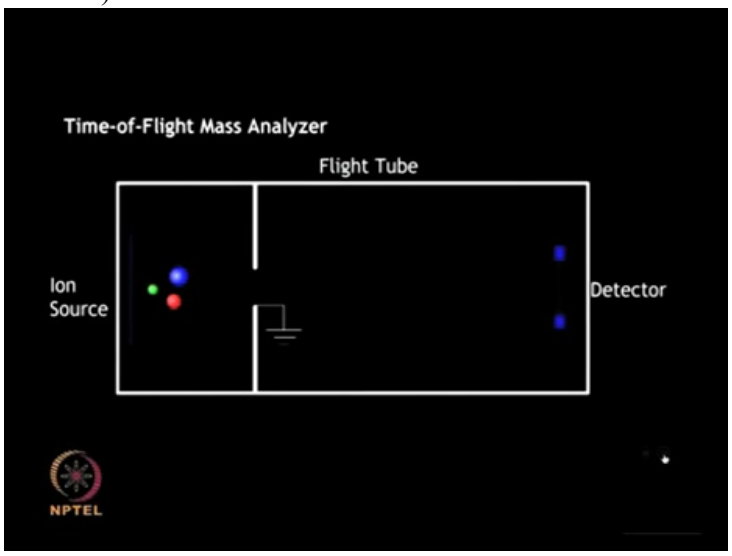

or the reflectron mode which depends on the sample to be analyzed. In case of small molecules this mode usually provides sufficient resolution.

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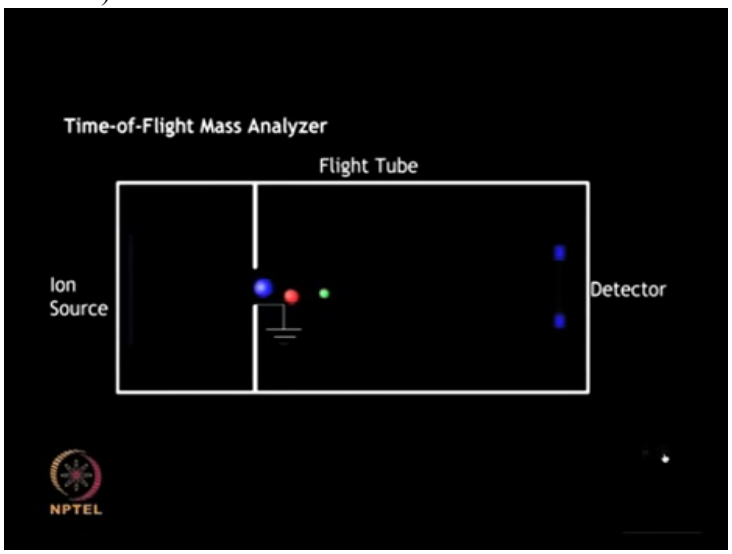

The generated ions are accelerated

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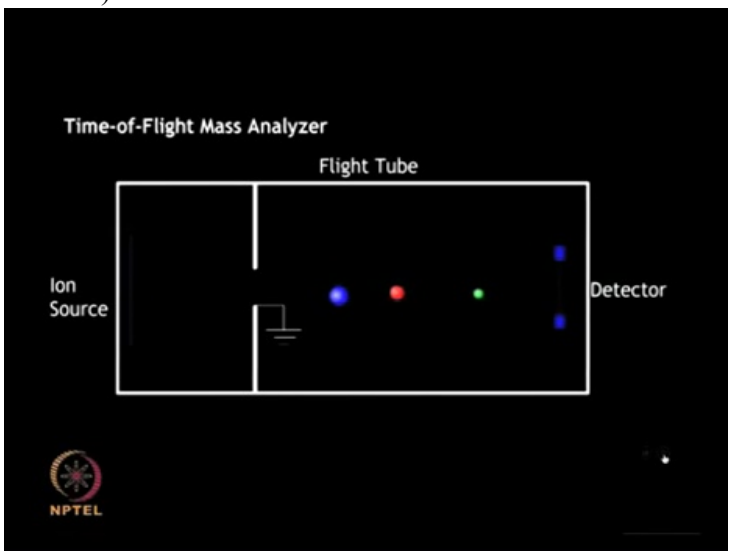

towards the detector, with the lighter ion

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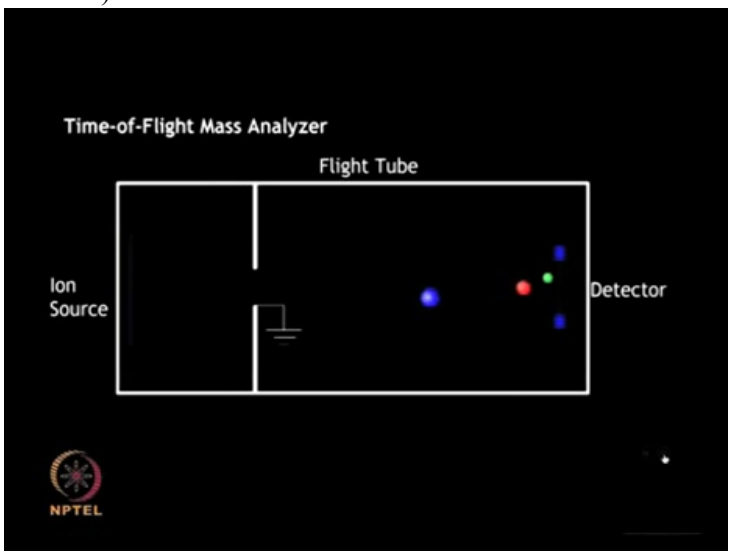

travelling through the TOF tube faster than …

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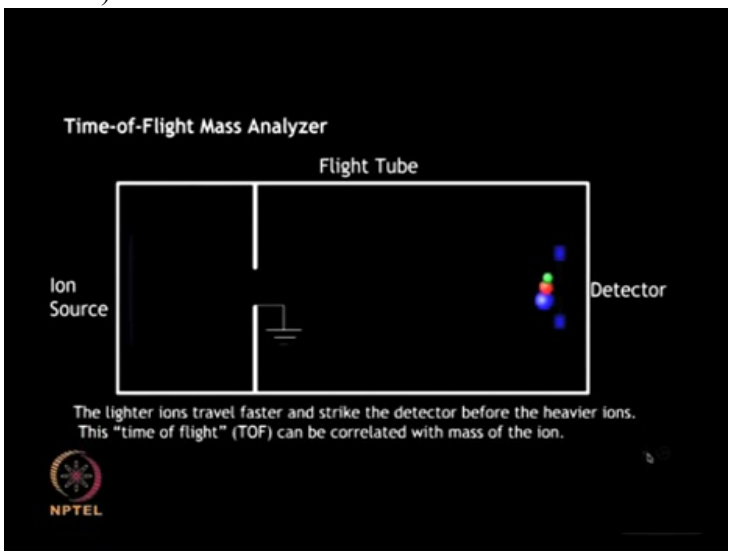

…the heavier ions.

So, the lighter ion travels faster and it strikes the detector before the heavier ion reaches to the detector. The Time Of Flight 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 ratio.

As we talked earlier the TOF analyzer can also be operated in the reflectron mode.

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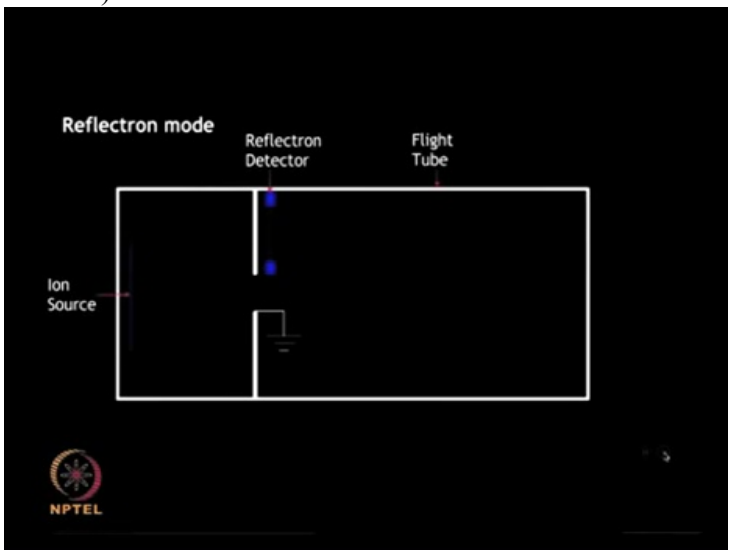

So, this is more commonly used for the proteomic studies.

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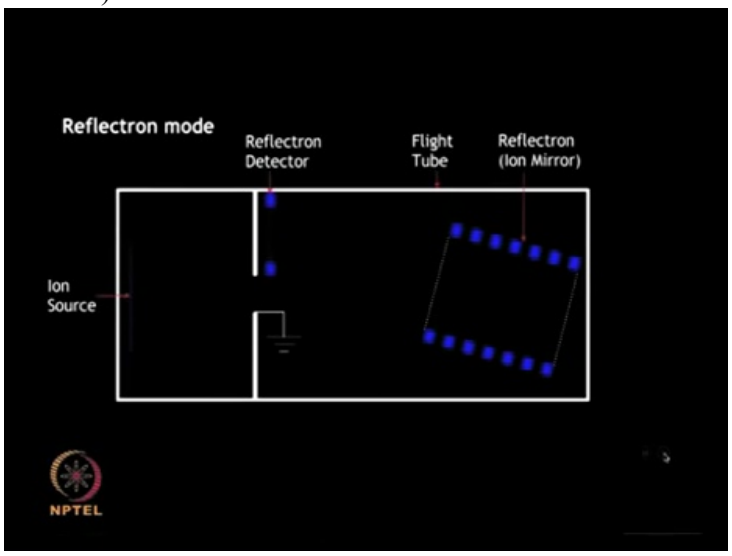

A reflectron which acts as a ion mirror is incorporated at one end of the time of flight tube.

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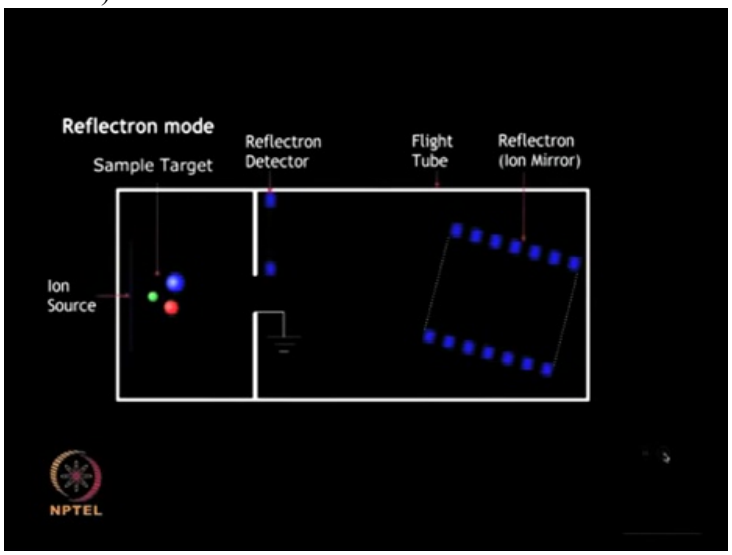

This helps in extending the path length

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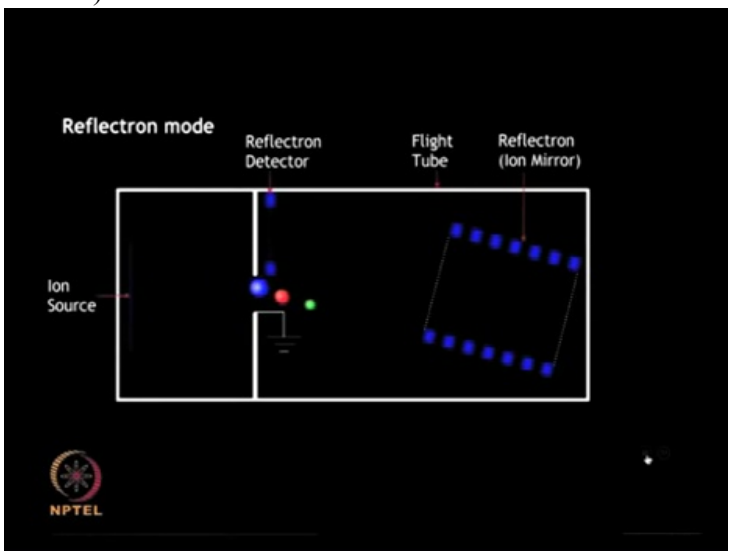

and in turn …

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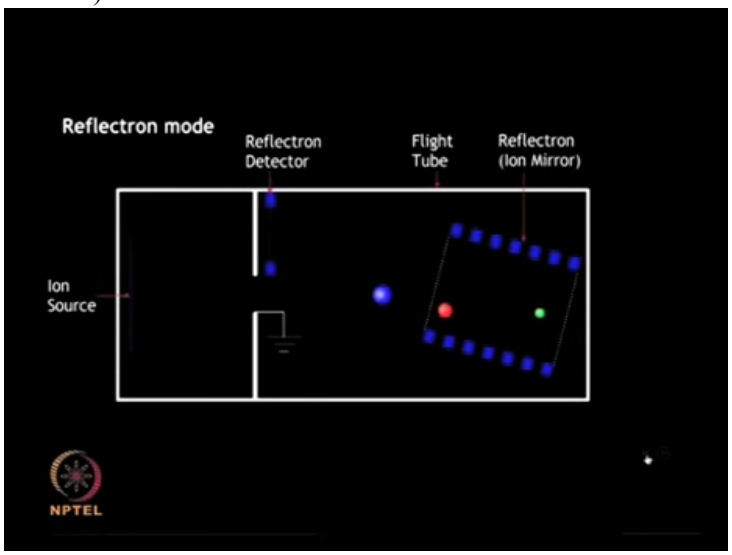

the flight time of the ion without having to increase the

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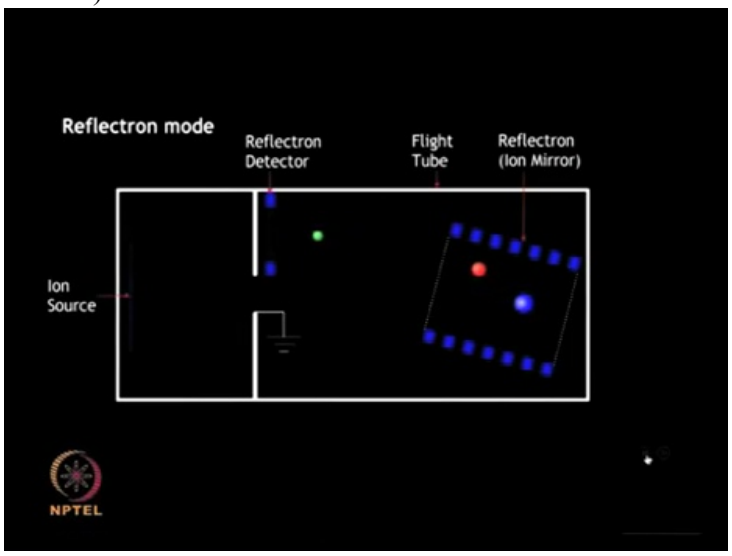

actual size of the instrument.

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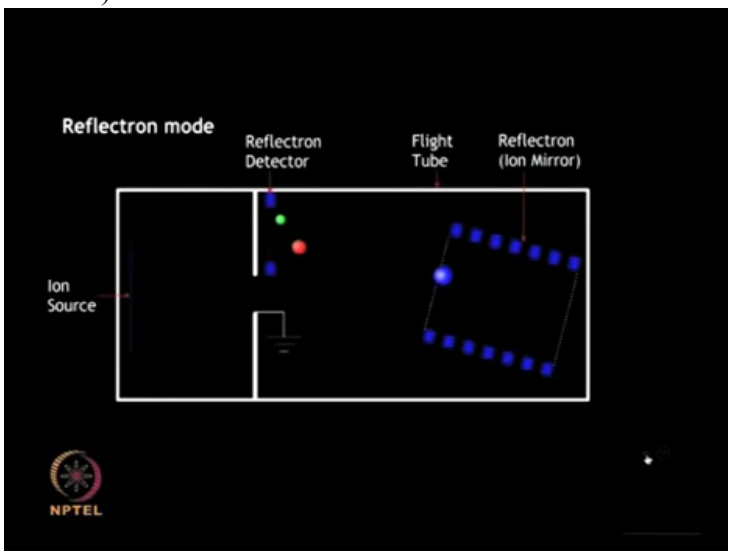

So, rather than using very long time of flight tubes; by including the reflectron ion mirrors, we can increase the path length. This helps to even out any kinetic energy differences between ions having the same mass and thereby improving the resolution.

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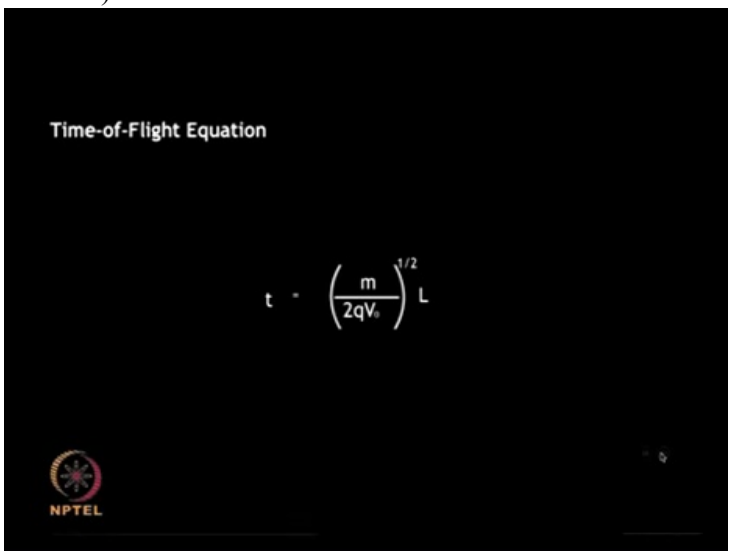

The time of flight of a charged ion can be calculated by means of the equation shown here. The flight time is directly proportional to the square root of mass of the ion.

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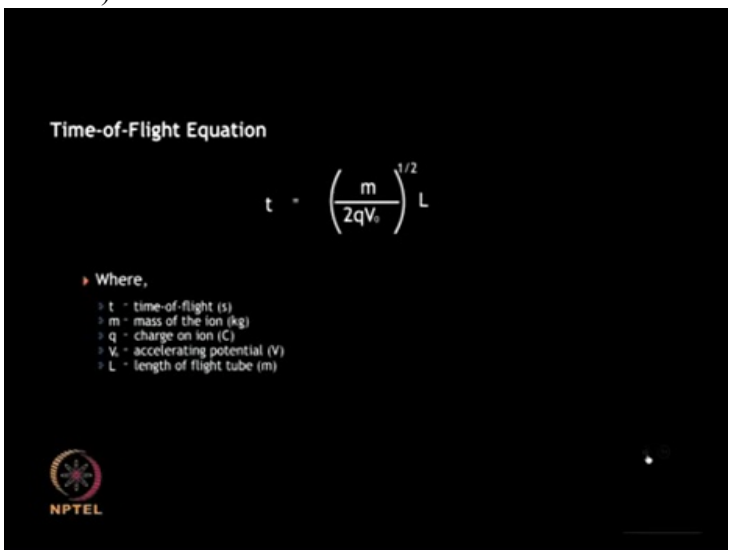

So we have discussed all the important components of liquid chromatography mass spectrometry. Now, one can apply these configurations in tandem. One can select different types of mass analyzers and use it based on their applications. Now we will look at some of the popular Hybrid-MS and MS/MS configurations.

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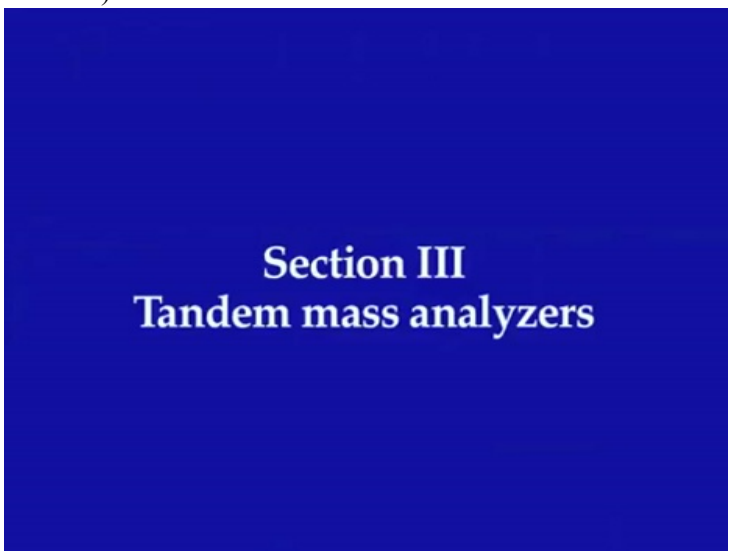

So we have discussed all the important components of liquid chromatography mass spectrometry. Now, one can apply these configurations in tandem. One can select different types of mass analyzers and use it based on their applications.

Now we will look at some of the popular Hybrid-MS and MS/MS configurations.

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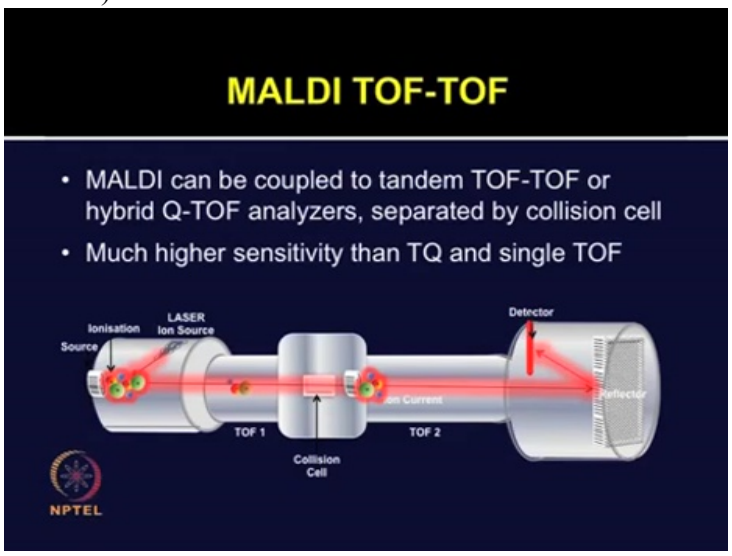

MALDI TOF-TOF; that is one of the widely used tandem-MS configuration. In this one the TOF-TOF or two time-of-flight tubes as well hybrid quadrupole time-of-flight analyzers can be used.

We have discussed the MALDI TOF-TOF system in some more detail in the previous lecture. So I will move on to some other configurations which is

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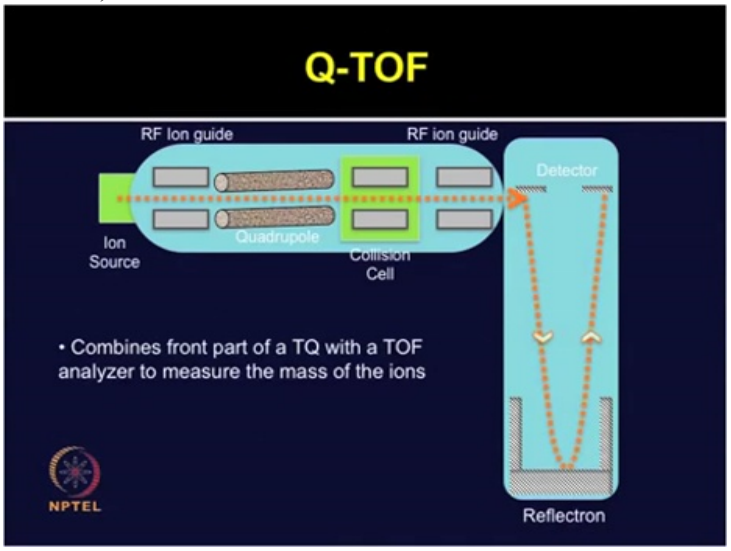

Q-TOF. The Q-TOF, it combines front part with quadrupole or it can be triple quad TQ along with the TOF analyzers to measure the mass of ion.

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Some of the important concepts involved in ionization, mass analyzers and tandem-MS

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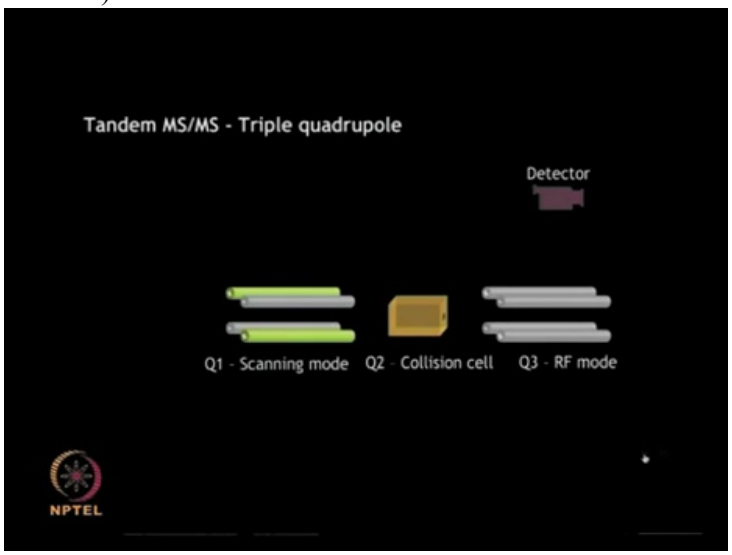
, I will describe those in the following animation.

(Refer Slide Time 18:49)



The triple quadrupole consists of two sets of parallel metallic rods interspersed by a collision cell. The first quadrupole scans the ions coming from the ionization source and allows only ions of a particular m by z ratio to pass through.

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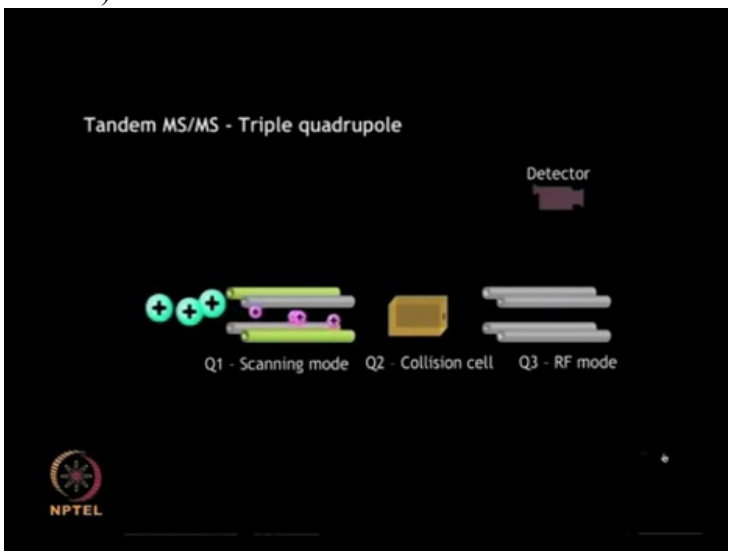

Once the ions are selected,

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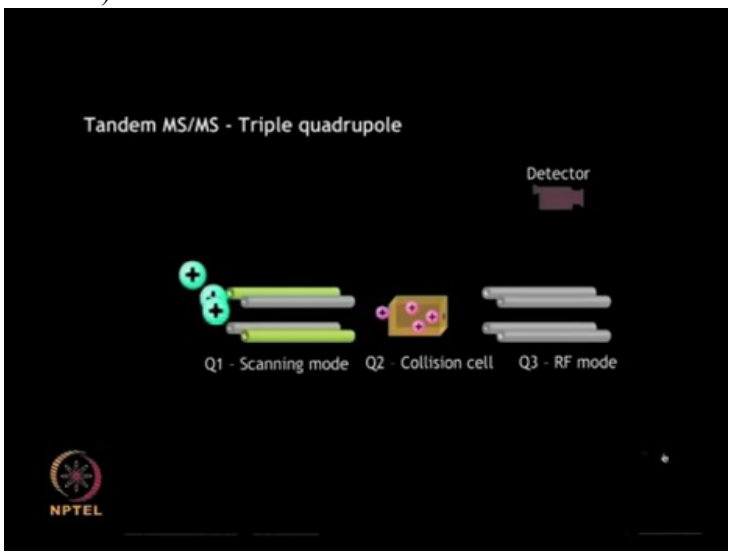

ions enter the collision cell where they are fragmented by collision against an inert gas like argon.

The smaller fragments then enter the third quadrupole, which scans all the ions in

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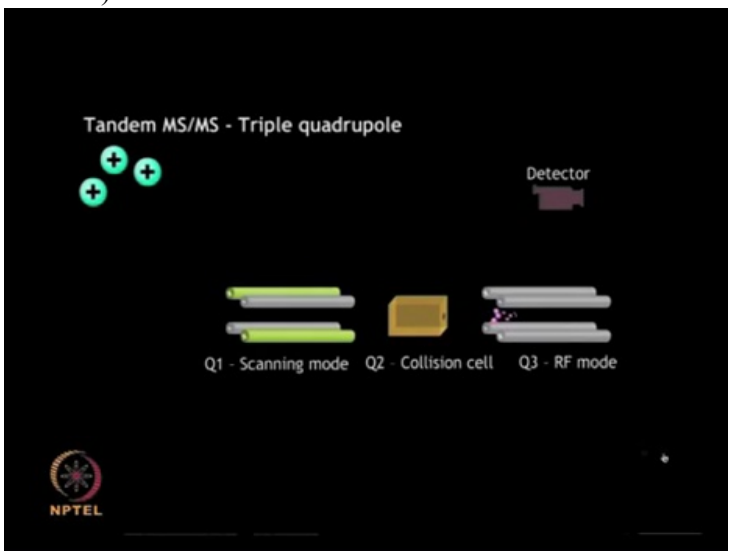

the radio frequency or RF mode

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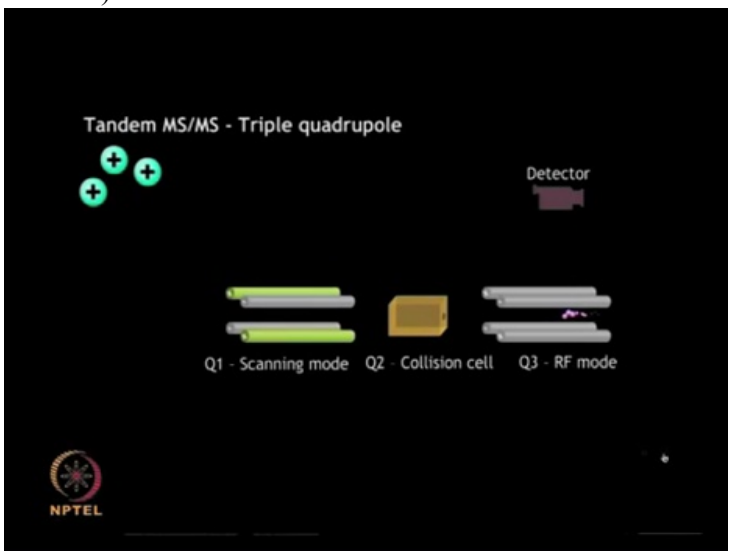

to generate a spectrum

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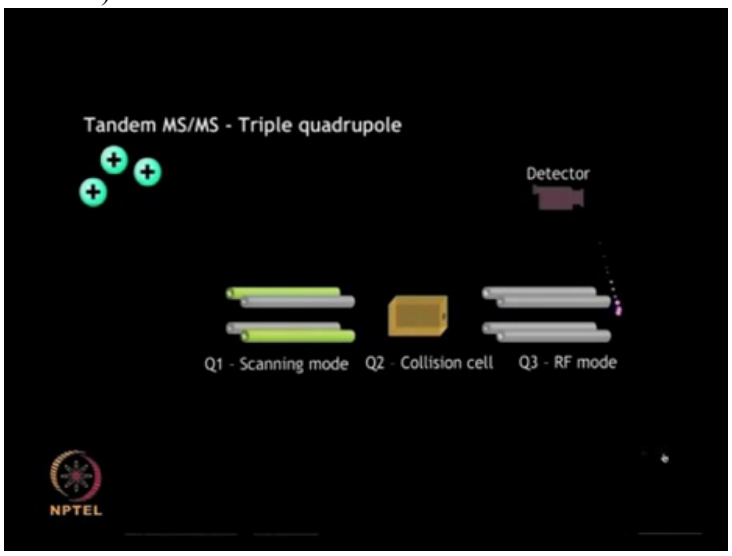

based on the varying behavior of ions in an oscillating electrical field.

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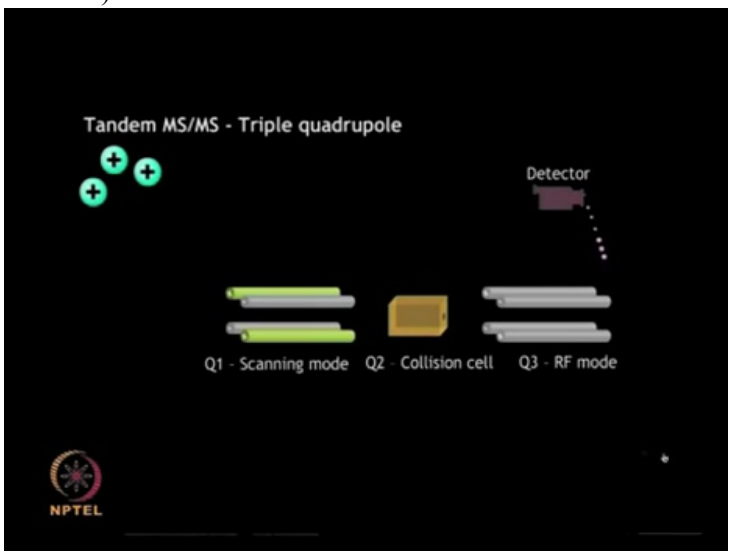

There are different types of tandem MS/MS configurations

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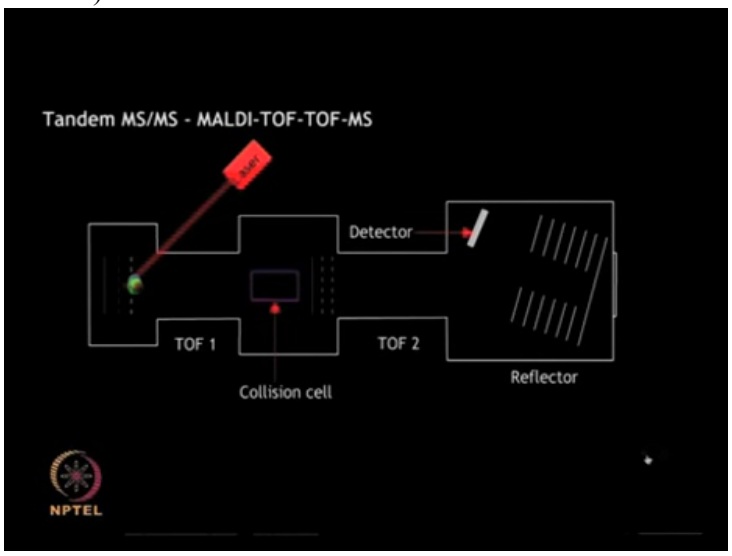

MALDI-TOF-TOF.

MALDI-TOF-TOF MS is a common tandem MS configuration in which the ions are first resolved on the basis of their time of flight in the first TOF analyzer.

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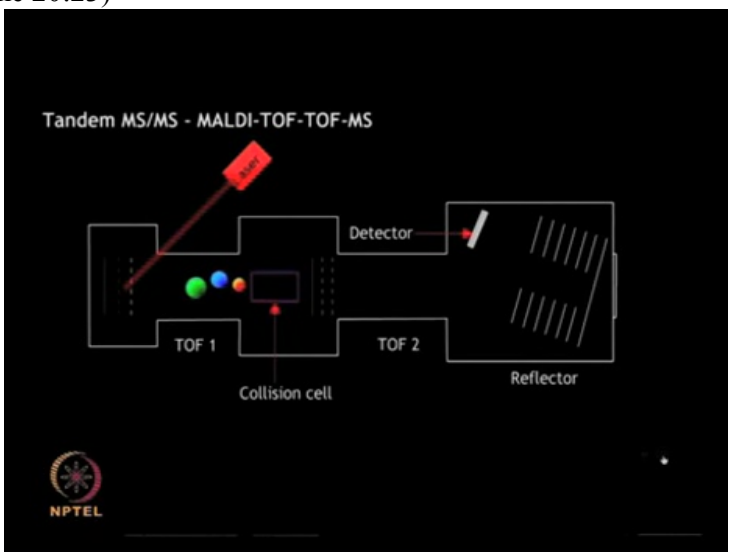

The selected ions enter the collision cell where they are further fragmented.

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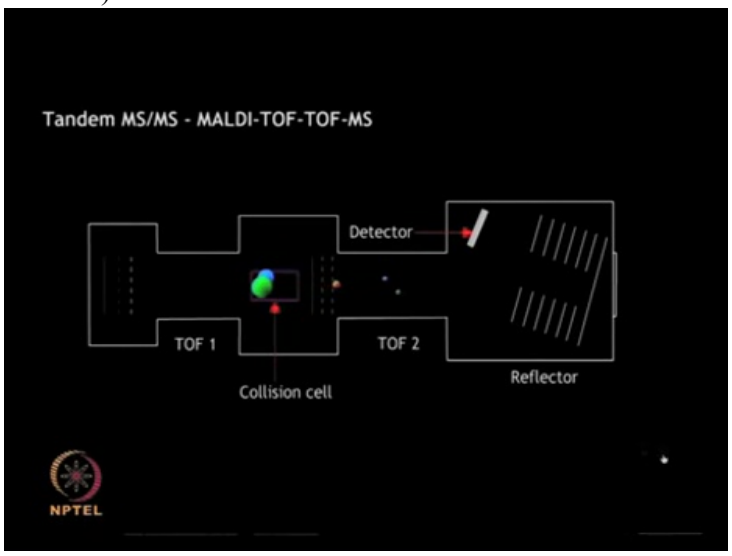

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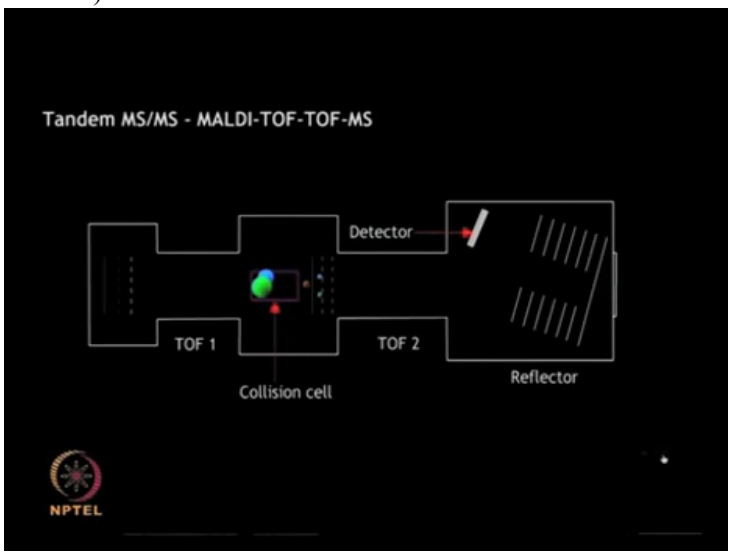

The fragmented ions are accelerated

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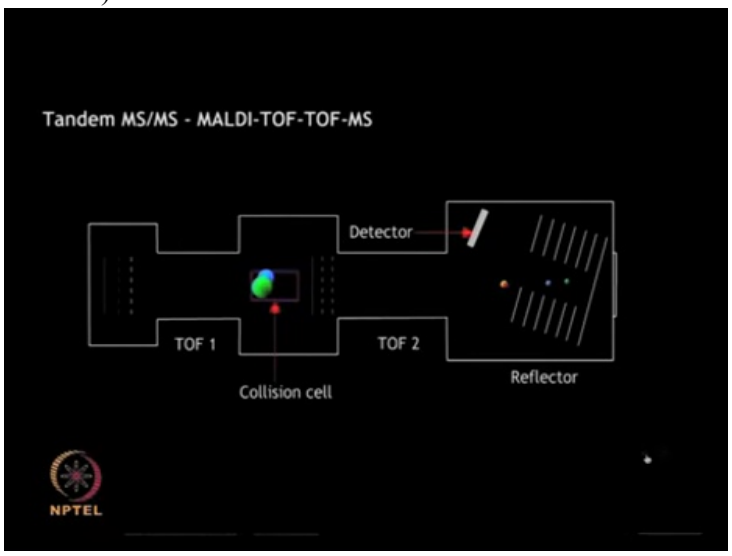

and further resolved on the basis of their m by z values in the second Time Of Flight tube, after which they can be detected.

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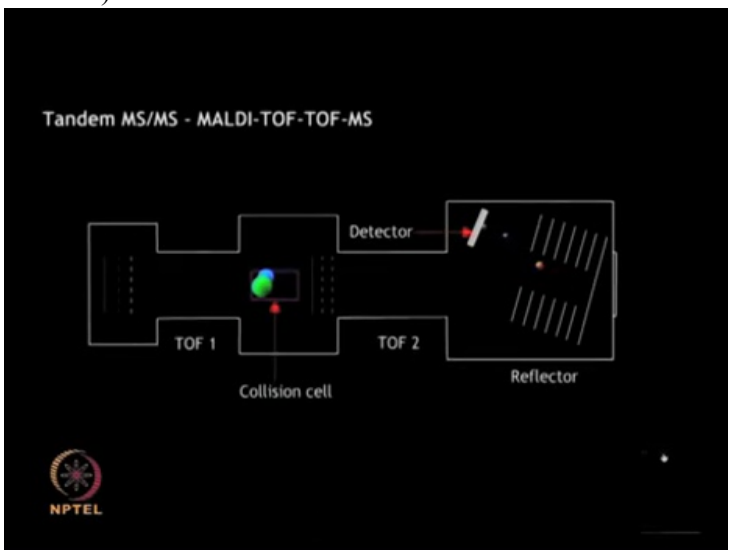

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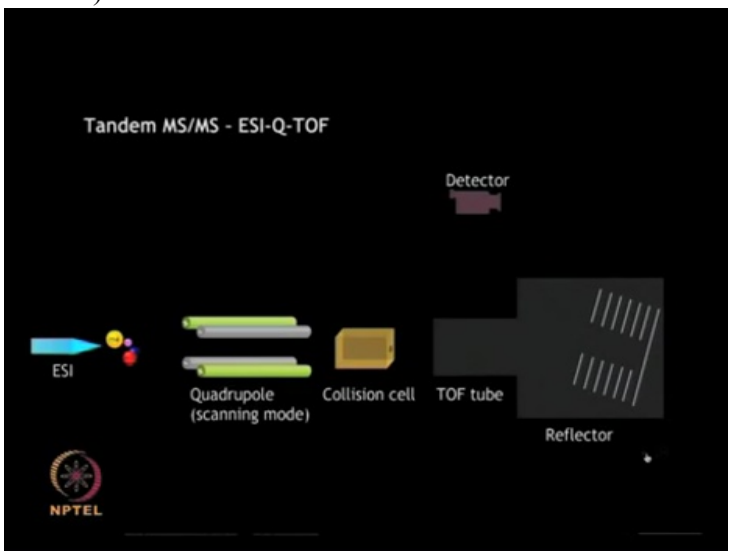

ESI-Q-TOF is another common tandem MS configuration that first selects ions in the radio frequency mode.

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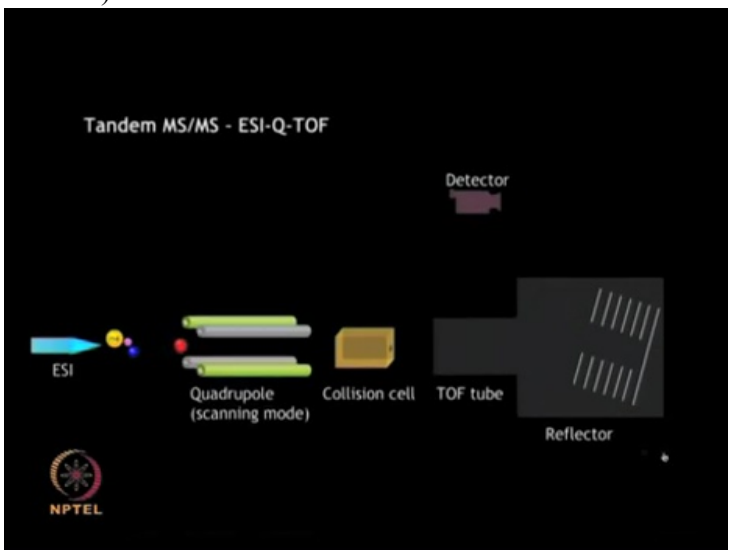

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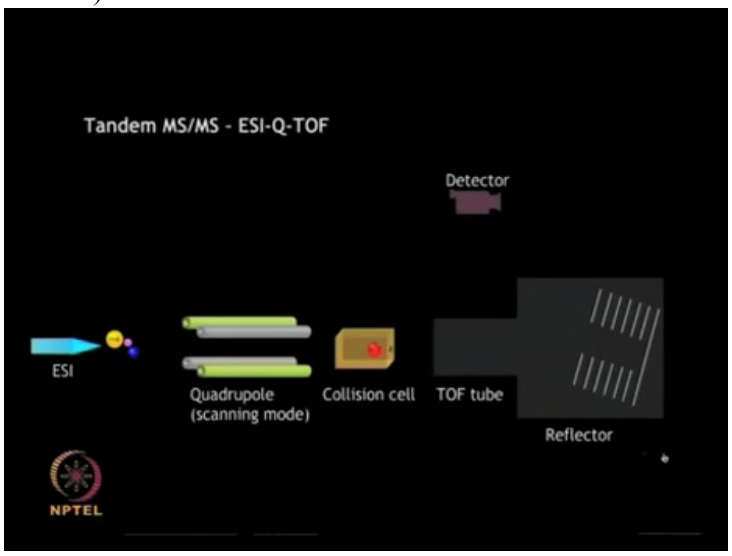

Selected peptides are fragmented in collision cells and resulting ions are accelerated and resolved on the basis of their time of flight.

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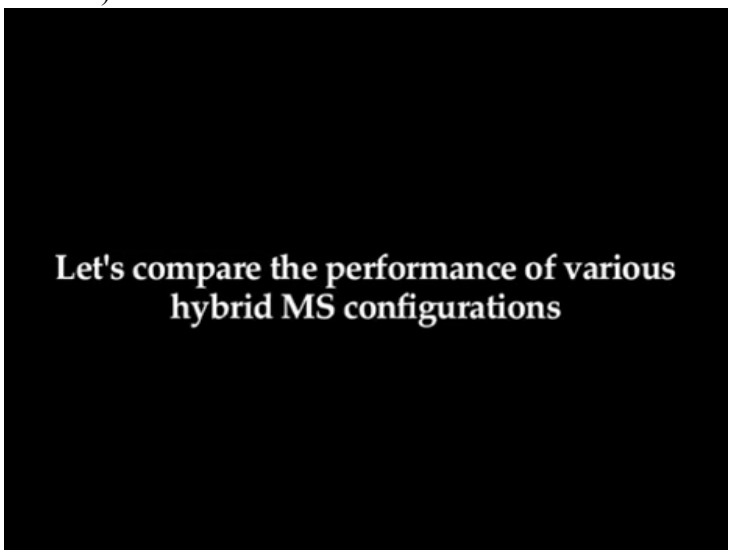

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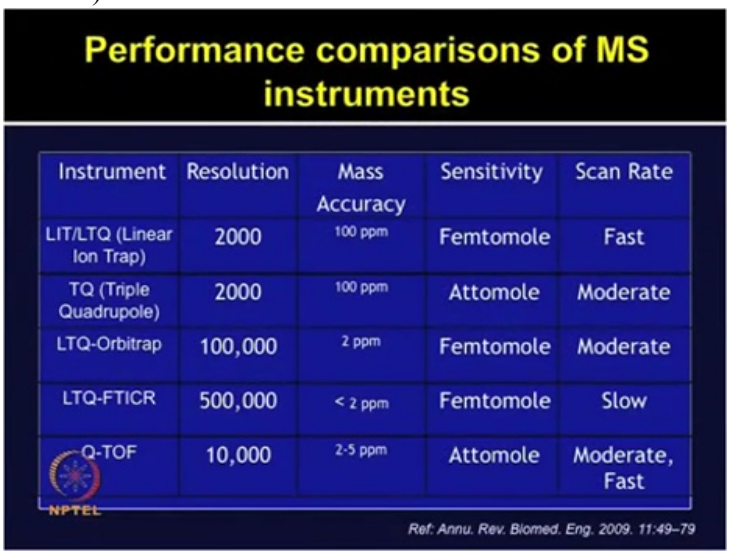

So finally, there are so many mass spectrometers currently available commercially. So now depending on individual's application, one can select different type of configuration. Based on an excellent review from Yates and colleagues I provided this performance comparison of MS instruments in following slide.

Here you can see the Linear Ion Traps or LIT or LTQs, they have resolution of 2000, mass accuracy 100 ppm, sensitivity femtomole and the scan rate is very fast.

The triple quadrupoles or TQs with resolution of 2000, mass accuracy 100 ppm, sensitivity in atomole and scan rate is moderate.

The LTQ-Orbitraps, they can provide high resolution of 100,000, mass accuracy 2 ppm, sensitivity in femtomole and scan rate is moderate to low.

LTQ-FT-ICR, they can provide very high resolution of 500,000, mass accuracy less than 2 ppm, sensitivity in femtomolar range, slow scan rate.

The Quadrapole Time of Flight, they provide resolution more than 10,000, mass accuracy 2 to 5 ppm, sensitivity in atomole range and scan rate is moderate to fast.

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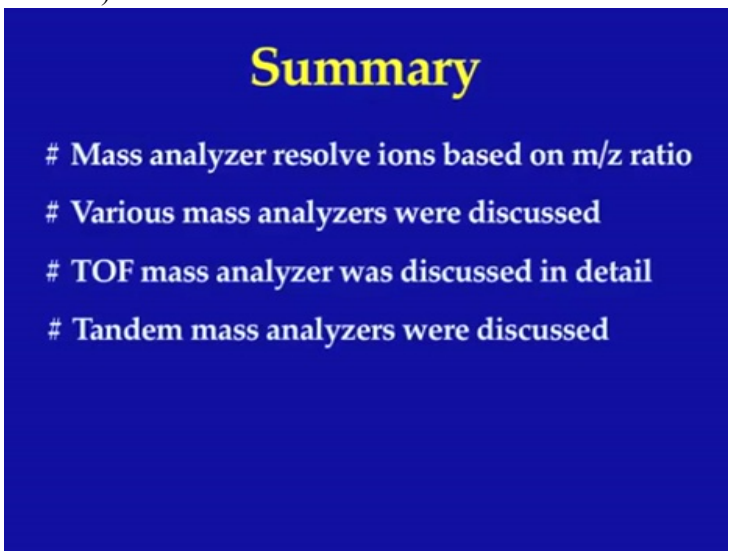

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