Mass Spectrometry Based Proteomics
Professor Sanjeeva Srivastava
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Indian Institute of Technology, Bombay
Mod 03 Lecture Number 12

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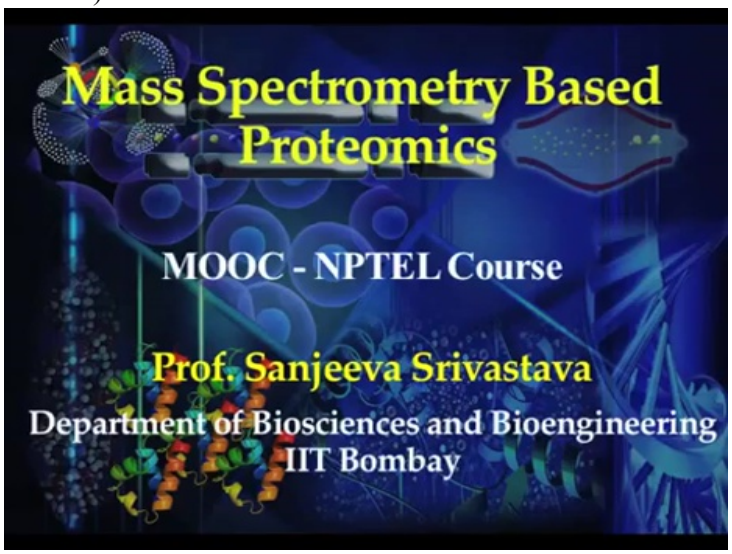

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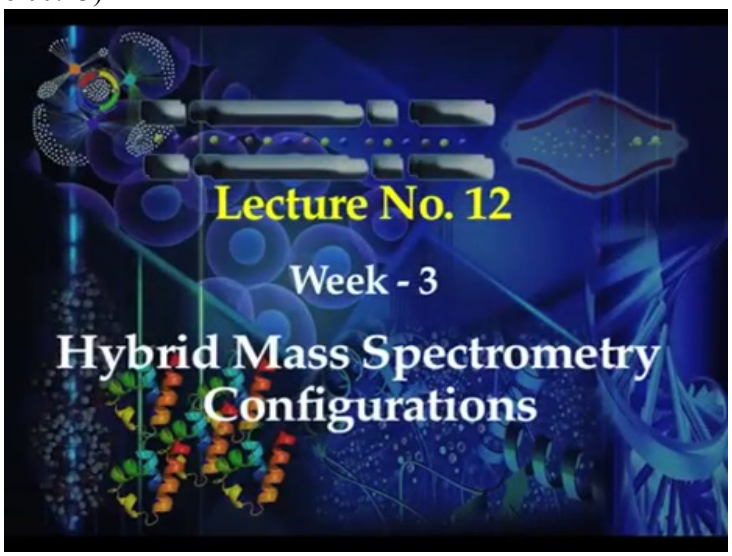

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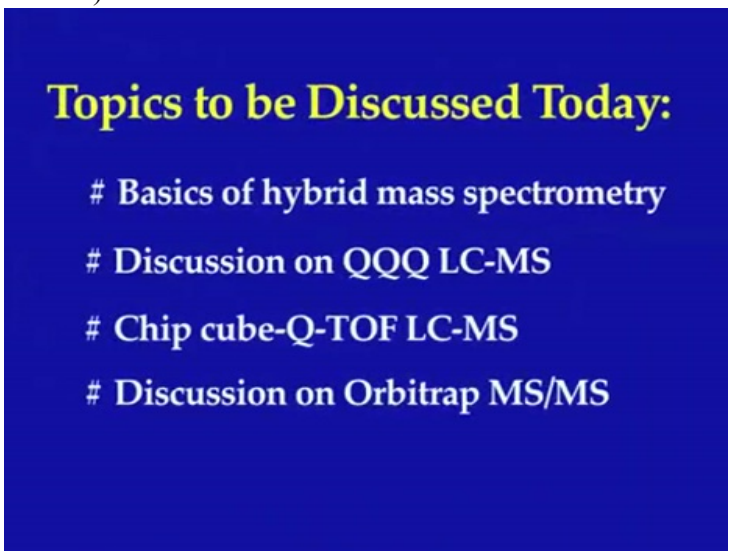

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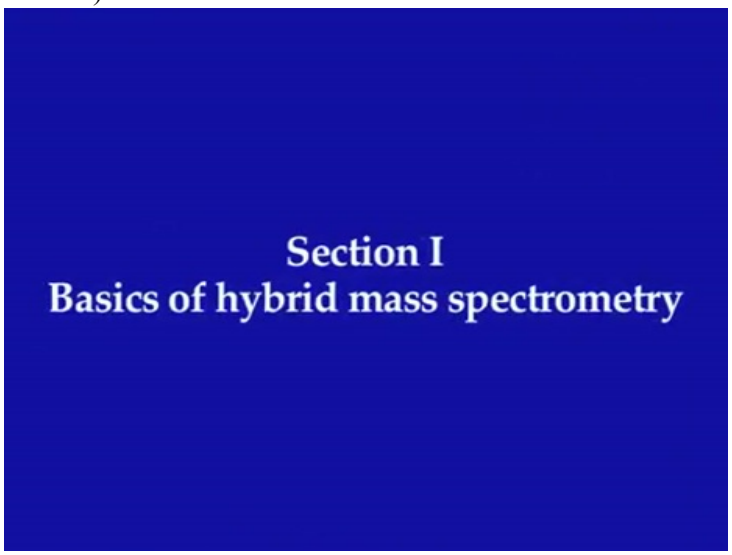

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Today we will talk about

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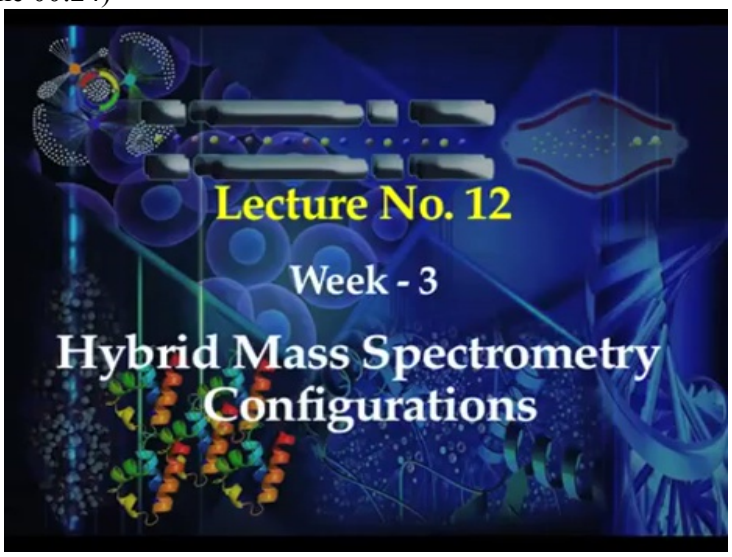

…hybrid MS/MS configurations

As we have discussed that there are different type of mass spectrometers available, and depending upon the mass analyzers how they can be used together, different type of MS/MS configurations hybrid MS/MS have emerged.

So, we have discussed that different type of hybrid MS or tandem MS can be used for various proteomic applications.

There are several new advancements which have happened in this field, and to keep up the pace for these recent advancement; in today's lecture…

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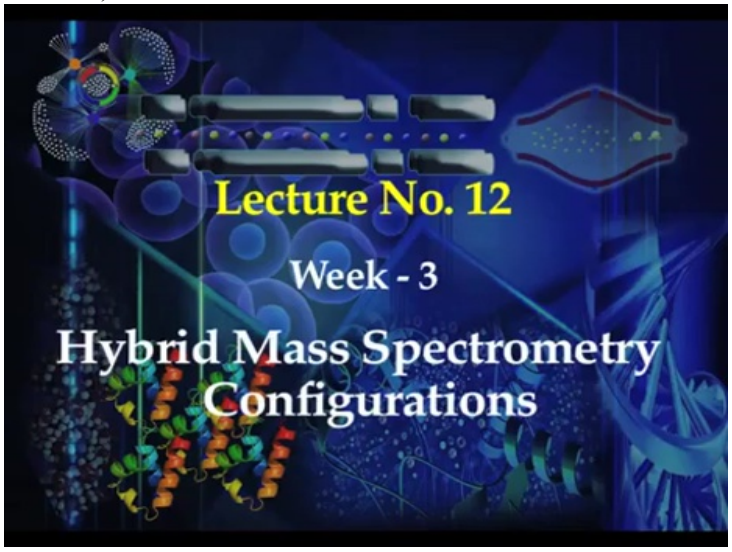

…I thought to involve a discussion on hybrid MS/MS configurations, and talk with two leading companies;

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…one with the Agilent technologies for Q-TOF and triple Quadrupole, as well as the chip technology,

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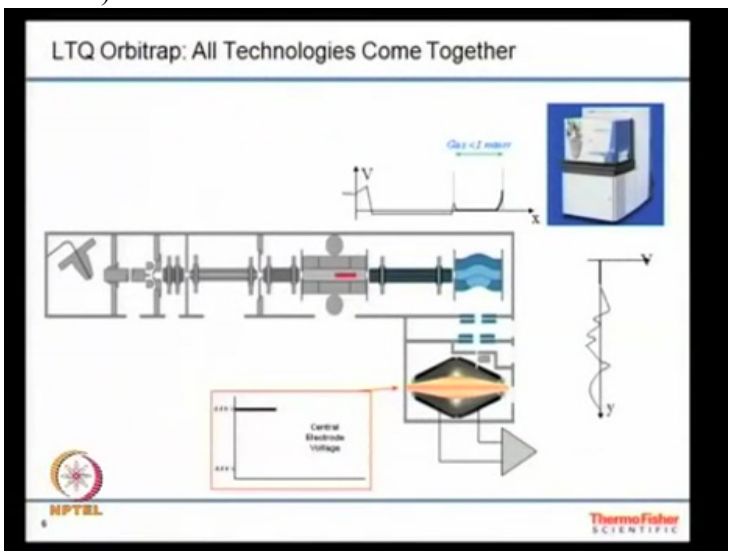

…and then with Thermo Fisher about the Orbitrap technology So, we will discuss…

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…about Quadrupole Time Of Flight and triple Quadrupoles

What are the advantages of using these types of hybrid configurations? What is the latest chip technology? How it can be used to overcome several limitations of HPLC based method liquid chromatography which is used prior to the ionization methods?

So, I will discuss this thing with one of the leading application expert from Agilent, Mr. Abhijeet. And during this short discussion and interview, we will try to provide you an overview of different type of latest configurations available, and what are their advantages.

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Professor Expert conversation start

Professor: Hello, it is my pleasure to introduce Mr. Abhijeet Kanungo, who is product specialist in the mass spectrometry division of Agilent technologies in India.

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Professor: So, welcome Abhijeet…

Expert: Hello

Professor: ... for this brief discussion session on mass spectrometry and your experience in this area.

Expert: Yeah

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Professor: So, first of all how long have you been working in the mass spectrometry division of Agilent?

Expert: I have spent almost my nine years in mass spectrometric field, out of which I am completing almost four years in Agilent technologies to take care of Agilent mass spectrometry-based product line to support entire sales team for the technical support

Professor: So, can you mention what are the major applications of mass spectrometry, on both small molecules and large molecules such as proteomics?

Expert: Yeah. If you ask me, mass spectrometry is wonderful technique, and I majorly classify into two types of application; one is the small molecule applications, second is the large molecule application.

Small molecule applications involves many, many applications starting from the drug discovery, drug development, forensic applications, food safety analysis, dope analysis, many other applications are available with this small molecule.

But when you look for the proteomics application, that is one of the... for large bio-molecule application; proteomics is one of the application where you really need to explore the possibilities and features what mass spec can support for this application.

If you look at for the, starting from intact information, then peptide mass fingerprinting, or a post transitional modifications, or drug discovery or in terms of biomarker discoveries, or protein-protein interactions, drug protein interactions, all such applications are very well possible with mass spectrometry-based solutions.

Professor: What is some of the major shortcomings you foresee in a mass spectrometry based applications which are currently being used?

Expert: If you ask me, in principle mass spectrometry-based proteomics particularly, liquid chromatography coupled to electro spray ionization technique has provides very high throughput application, unbiased identification and characterization of proteins in biological samples.

Moreover, multiple techniques are available today, to monitor these changes in protein expression as well as post transition modification studies.

However, people think that mass spectrometry suffers from limited dynamic range or a finite acquisition rate, but it is not true. It is not really true.

Many new innovations have been implemented to overcome all these issues, and all these innovations with the goal to improve detection of low abundance proteins and rare post transition modification studies.

Professor: So, what MS based instrumentation and technologies Agilent is able to provide in the field of mass spectrometry?

Expert: Mass spectrometry, the field of proteomics is rapidly expanding, and it is just about every aspect; any scientist we are looking for each and every aspect which protein research involves from detection and characterization, or to biomarker discovery and the quantitation studies.

Protein analysis has many challenges, so Agilent has a complete solution, if you look at from Agilent offers that chip based Q-TOF proteomics; integrated proteomic solutions. And integrated proteomic solution is include; it includes advanced LC MS platforms with unprecedented plug and play flexibility, if you look at its not only instrument you have to take care of many things.

Starting from the best of mass spectrometer detector, the software which assist you to get the desired information's and the sample preparation, so Agilent, in nut shell, Agilent has complete end-to-end solutions for all your proteomics analysis needs.

Professor: So, what is this chip-based technology which you just mentioned?

Expert: If you ask me about the chip-based technique, the very biggest problem comes when you work with the protein sample is, you have a very low concentration and low volume of samples.

Professor: Right.

Expert: And to work with all these low volume, low concentration samples you have to work with the nano-HPLCs, and traditional or conventional nano-LCs has a biggest problem of the leakage. As soon as the leaks is out, it is very different to identify the where the leak is going to happen.

And what is the problem? Why the leakages cannot be detected? The reason is it has lot of nuts, ferrules, tubings, fittings; because of that you cannot detect the leakages.

In fact, I am carrying a chip with me. If you look at conventional nano-LCs you have lot of fittings, columns, consumables, because of that any leakages are there you cannot detect the leakages, right? So, Agilent has come up with the excellent solution

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Expert: This is the chip technology where you have a sample enrichment capability over here, so once you sample is enriched by the capillary pump, then the nano-flow pump comes and takes a sample to the nano columns, and in fact you have a ionization source itself on the

chip. So, it is completely integrated to avoid all the complications of conventional nano-LC, this…

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Expert: …This chip technology has the best solution available today, and it is one of the best thing available for any of the proteomics lab.

Professor: So, now as mass spectrometry technology is very much revolutionizing all the aspects of life science research, and it is heavily used in the clinical proteomics and clinical research. How do you foresee, why is still the mass spectrometry is not so much used in the clinical set exist al, in the clinical hospitals?

Expert: In fact, I think the mass spectrometry is waiting to make the changes in the industry; it will have a huge impact especially on the infectious disease. If you look at, instead of many biological based methods one should use the mass spectrometry.

Mass spectrometry is very, very simple, it gives you information in a minutes and saves time, money and more accurate results, the mass spectrometry is the basically a transformative technique.

But the only question is how fast it will be adopted by the scientific community? That is the one thing analysts have, and most of the analyst have perceptions that this instrument has some limitations, but it is not true. This is not user friendly technique, but it is not true.

This instrument is very simple, easy to use; get results in a minutes and more accurate results.

Professor: So Abhijeet you rightly mentioned, and in fact I will mention here that although the cost is one of the limiting factor for adopting these mass spectrometry-based technologies in the hospitals and clinical setting.

But nevertheless even in Mumbai, in different hospitals when I visited, I saw like lot of these mass spectrometers are actually being integrated for various type of diagnosis. So, that is actually I see one of the very good change

Expert: Yes

Professor: In terms of using the technology, and directly providing the results for the deciding what type of treatment and drug or dose patient should get.

Expert: Yes, I am 100% agree with you. Even I have seen most of the hospitals nowadays having a mass spectrometer for the clinical applications.

Professor: Right.

Expert: And majorly, I have seen for the small molecule application, but nowadays they are looking for the proteomics-based or clinical trials applications, and it starts from various research institutes. Most of the research institutes these who are doing research on the clinical-based proteomics, they started using mass spectrometry

Professor: Right. Can you briefly describe about some of these technologies in which you mentioned as a triple Quad and chip-based technologies?

Expert: We, Agilent has a very good solutions, and if you look at the point of triple Quadrupole. In fact, I have some videos to show you. Look at here, these videos explains you what all techniques are available and how it is useful for proteomics applications.

Professor Expert conversation end

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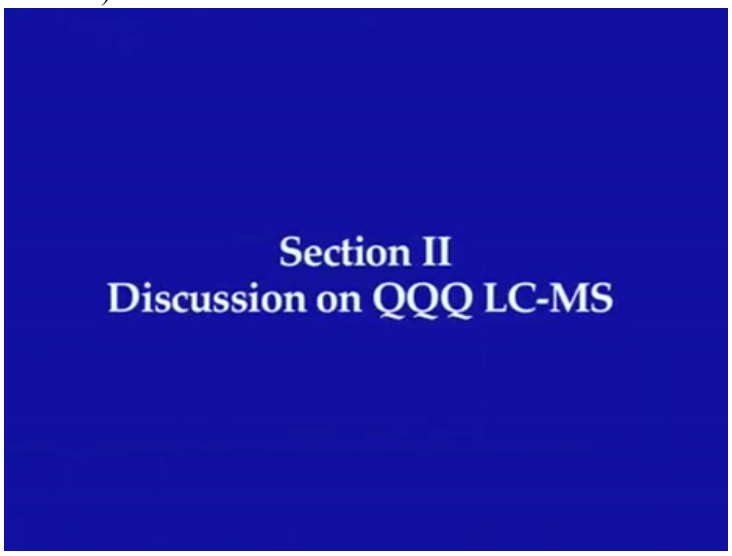

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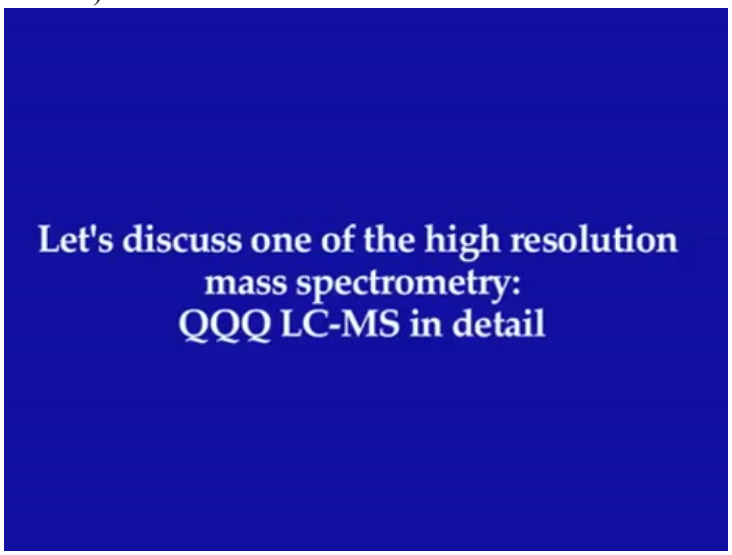

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Video narration starts

Whether you quantitate drug metabolites…

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…measure herbicide levels in fluids or….

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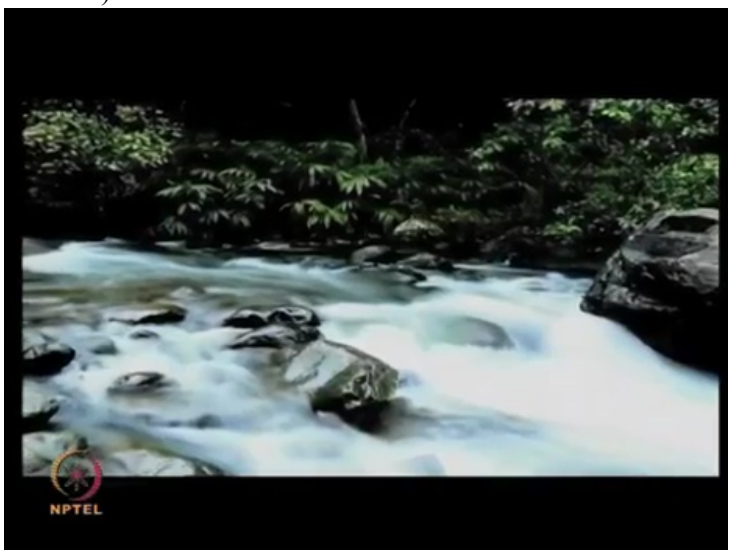

…determinate contaminant levels in ground water…

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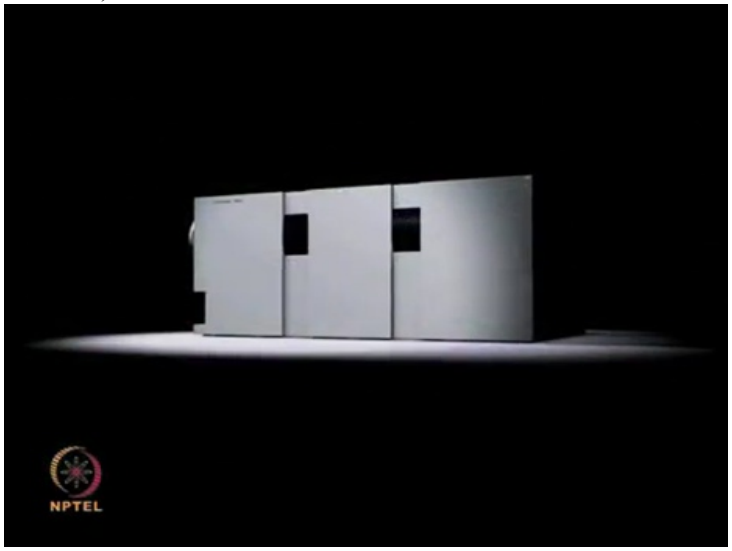

…the triple Quadrupole Mass spectrometer is unequalled for quantitating trace organic compounds in complex matrices.

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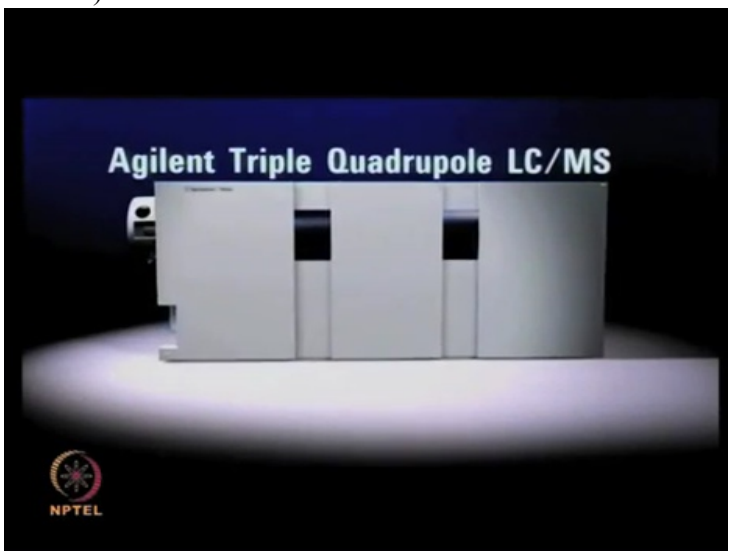

The Agilent triple quadruple LC/MS delivers outstanding sensitivity, great ease of use and legendary Agilent reliability…

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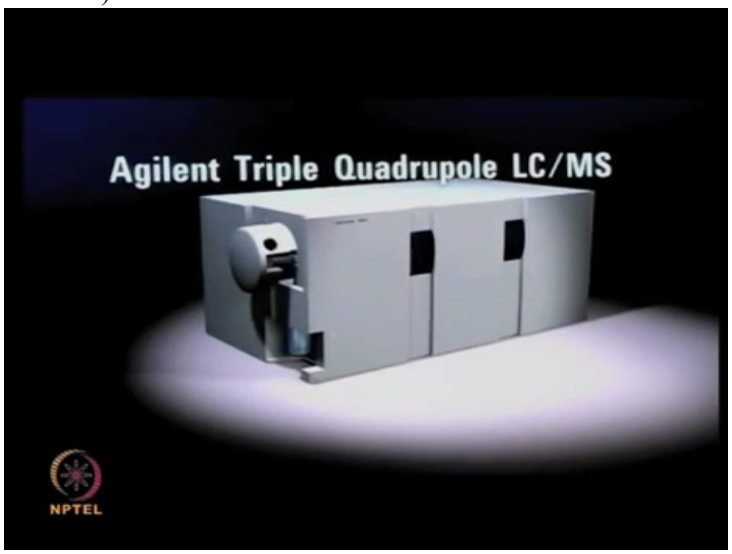

… all at a very attractive price

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Great sensitivity starts with superior ionization technology. Agilent's LC/MS ion sources…

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…use our patented nebulization technology and high-volume counter-flow drying Gas …

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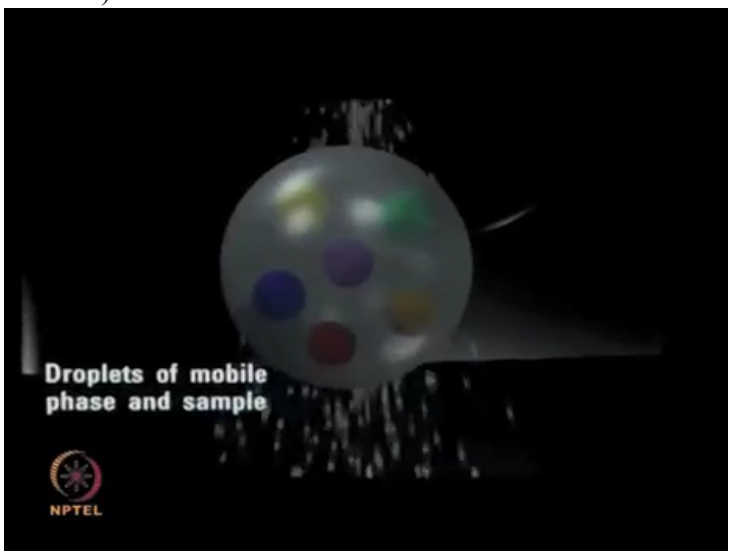

… together they reduce noise related to incomplete drying of solvent droplets …

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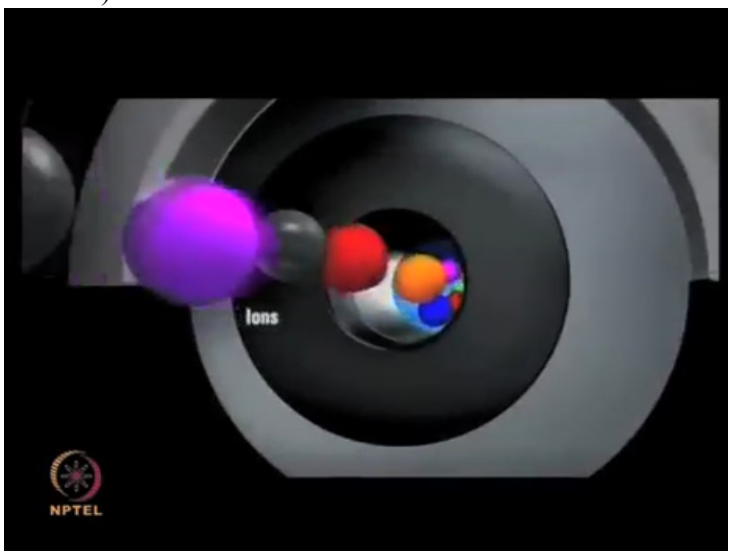

…and keep the …

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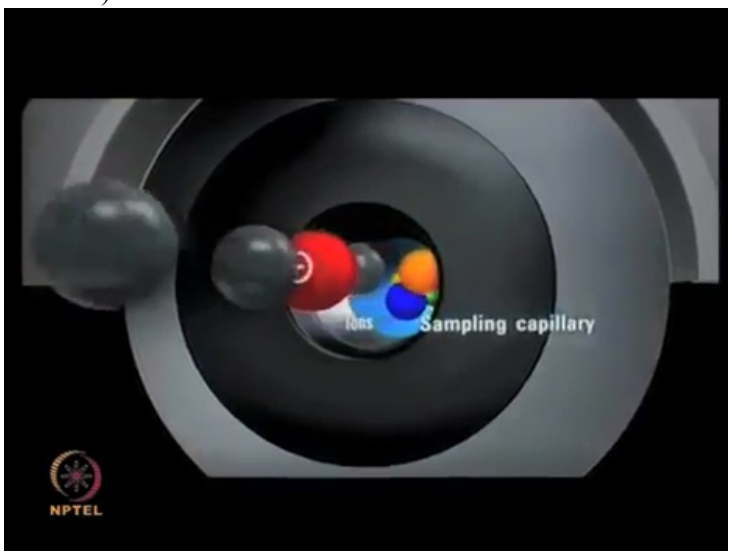

… sampling capillary and ion optics cleaner for…

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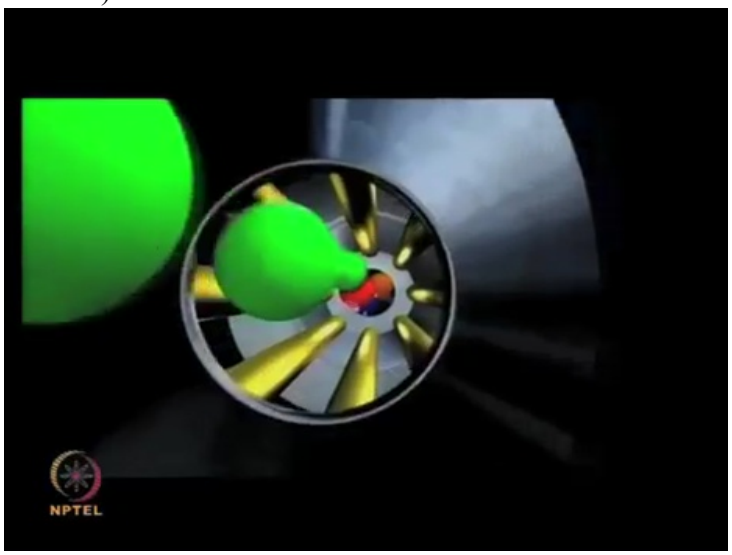

…reduced maintenance

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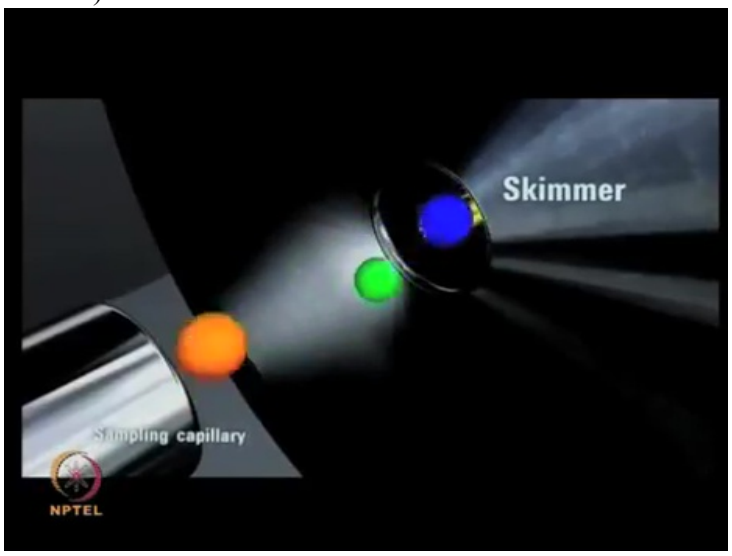

A thin Skimmer aperture, carefully matched hole size, and short capillary-to-Skimmer distance reduce beam broadening.

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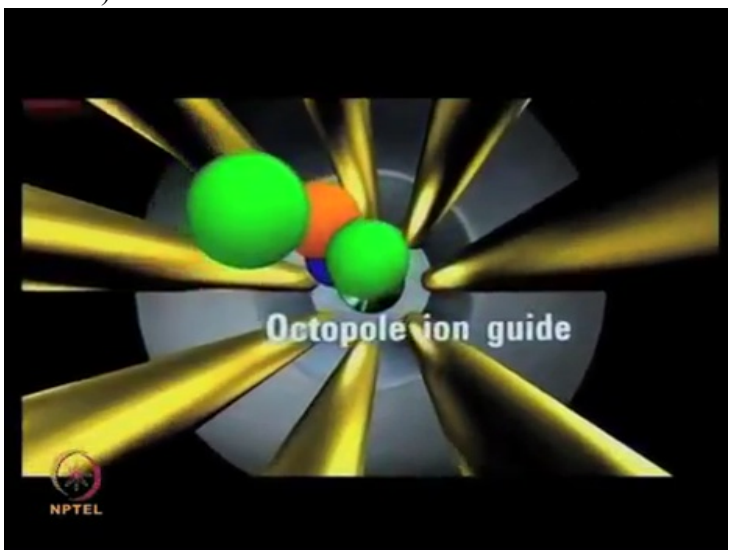

An Octopole ion guide provides better ion transmission over a wider mass range…

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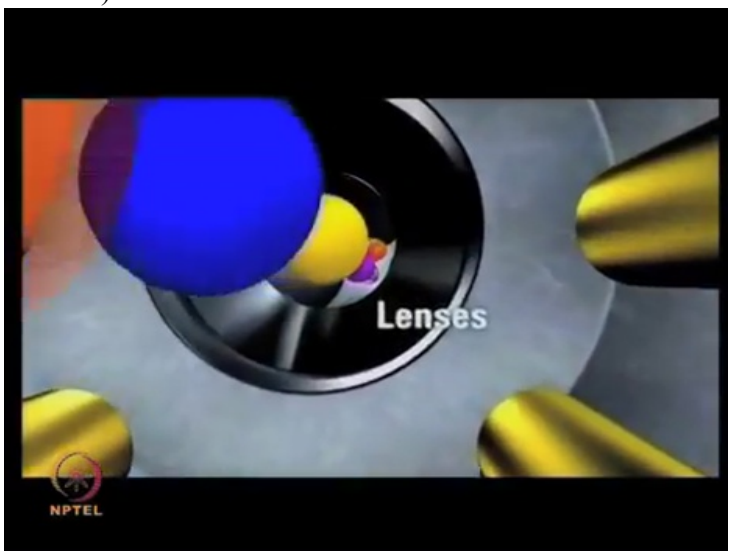

…patented lenses enhance high mass ions transmission and increase sensitivity over a wider mass range.

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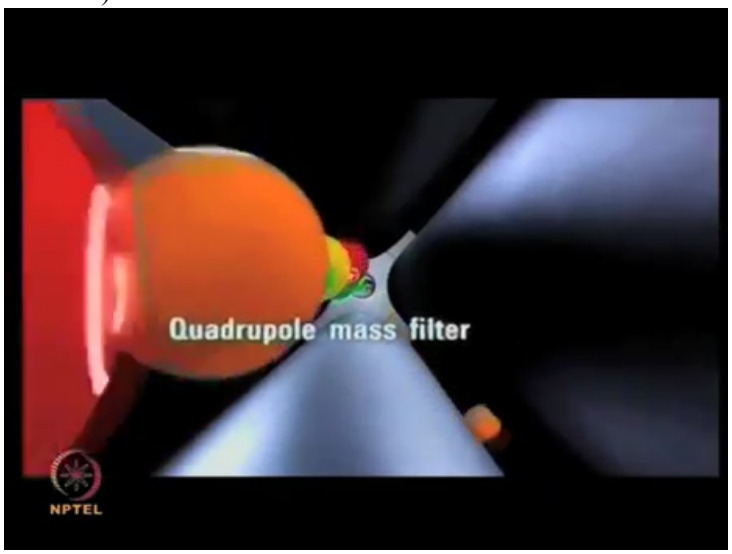

The first Quadrupole mass filter allows only ions of the target mass to pass through, the hyperbolic shape of the rods enhances ion transmission and spectral resolution.

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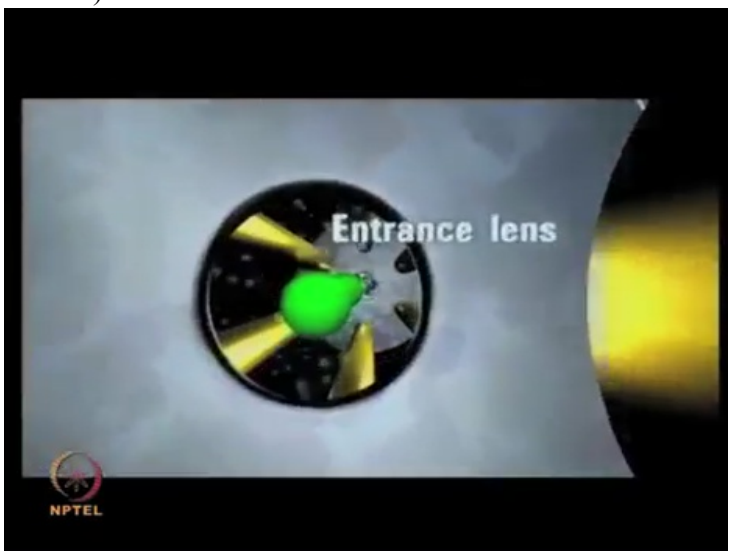

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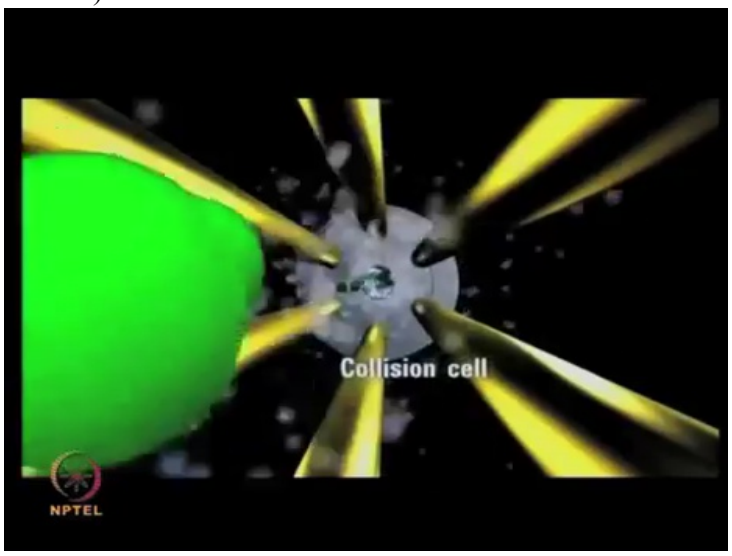

In the hexapole collision cell, precursor ions strike collusion gas molecules generating product ions and neutral fragments.

Linear axial acceleration and high collision gas pressure simplify operation, and ensure fast sensitive MS/MS without cross-experiment memory effects.

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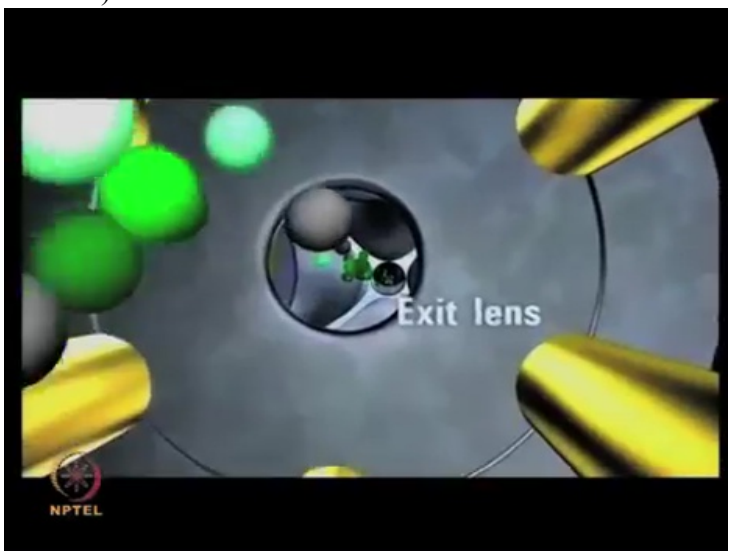

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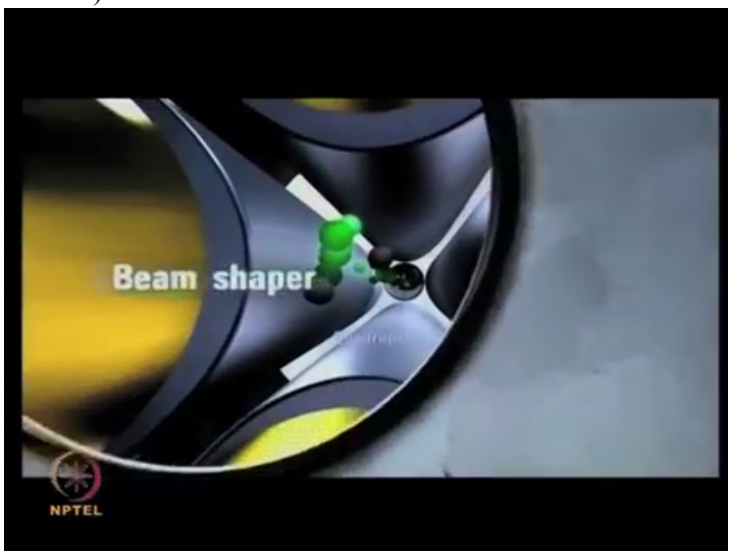

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The second Quadrupole serves as a mass filter for the product ions produced in the collusion cell. For quantitative analysis of the target compound, the second mass filter is operated in a selecting ion monitoring mode.

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In the detector…

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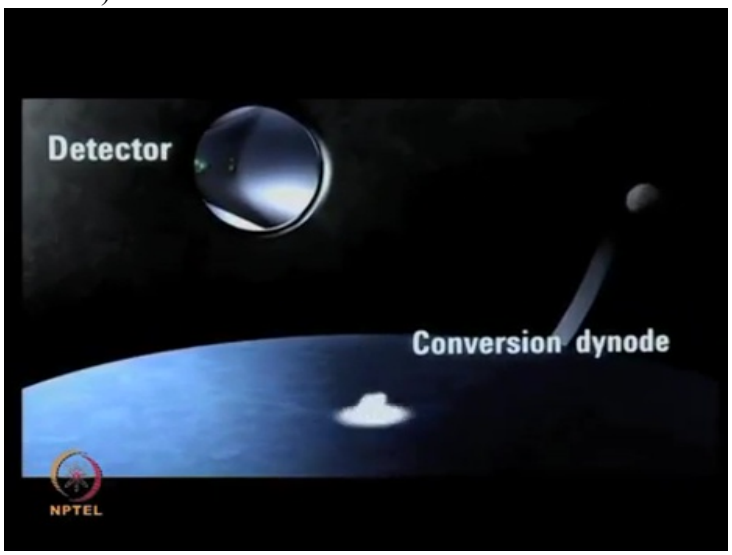

…Conversion dynode operates at 10000 volts to improve sensitivity; because the conversion dynode is off the main axis of the ion path…

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…neutral molecules miss the dynode thus eliminating neutral noise.

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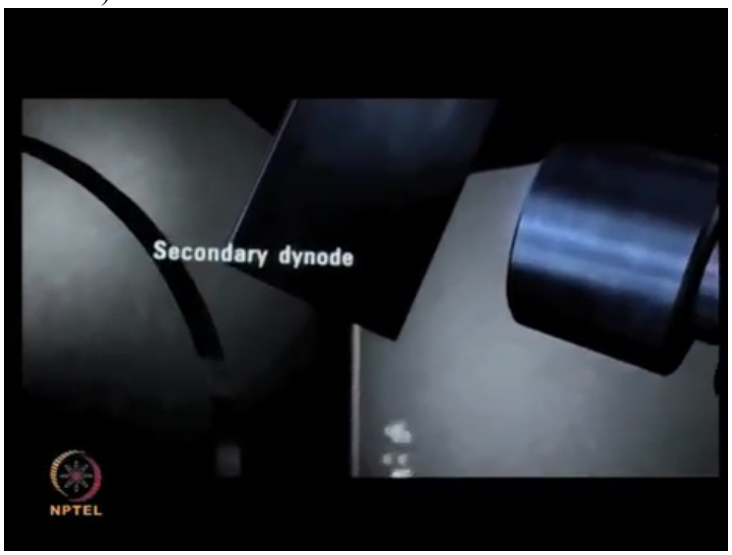

A secondary dynode helps to extend the useful life of the electron multiplier.

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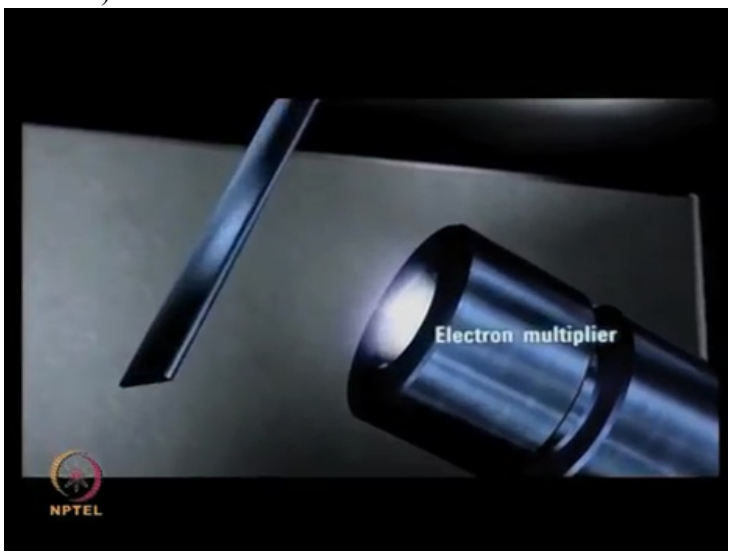

The electron multiplier has a long life but it is also easily replaced.

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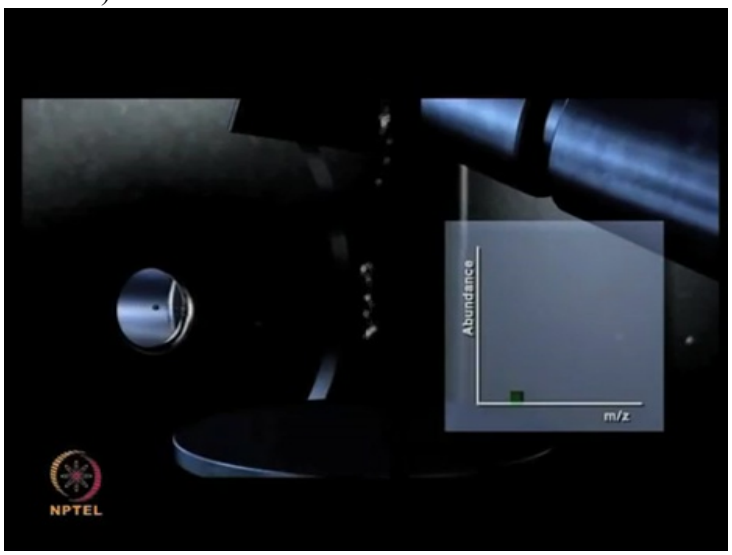

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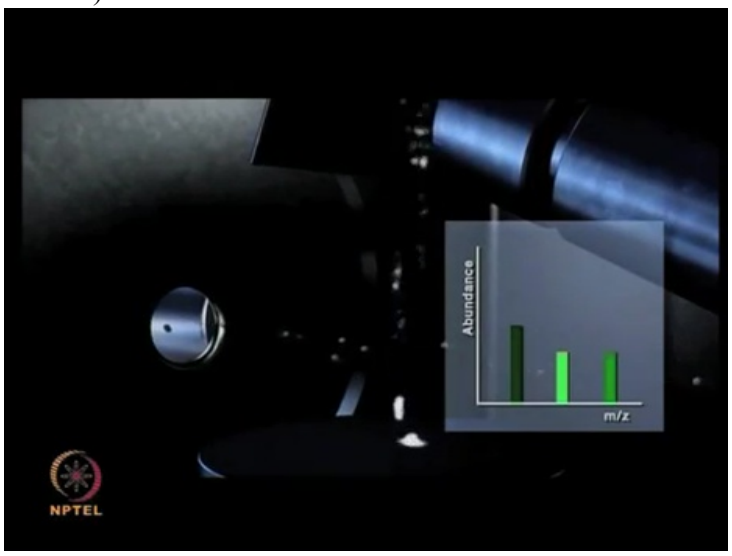

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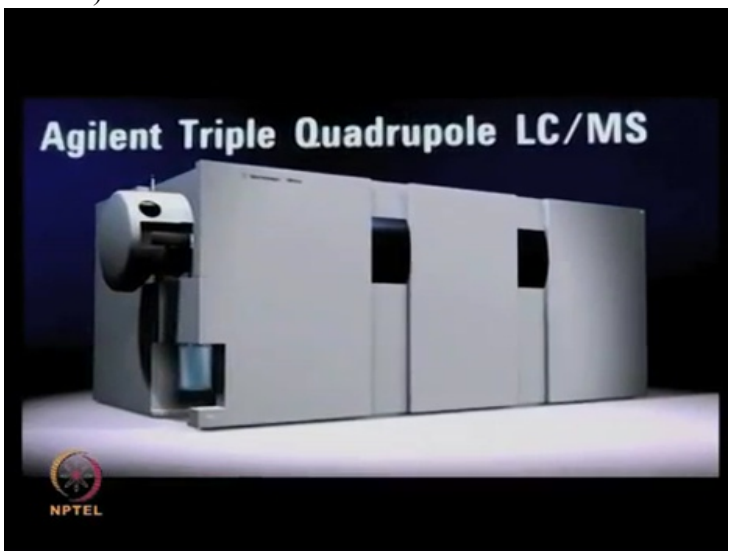

The Agilent 6410, Triple Quadrupole LC/MS establishes a new standard for value in a triple Quadrupole mass spectrometer.

It delivers outstanding sensitivity, great ease of use, and legendary Agilent reliability, all at a very attractive price.

Video narration ends

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Professor Expert conversation start

Expert: Now, we have seen how the triple Quadrupole is the best technique for the quantitative application, now let us look for the Q-TOF technology; how it is useful for the proteomics application...

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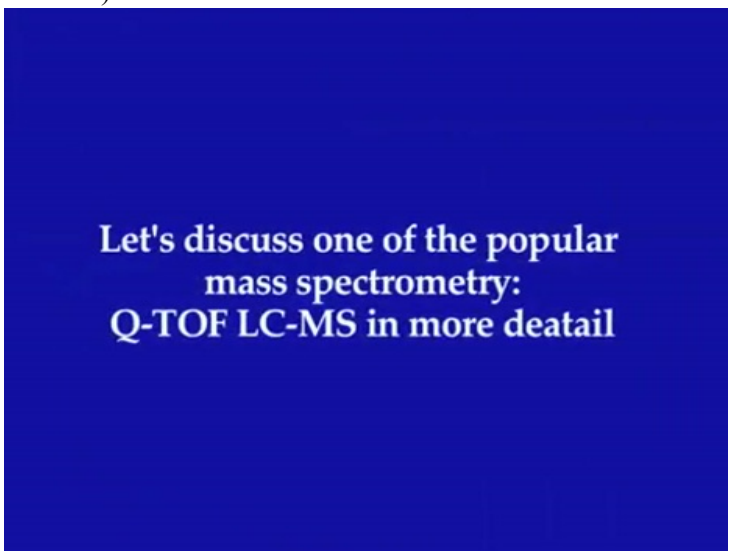

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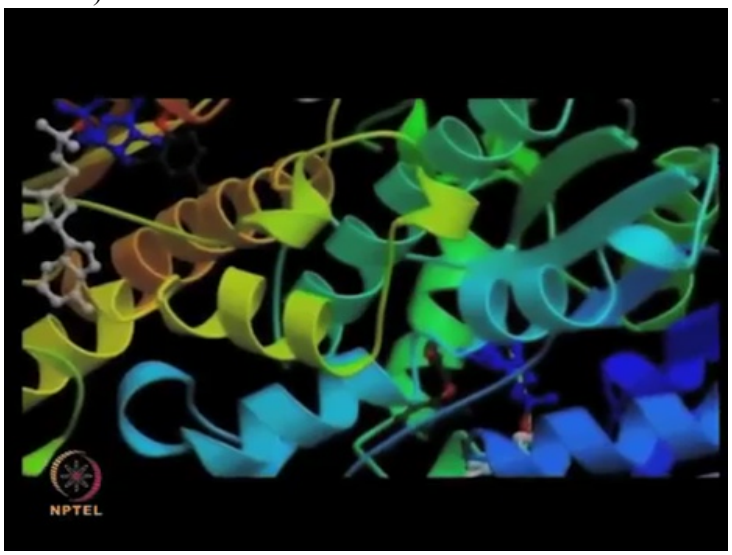

Video narration starts

Whether you are identifying proteins ….

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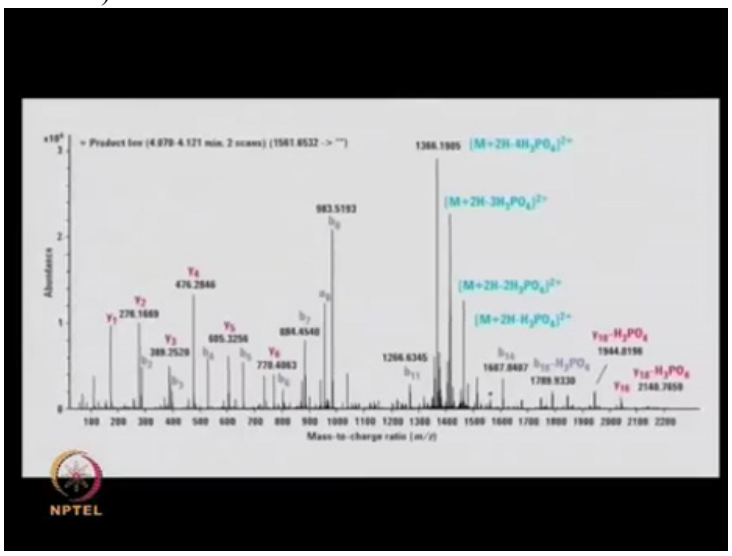

…and characterizing post-translational modifications…

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…, searching for metabolite biomarkers or …

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…finding impurities in pharmaceuticals or fluid …

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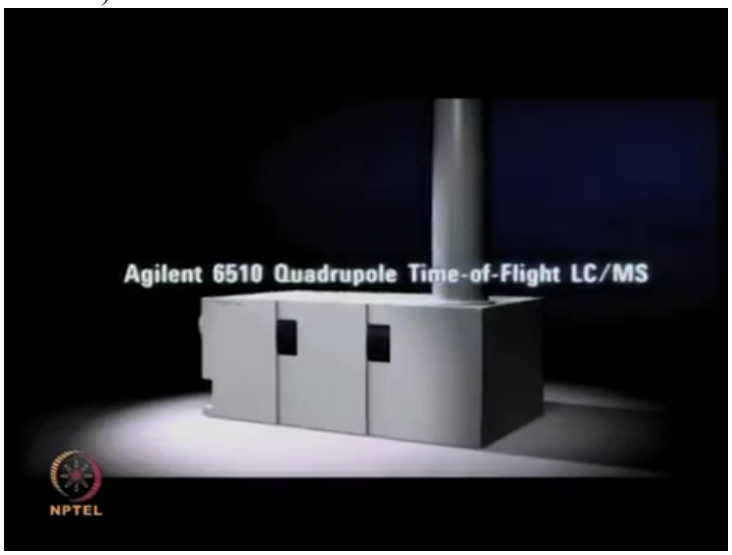

…the Agilent 6510 Quadrupole Time-Of-Flight LC/MS is an outstanding choice.

It delivers better than two ppm mass accuracy for MS, and better than five ppm mass accuracy for MS/MS. It also delivers wide in-spectrum dynamic range and unsurpassed Q-TOF sensitivity, all in reliable and easy to use system.

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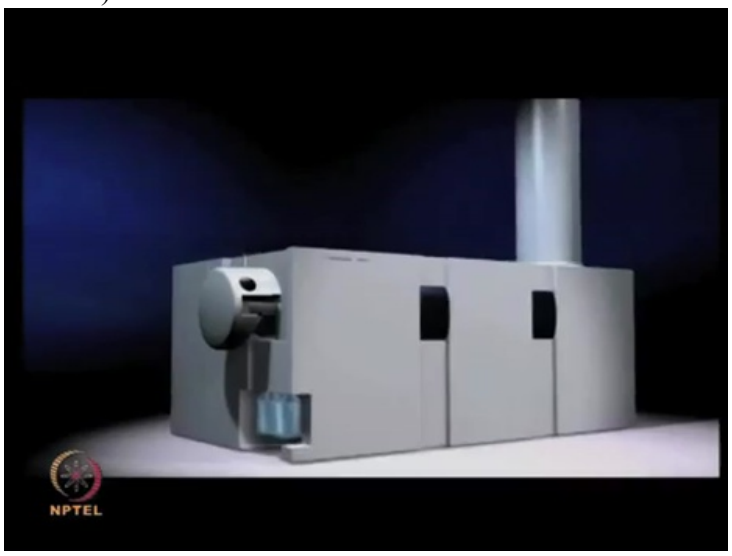

Great sensitivity …

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…starts with superior ionization technology

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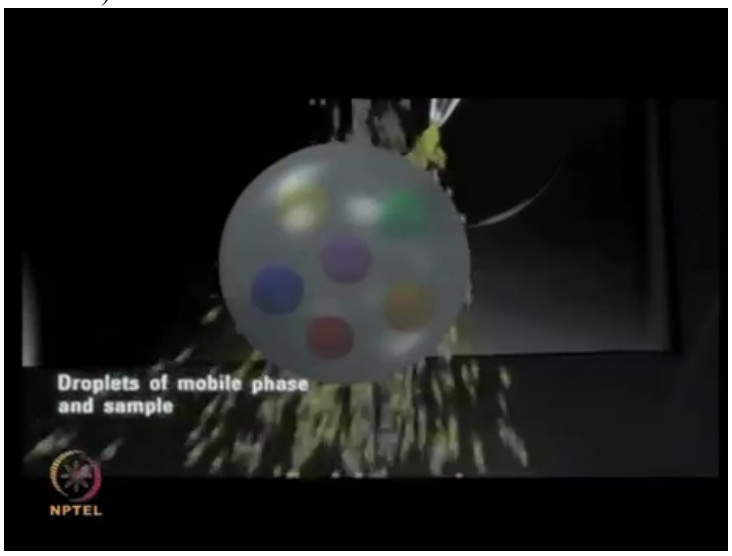
Agilent's patented nebulization technology produces finer droplets and delivers more ions to the mass spectrometer.

(Refer Slide Time 16:00)

A second nebulizer introduces reference mass solution that ensures continual mass access correction to the best possible mass accuracy.

(Refer Slide Time 16:10)



High-volume counter-flow drying gas reduces noise related …

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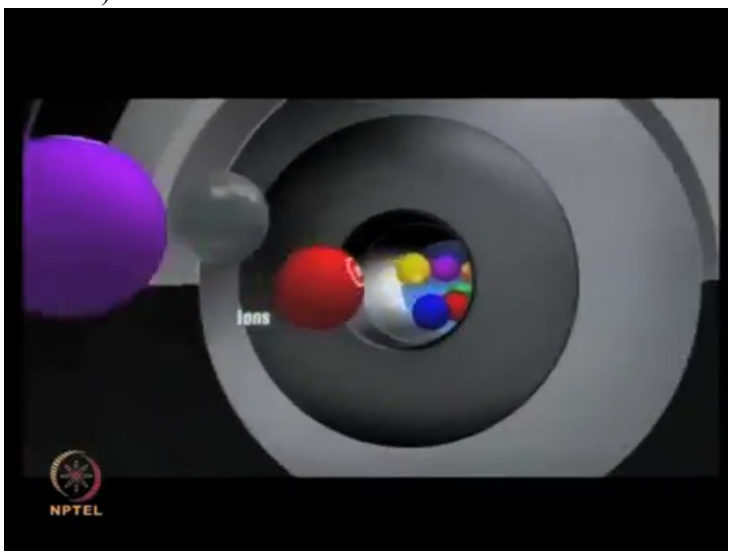

…to incomplete drying of solvent droplets and …

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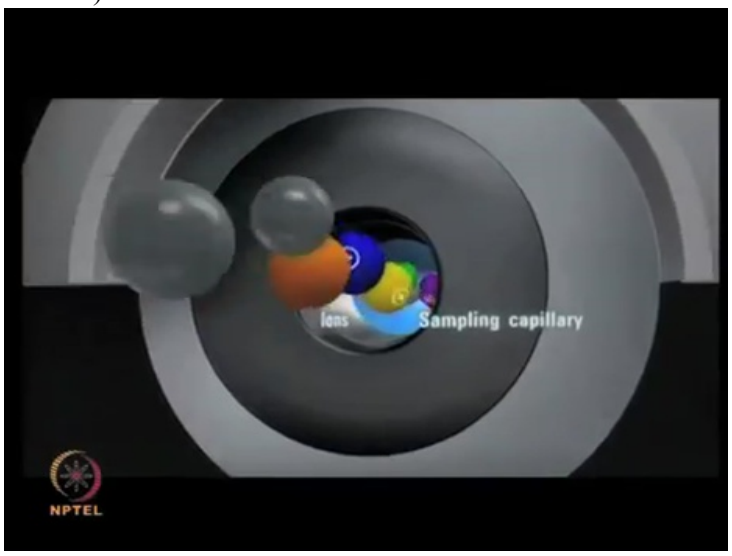

…keeps the sampling capillary and ion optics cleaner for reduced maintenance.

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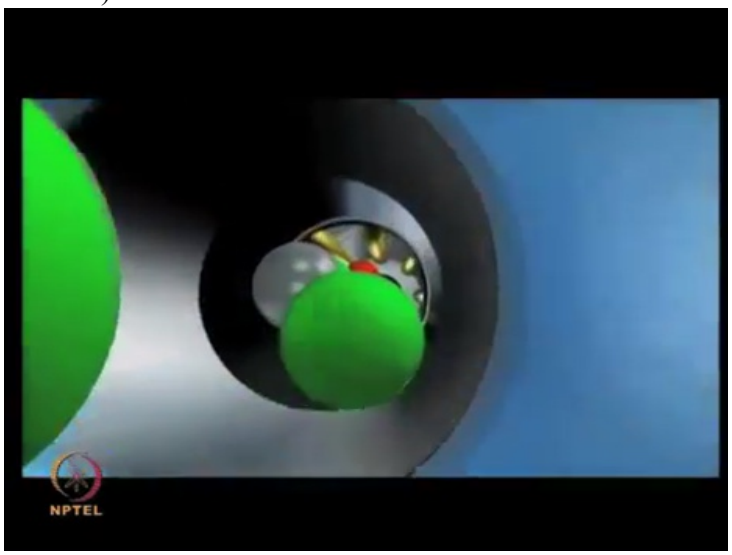

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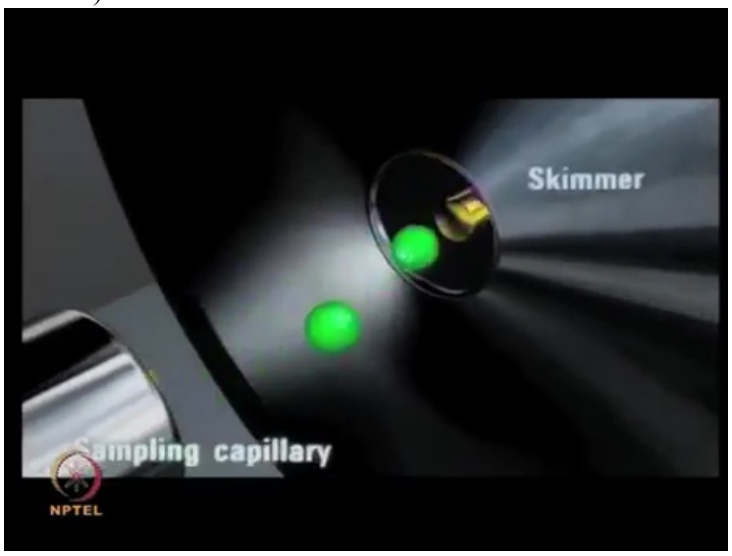

A thin skimmer aperture, carefully matched hole size and short capillary-to-skimmer distance reduce beam broadening.

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And on-access octopole ion guide provides nearly 100% ion transmission over a wider mass range.

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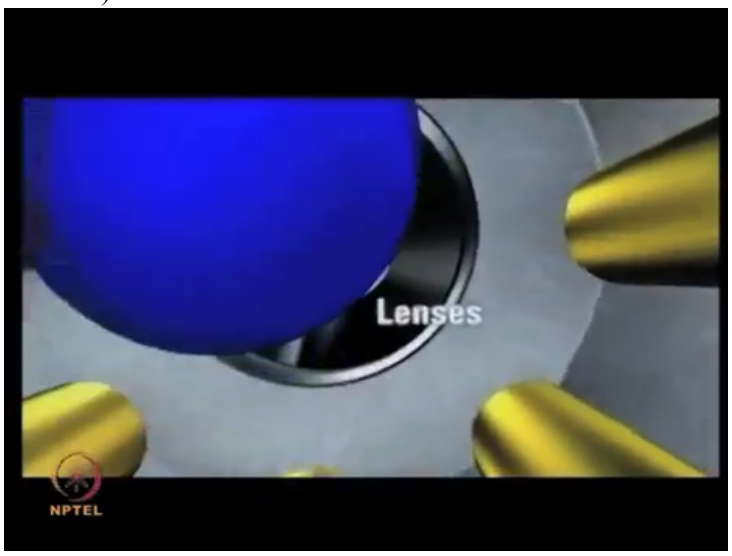

Patented lenses enhance high mass ion transmission and increase sensitivity over a wider mass range.

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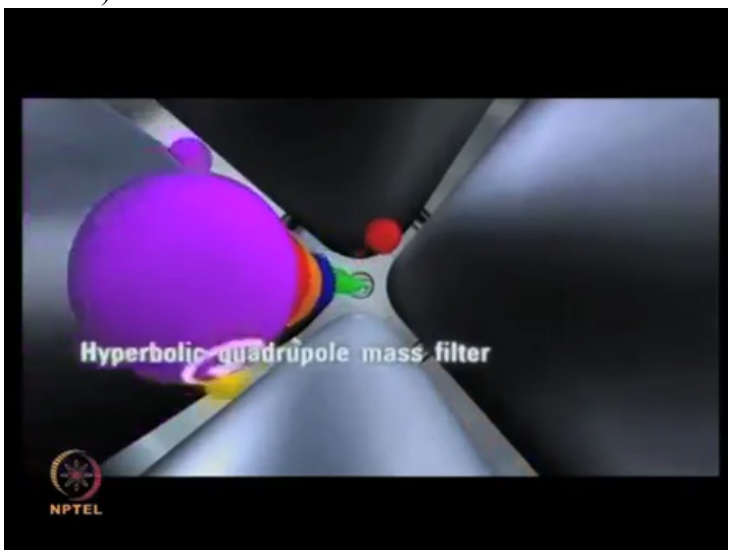

The Quadrupole mass filter allows only ions of the target mass to pass through, the hyperbolic shape of the rods enhances ion transmission …

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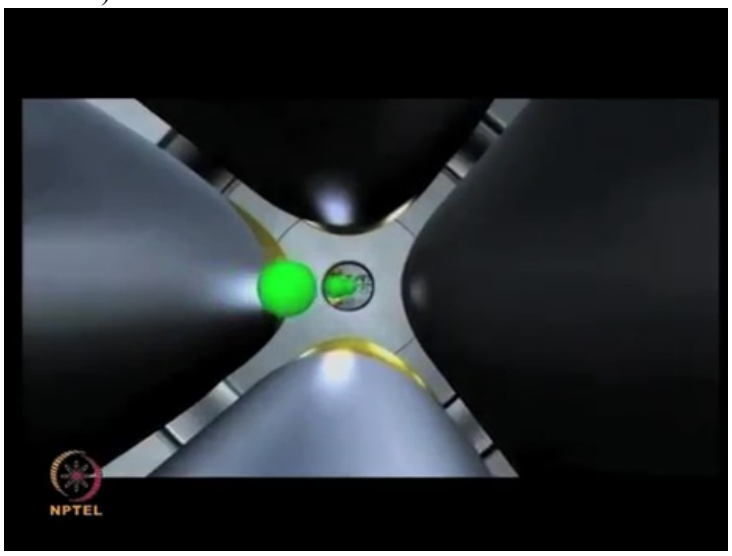

…and spectral resolution

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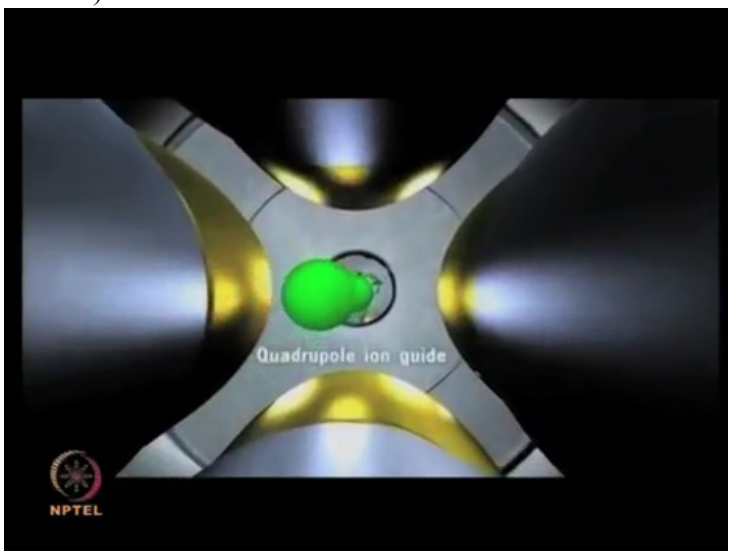

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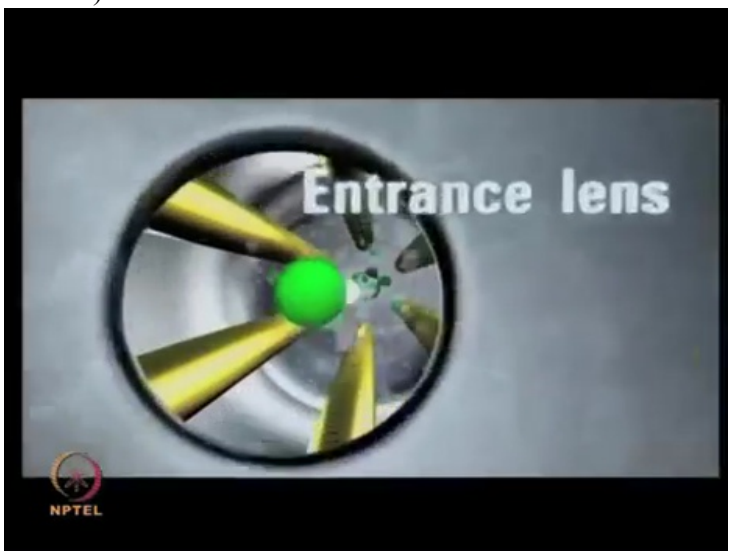

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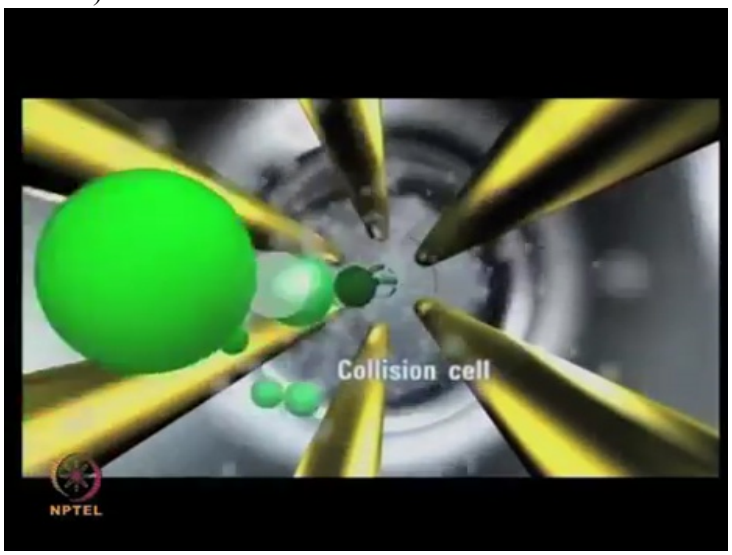

In the hexapole collision cell, precursor ions strike collision gas molecules generating product ions and neutral fragments.

Linear axial acceleration and high collision gas pressure ensures that all ions exit the collision cell with nearly identical energy.

This allows the same mass calibration factors to be applied to MS and MS/MS ions. Result is better than 5 ppm mass accuracy for MS/MS ions.

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Another Octopole ion guide keeps the ions together while allowing excess collision gas…

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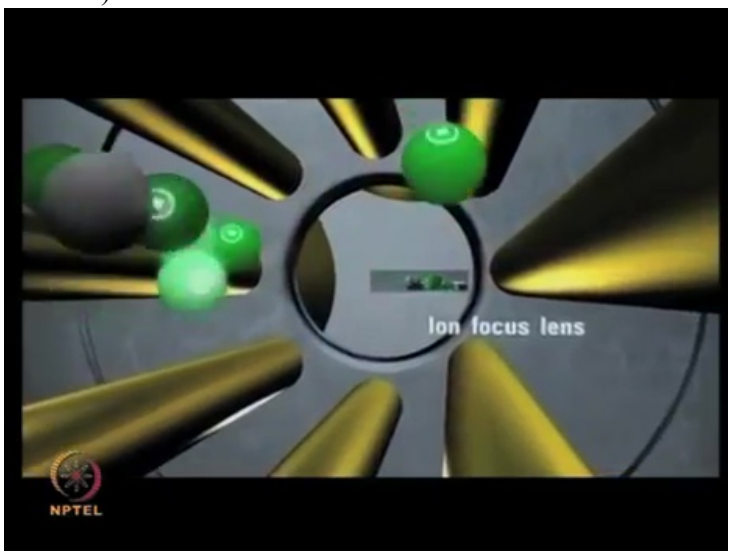

…to be pumped away. A Quadrupole ion guide ….

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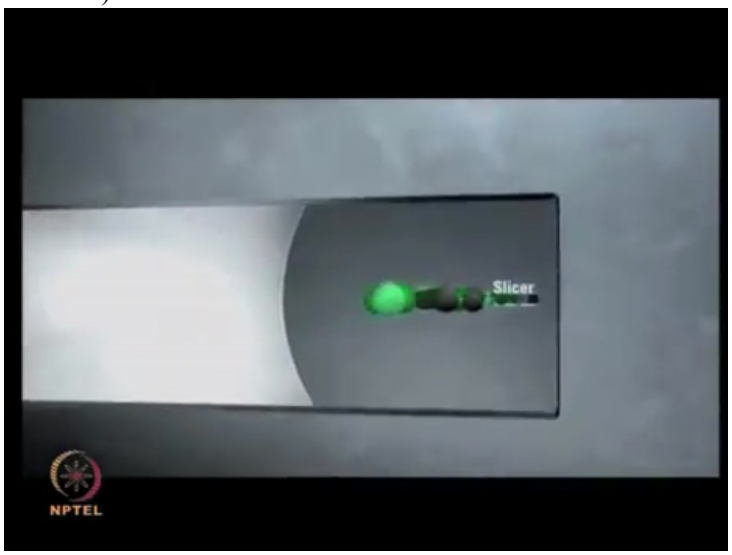
…flattens the stream of ions …

(Refer Slide Time 17:42)



..for better transmission through the slicer The slicer reduces variations in the vertical momentum of the ions.

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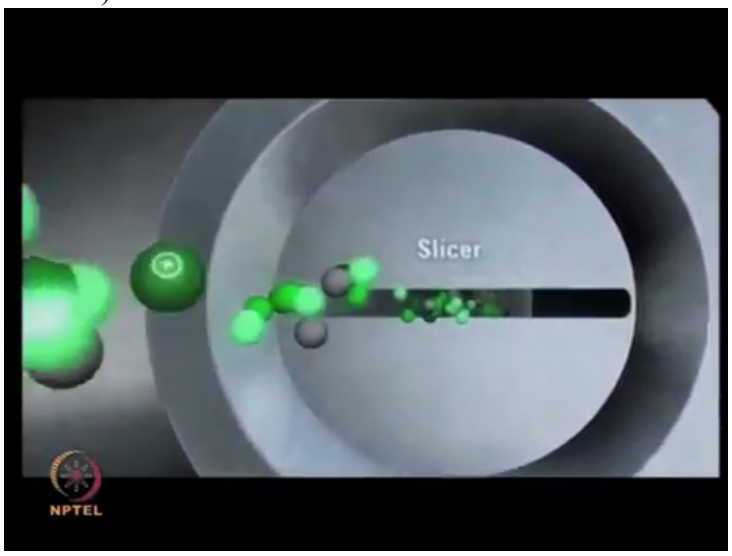

Ions with too much vertical momentum do not reach the pulser.

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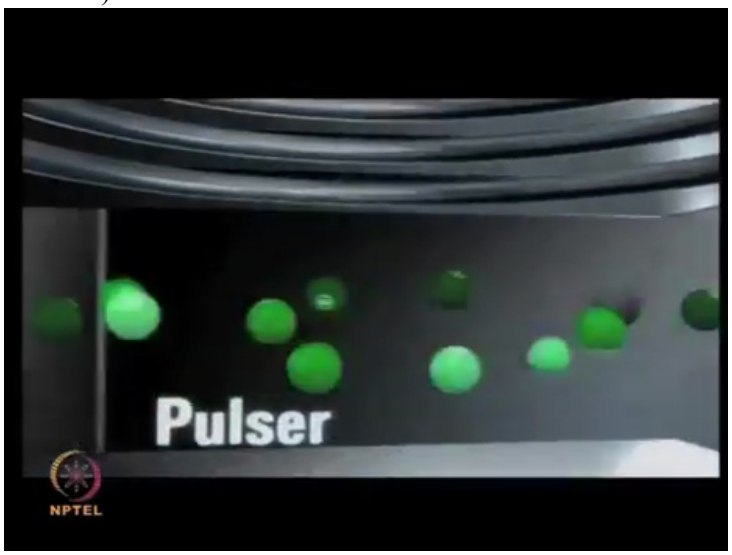

This improves mass accuracy for all ions.

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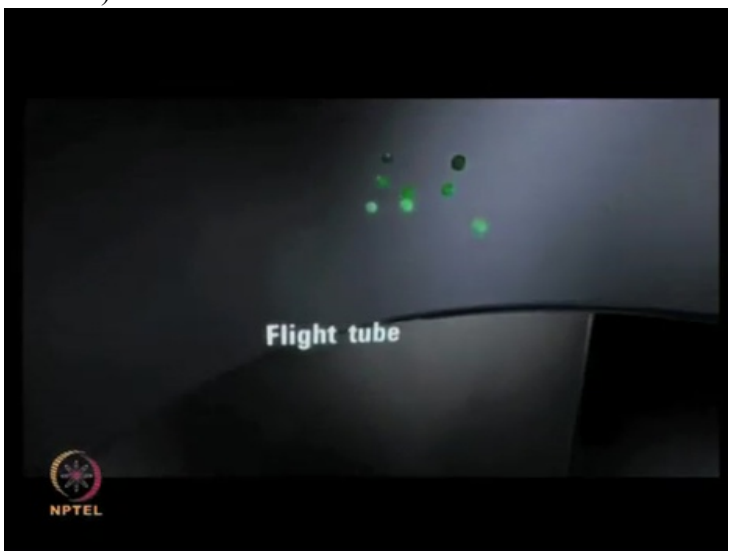

The flight tube is constructed of special materials with very low coefficients of the thermal expansion, so it is less sensitive to temperature changes.

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The Reflectron compensates for minor velocity differences improving the resolving power of the 6510.

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The micro-channel plate detector converts the ions signal from the ….

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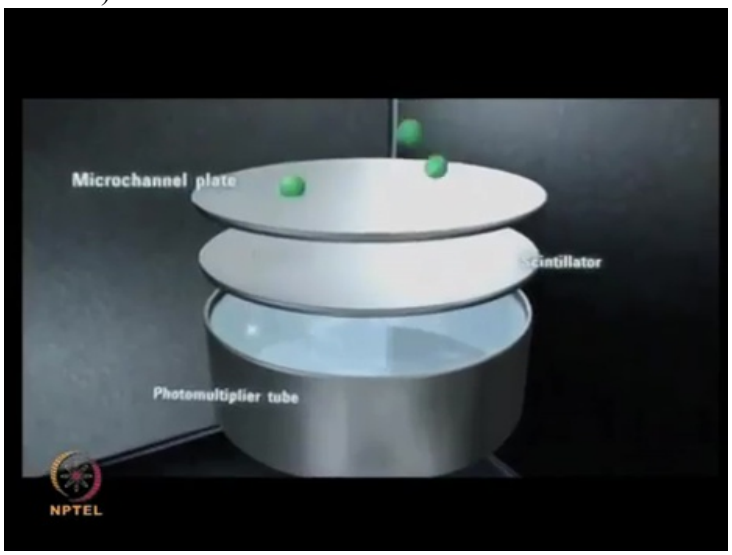

…electrons to photons and back to electrons This electrically isolates the high voltage flight tube and front of the detector from a signal pass to the electronics.

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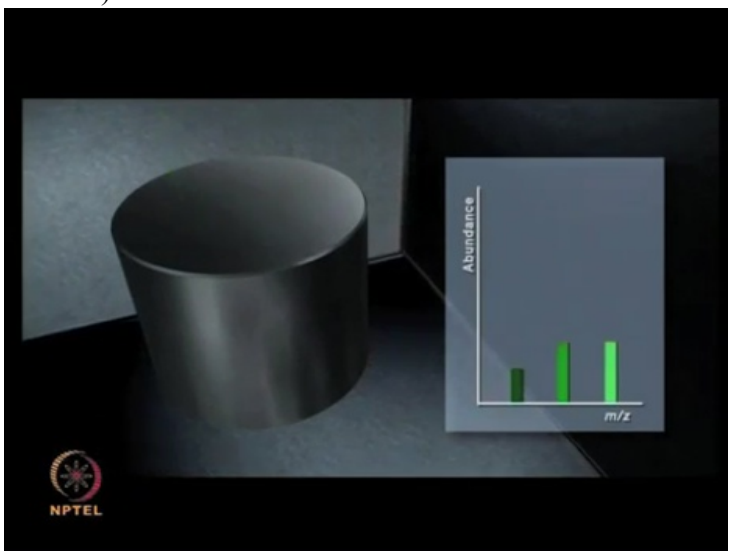

ADC digitizer electronics provide extremely high mass accuracy over a broader dynamic range. The 6510 offers outstanding in-spectrum dynamic range for a time of flight instrument.

When you need the ultimate in LC MS/MS power and versatility…

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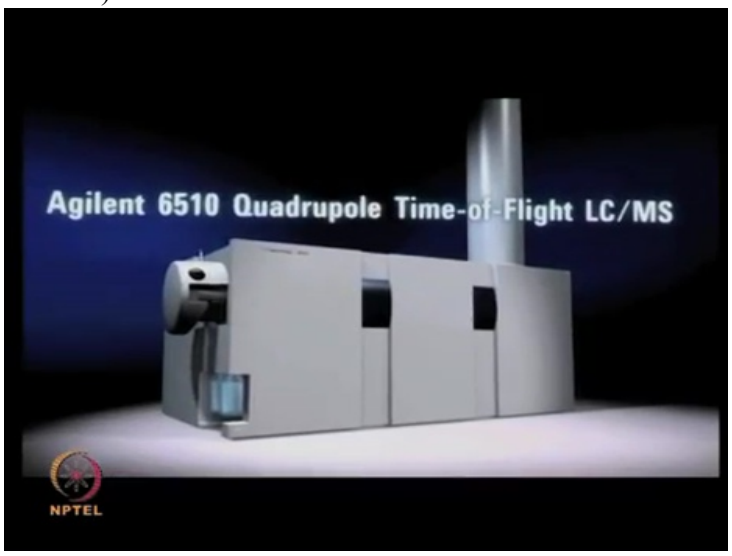

, the Agilent 6510 Q-TOF provides it, and ease of use and reliability, really found in a research grade mass spectrometer.

Video narration ends

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Professor Expert conversation start

Expert: Isn't it good? The Q-TOF technology which we have seen has a lot of tremendous advantages for the proteomics application. We have seen how triple Quadrupole and Q-TOF works for your application, but what about chromatography?

I do have some videos which explain you how chromatography technique; the conventional chromatography technique, and how chip technology has an advantageous feature, look at the video.

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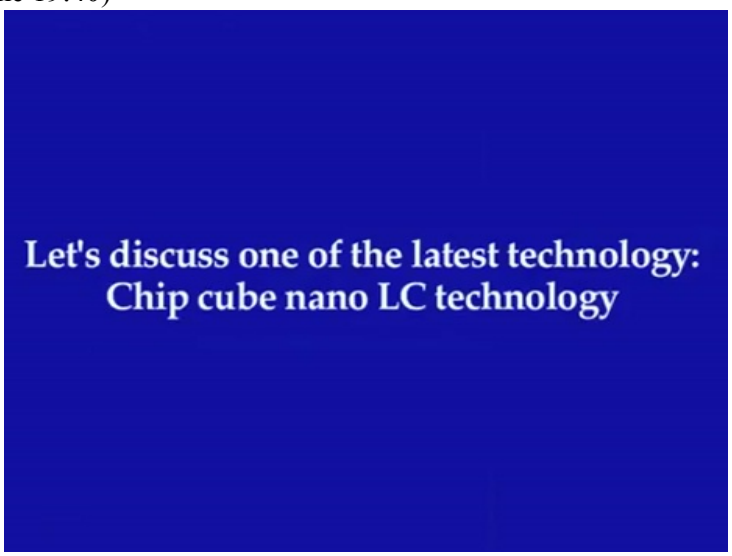

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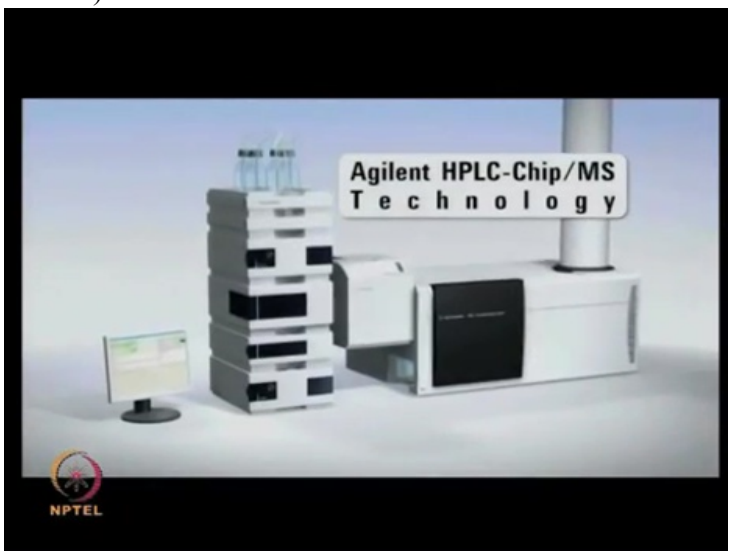

Video narration starts

It is time to prepare for a new generation LC/MS technology from Agilent, HPLC chip/MS.

The Agilent 1200 series HPLC chip/MS platform takes you to a new level of nano-flow LC/MS performance by combining microfluidics, an easy to use plug-and-play interface that lets you focus on your results.

The Agilent HPLC chip/MS platform is based on the Agilent HPLC chip and …

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…Agilent HPLC chip/MS interface, that is designed for use with all Agilent 6000 series mass spectrometers.

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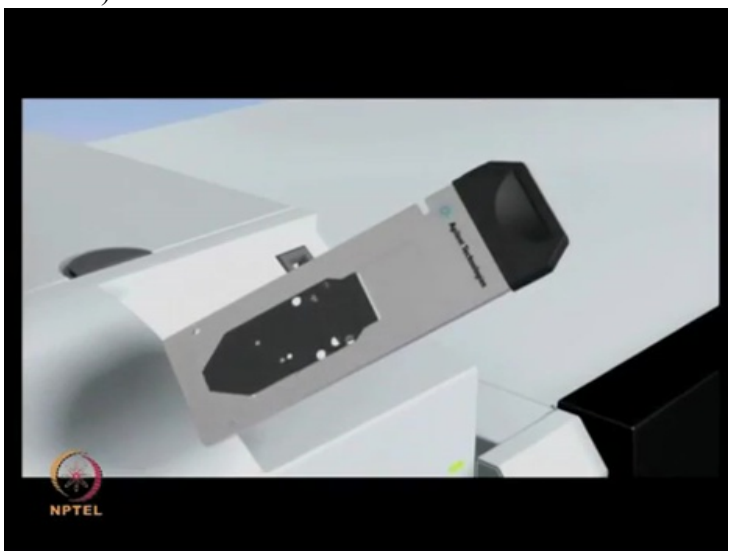

The Agilent HPLC chip integrates enrichment and analytical columns, micro-valve connections…

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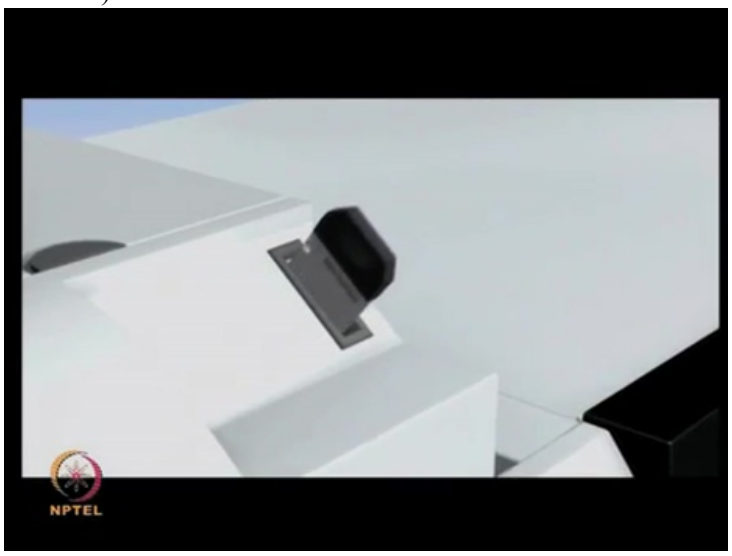
…and …

(Refer Slide Time 20:37)



…metal-coated nano-electrospray tip on an inert multilayer polyamide film and it is smaller than a credit card.

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The compact architecture of the Agilent HPLC chip reduces peak dispersion and combines all steps from sample loading through compound ionization for seamless operation.

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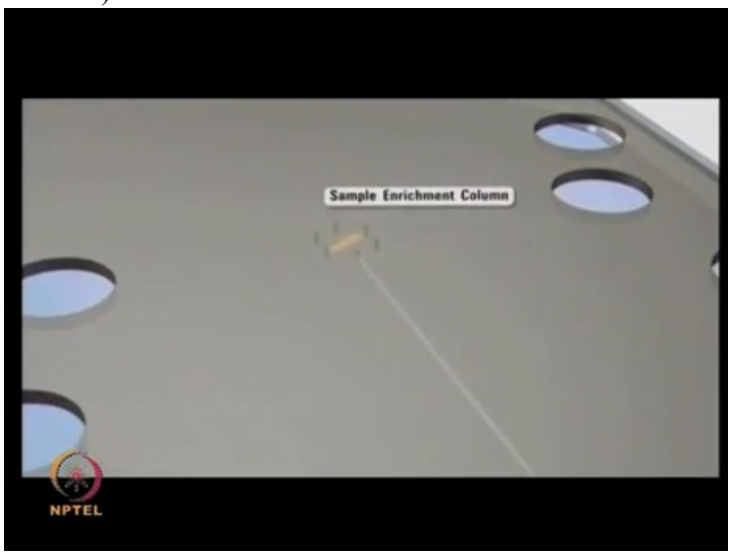

A closer look at the HPLC chip reveals that sample enrichment, and separation columns …

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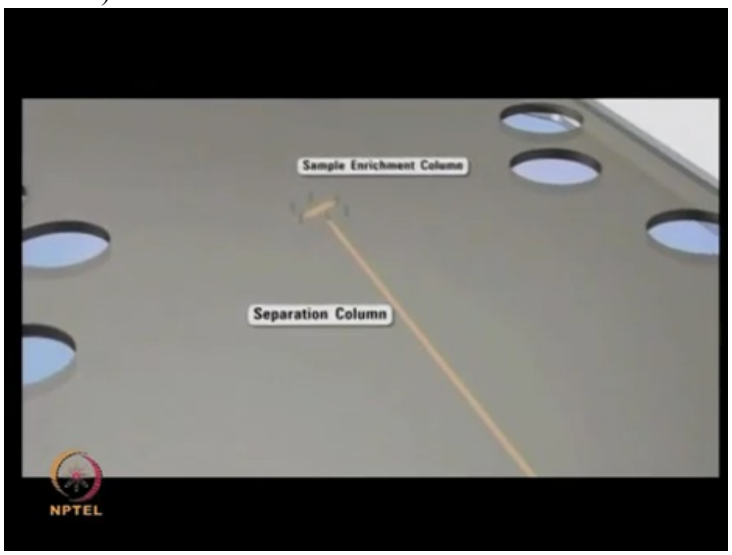
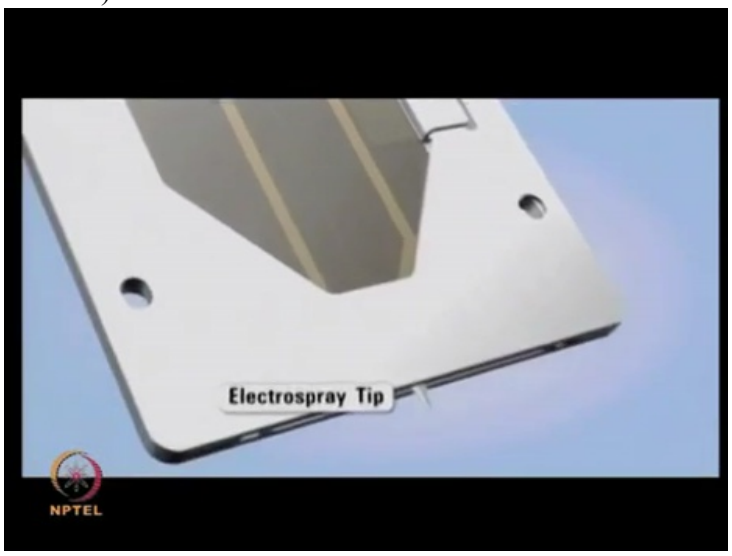


…of a nano-flow LC system are integrated with intricate connections …

(Refer Slide Time 21:06)



…and nano-electro spray tip for compound ionization in mass spectrometry

This eliminates 50% of the traditional fittings and connections typically required in nano-flow LC/MS system, which dramatically reduces the possibility of leaks and dead volumes, and significantly improves ease-of-use….

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…sensitivity, productivity and reliability

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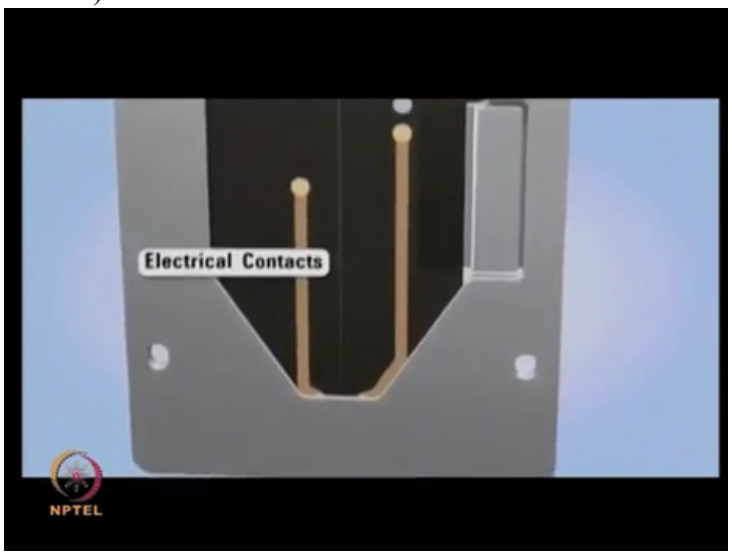

The HPLC chip also incorporates all electrical contacts for the nano-electro spray tip, and features …

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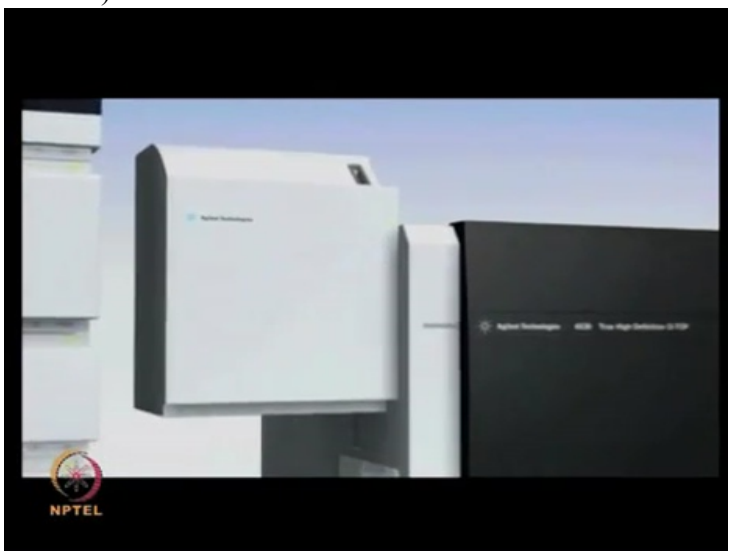
…an embedded radio frequency id tag; that tracks the usage and operating parameters of the chip. The HPLC chips are housed in

(Refer Slide Time 21:48)



… the Agilent 1200 series HPLC chip/MS interface; the chip cube. The chip cube includes an electrospray ion source with optics for spray visualization, HPLC chip loading and ejection mechanism, nano LC connections and micro-valve switching.

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The HPLC chip loading mechanism precisely and optimally positions the electrospray tip orthogonal to the MS inlet for maximum sensitivity and robustness, day-in, day-out.

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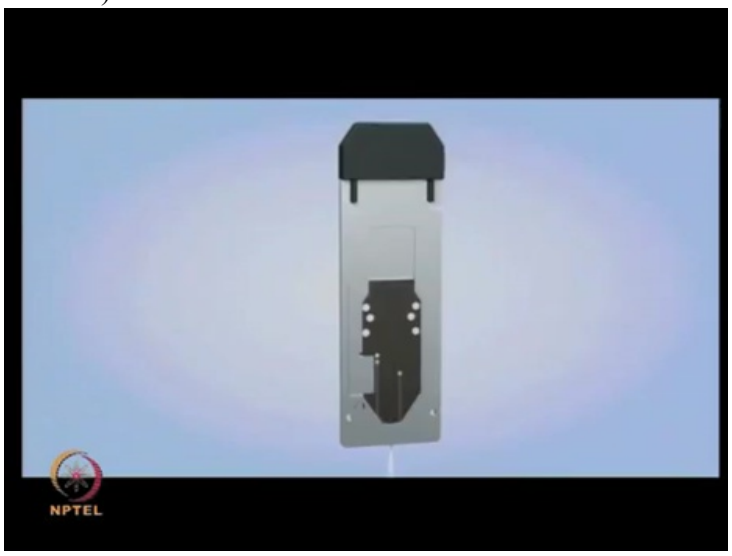

With the Agilent 1200 series nano LC system including microwell plate auto sampler and loading pump connected directly to the chip cube, an HPLC chip is loaded and …

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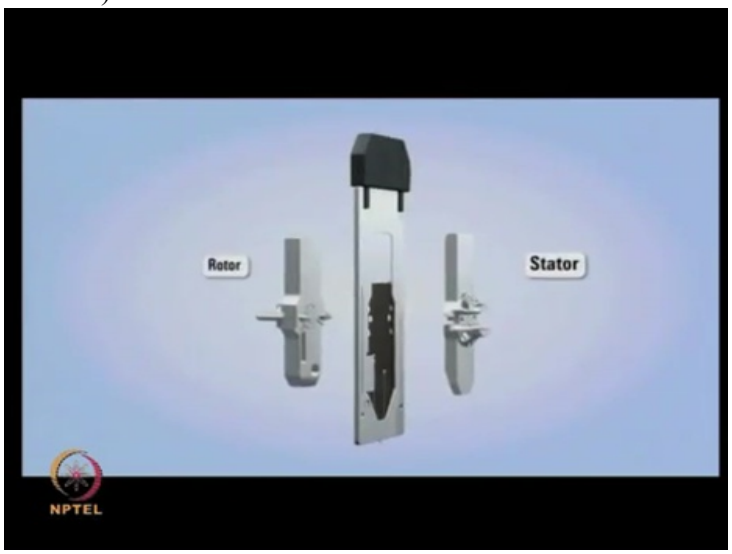

…leak-tight fluid connections are established automatically, by sandwiching the chip between the rotor and the stator of the built-in multiport micro-valve. The rotor and stator dock onto the chip, and establish a …

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…flow path from the nano LC to the ports on a chip surface. Fast movement of the rotor …

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…ensures reliable switching between sample loading and sample analysis positions …

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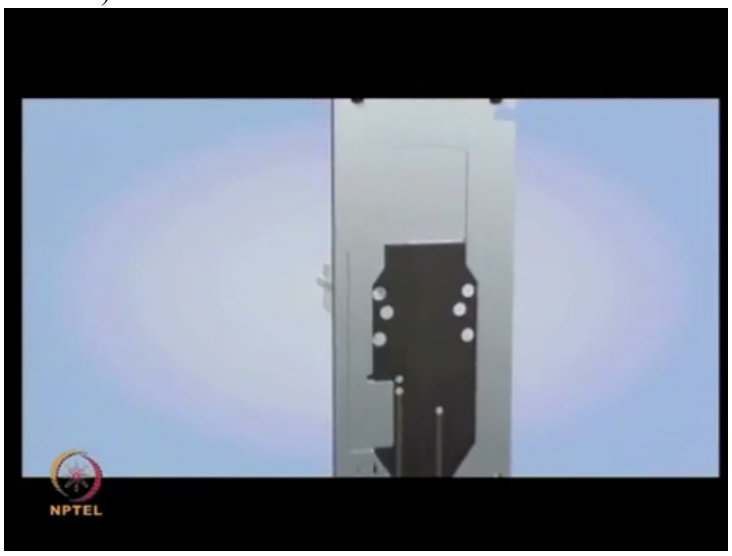

….on the HPLC chip

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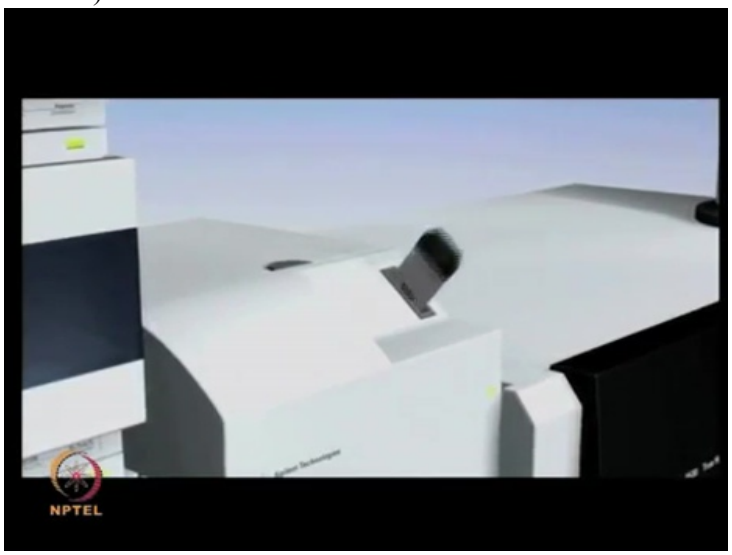

Replacement of the HPLC chip is simple and can be completed in a few seconds. Let us look at how the Agilent 1200 series HPLC chip/MS system …

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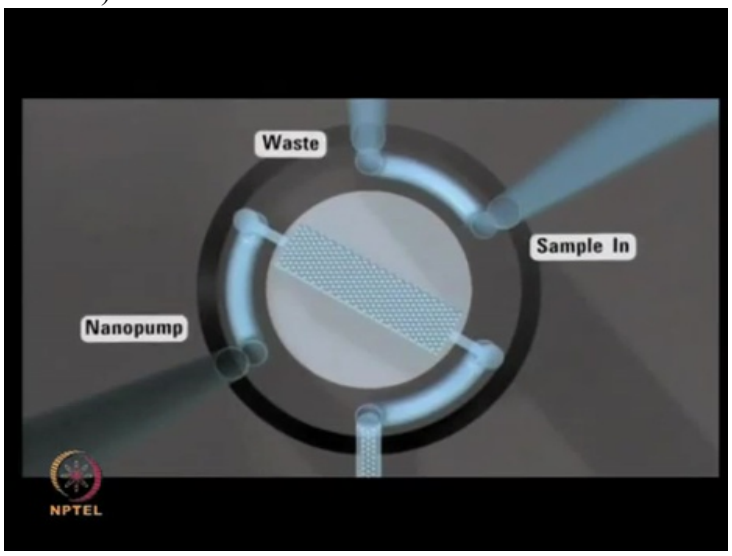
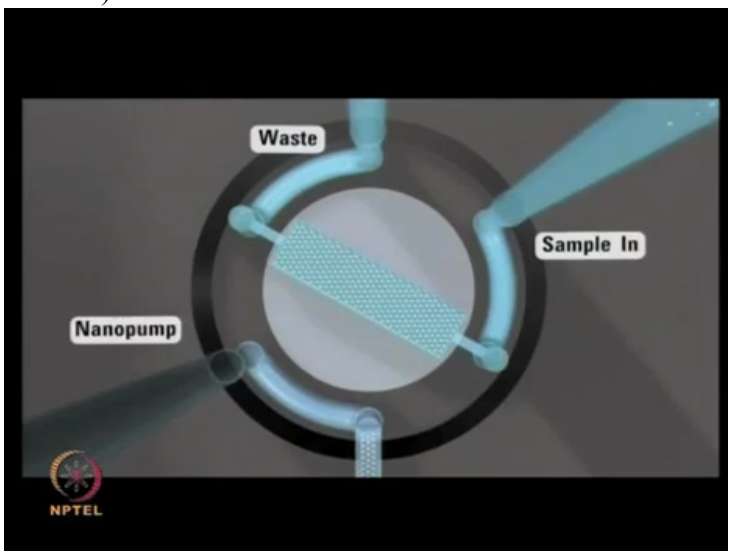


… can be applied to a typical protein identification analysis

(Refer Slide Time 23:10)



The Agilent microwell plate autosampler ….

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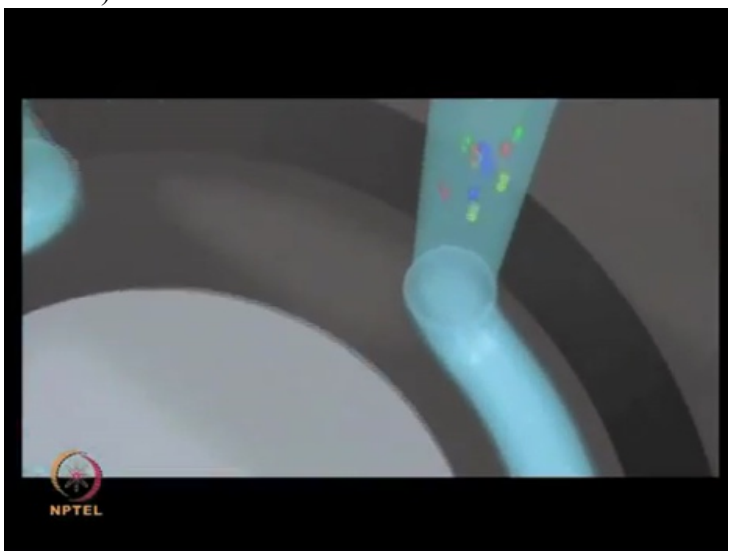

…loads the digested proteins…

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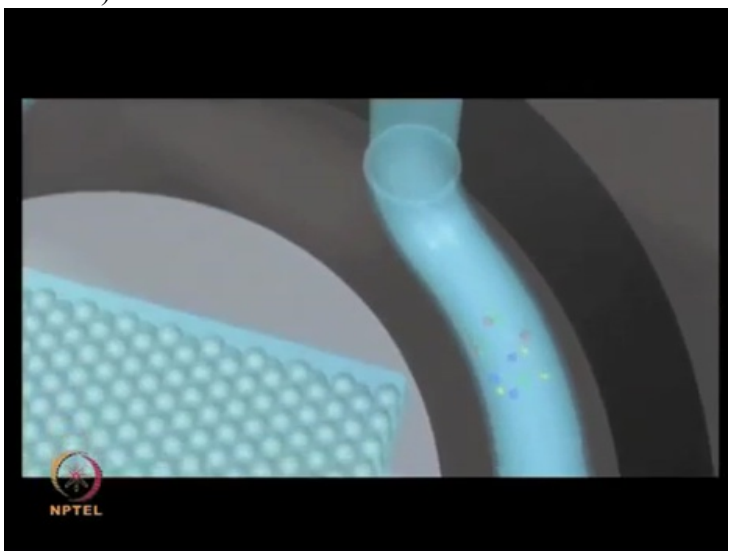

…a solvent flow ….

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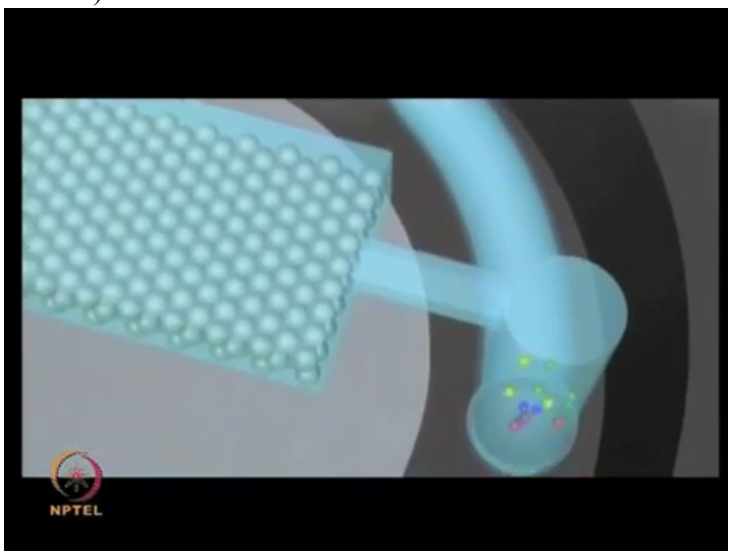

… moves the peptides ….

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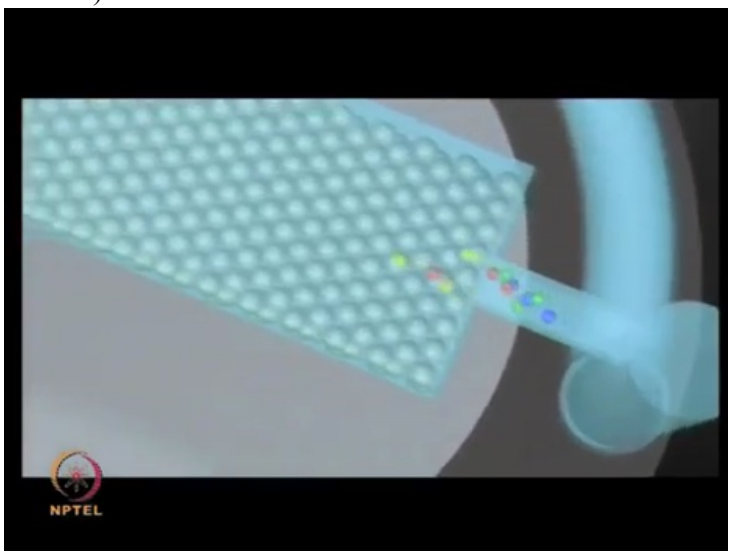

…into the trapping column, the micro-valve changes the flow path…

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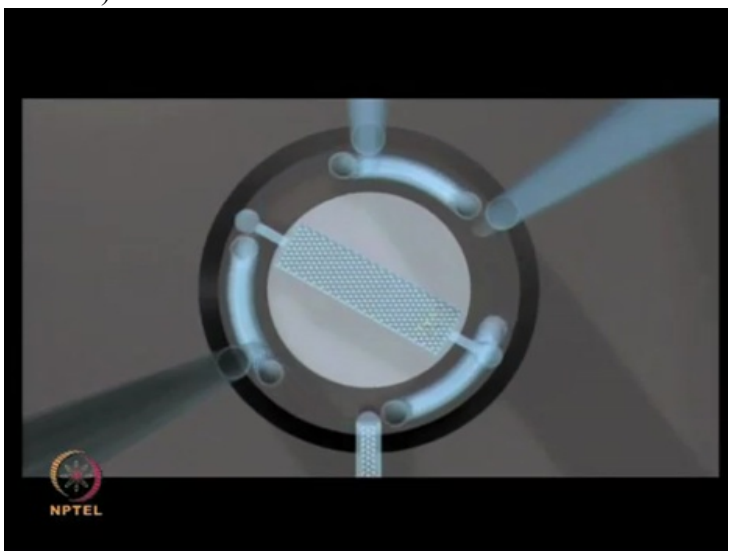

…the gradient flow from the nano-flow pump takes the enriched sample from the trapping column …

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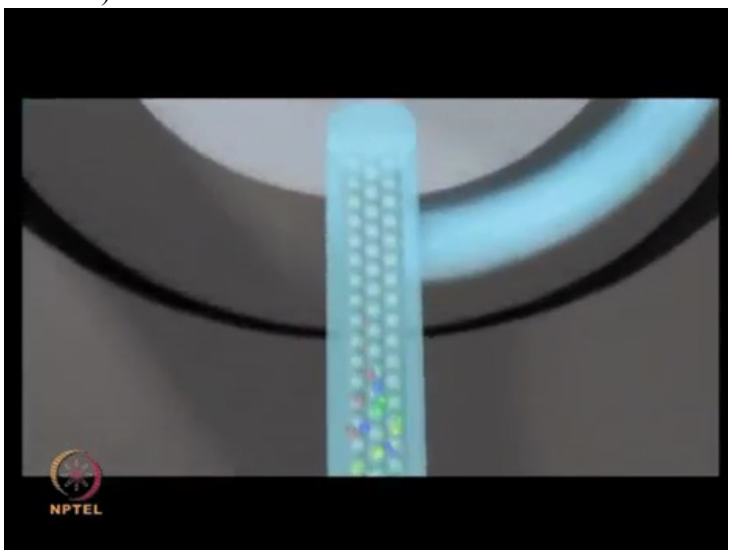

…to the separation column, the peptides are separated just like on a conventional nano-flow column…

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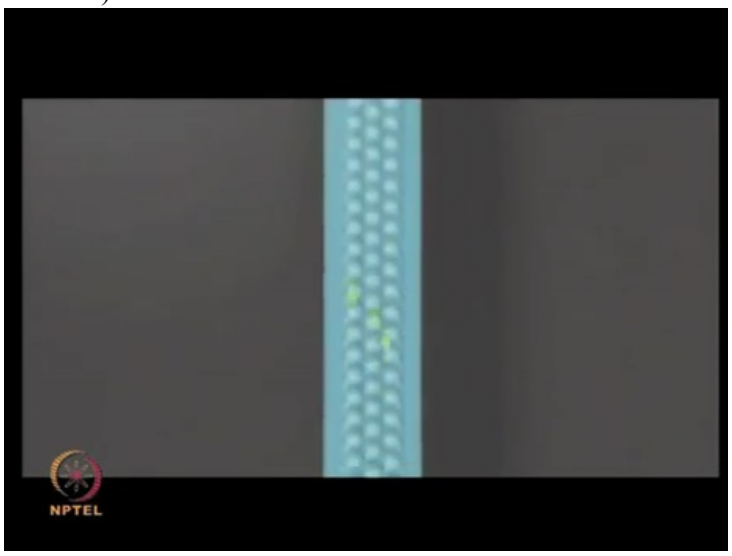

…reduced peak dispersion yields better separation efficiency

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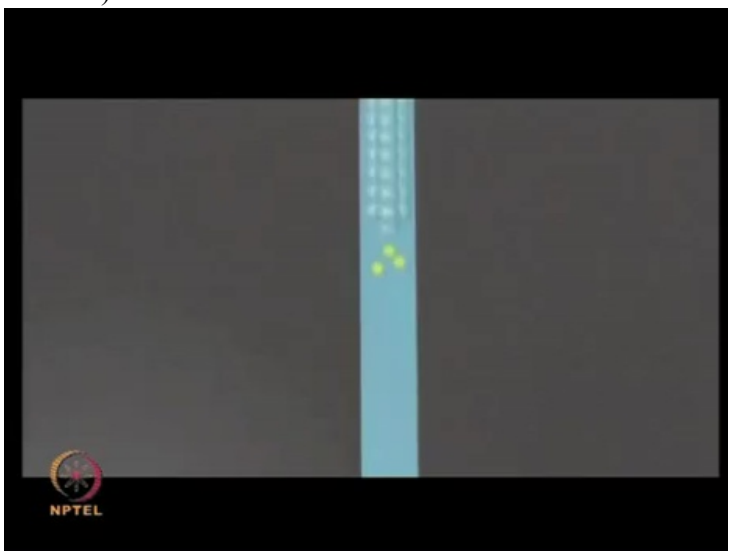

…and sensitivity

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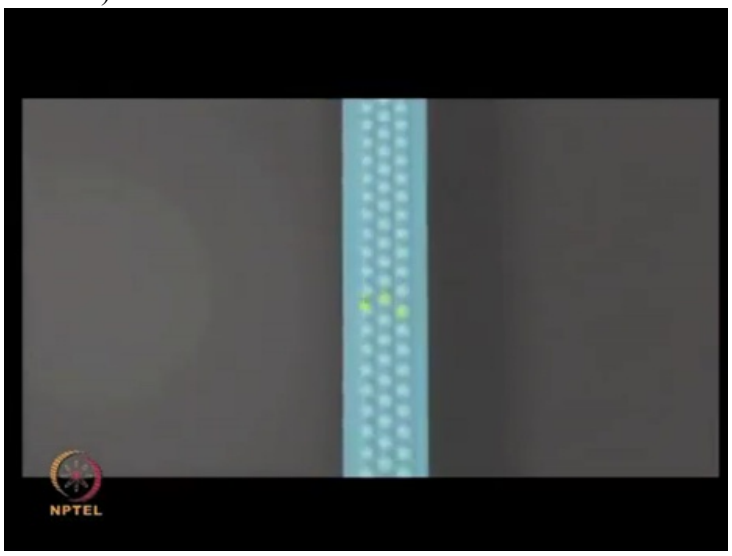

The integrated nano-spray tip …

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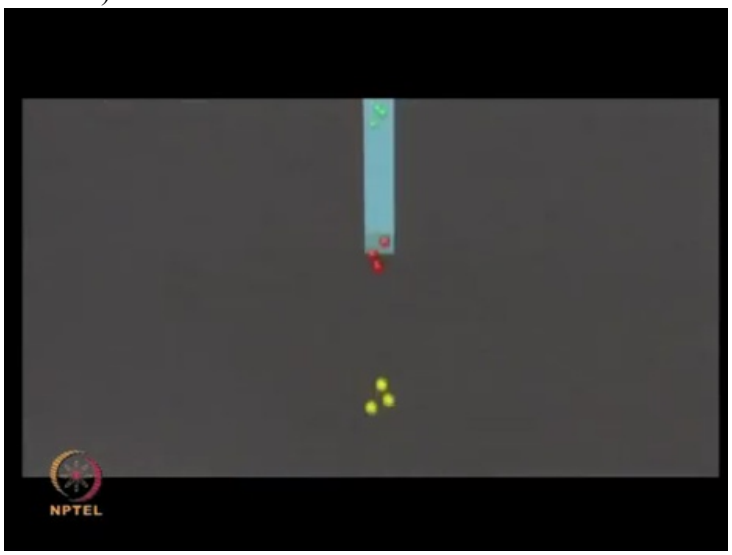

…ensures reproducible nebulization of the effluent, vital for …

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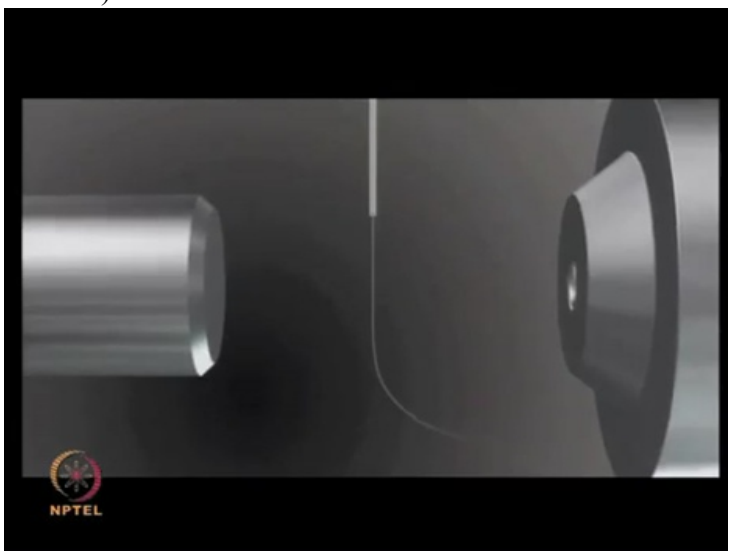

…optimum ionization of compounds and best results Proven and nano-flow LC/MS technology, and the new and exciting …

(Refer Slide Time 23:54)

…capabilities of microfluidics, combined to form a system that is easy to set up and …

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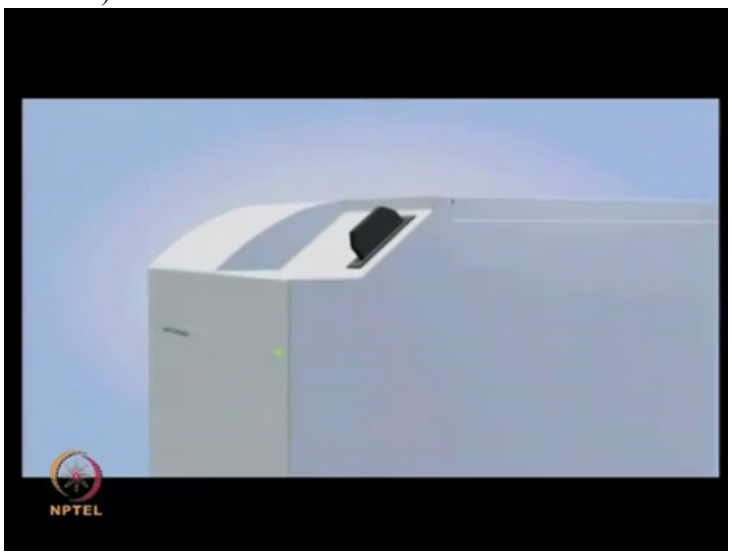

…easy to maintain

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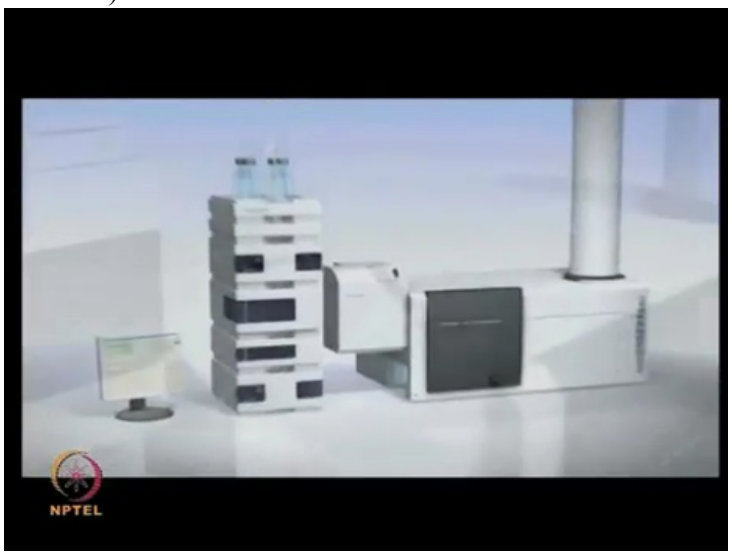

Scientists can now get more results, faster. The flexibility of the HPLC chip design and the HPLC chip/MS interface micro-valve technology in…

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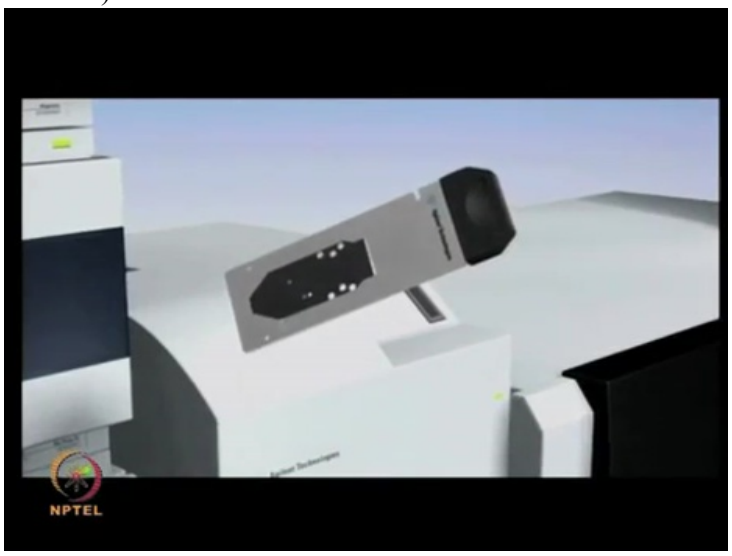

…integrating additional chemistries and separation strategies, opens up a wide range of potential solutions for ….

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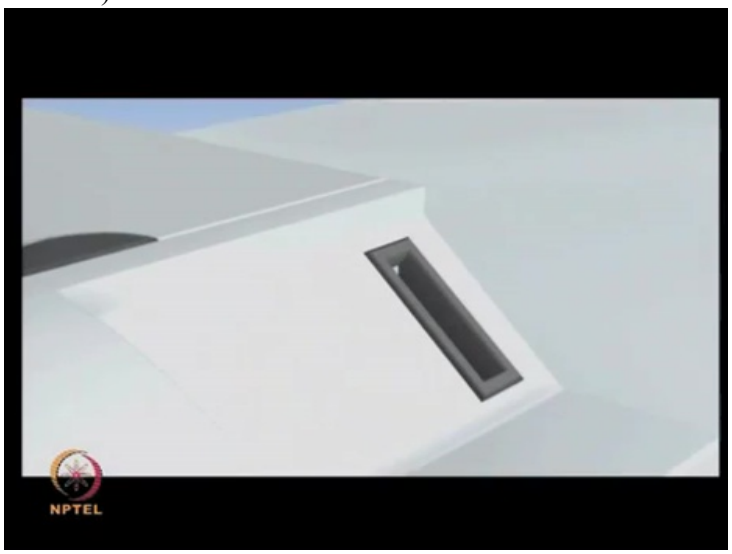

…many research challenges.

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On-chip multidimensional nano LC is….

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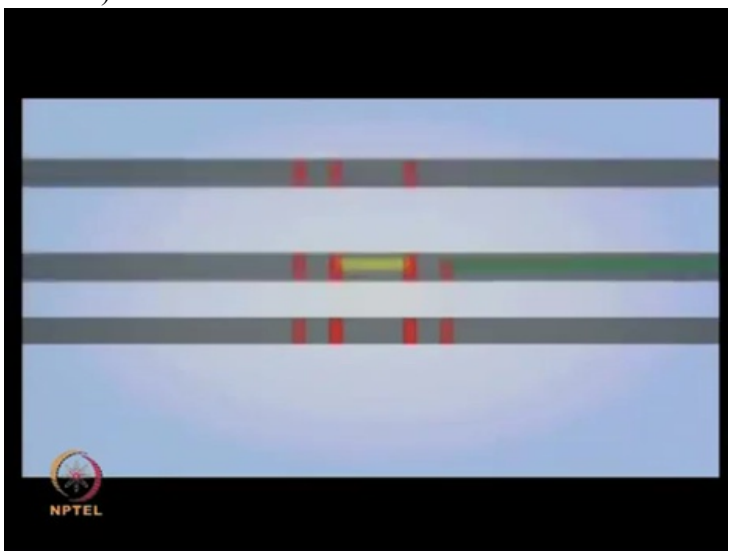

… one of many possible new applications. By adding more layers to the HPLC chip…

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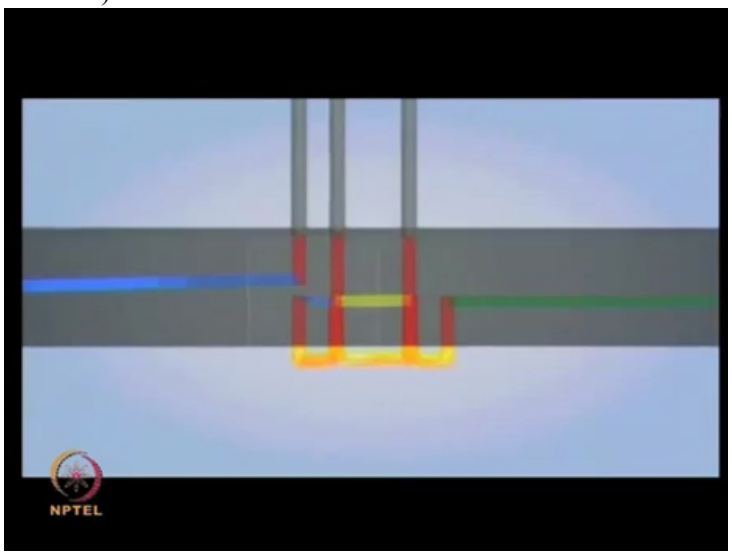

…additional capabilities such as two dimensional HPLC….

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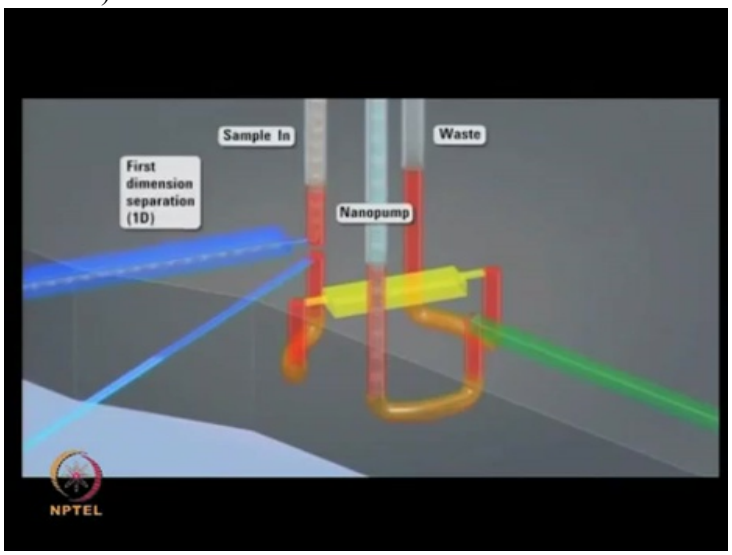

…the affinity chromatography and on-chip chemistries such as on-chip protein digestion are possible. These new applications and many others such as chips with different columns lengths, and packing materials are part of …

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….Agilent's exciting custom HPLC chip portfolio. Moving beyond protein identification…

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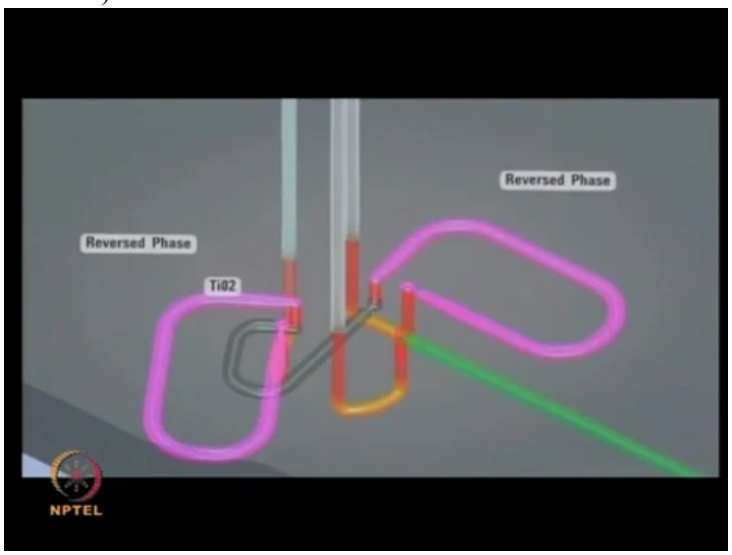

…the new phospho-chip with sandwiched reversed phase titanium dioxide trapping column provides researches working on post-translation modification with a convenient tool targeted at phosphorylated peptides. Pushing beyond proteomics…

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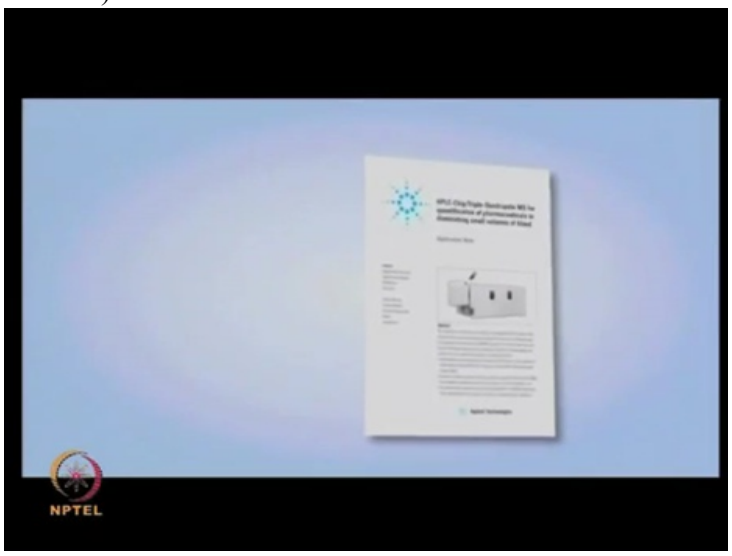

…the new ultra-high capacity chip with a 500 nano-liter trapping column facilitates analysis of pharmaceuticals; such as drug metabolism pharmaco-kinetics with better sensitivity and much lower sample requirement.

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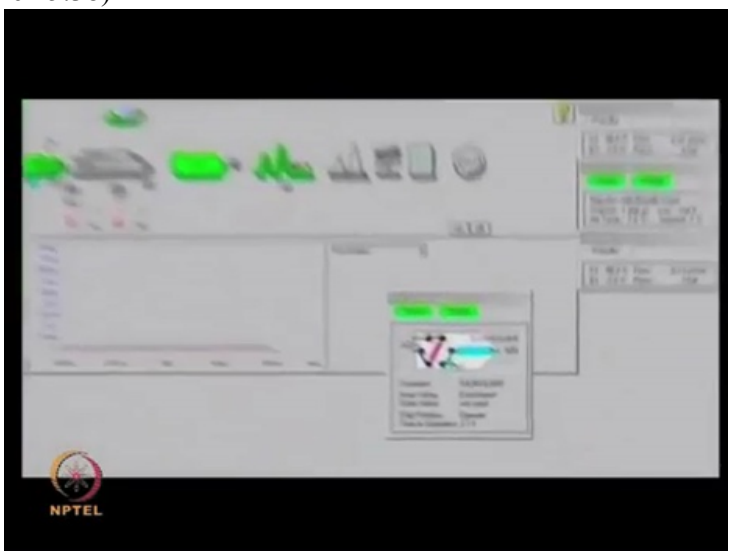

The HPLC chip/MS interface is standard module within …

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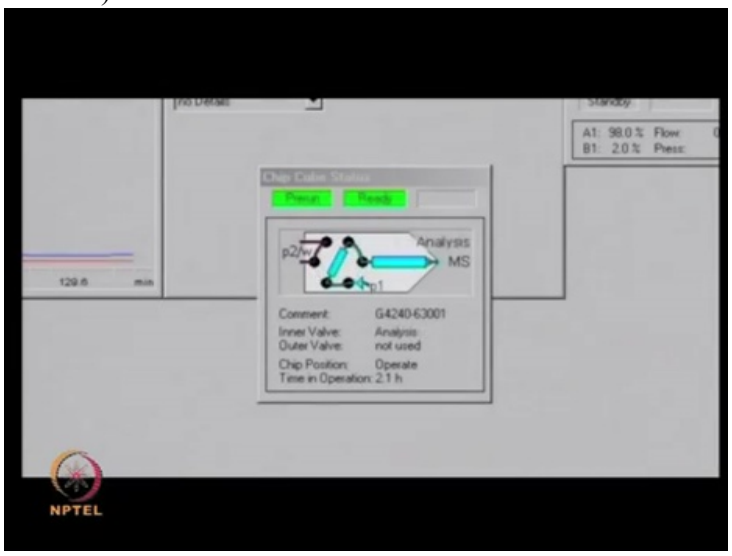

… the Agilent 1200 series LC portfolio, and is fully controllable …

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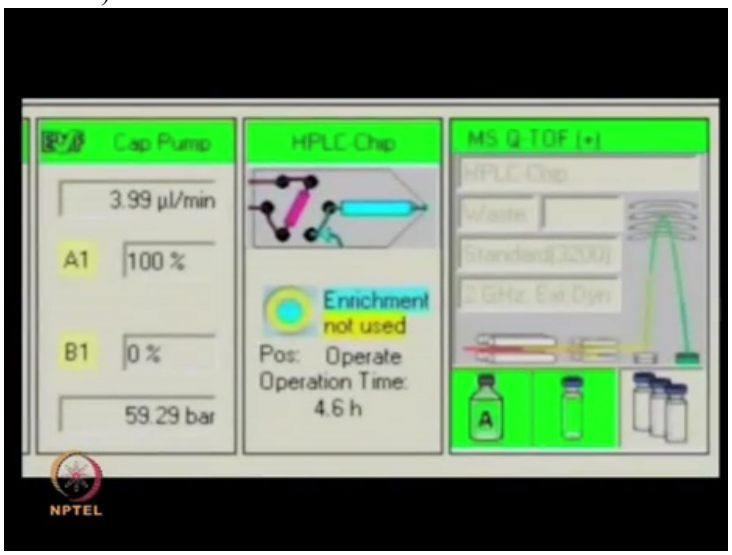

…through the Agilent ChemStation or …

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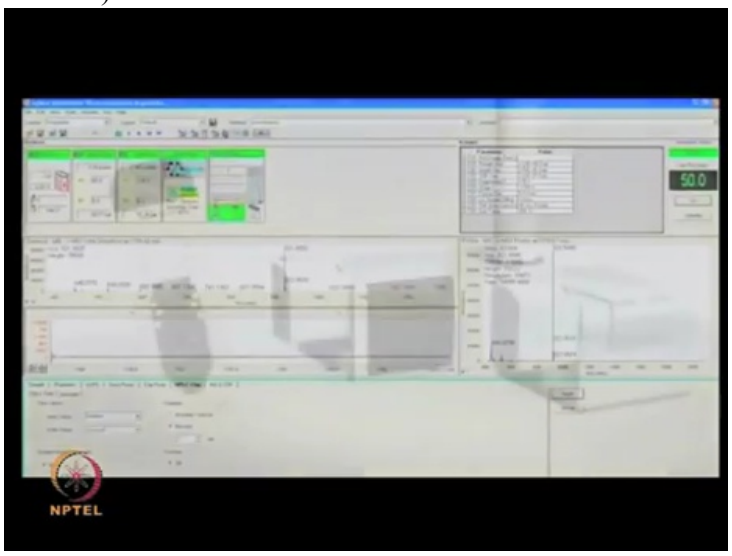

…Agilent MassHunter software. Step-by-step, chip-by-chip, Agilent facilitates new applications in life science, pharmaceutical and …

(Refer Slide Time 25:59)

…chemical analysis. HPLC chip MS, a growing trend in LC/MS technology.

Video narration end

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So, far discussion on the Quadrupole time of flight, the Q-TOF as well as triple Quadrupoles.

Now, let us move on to another latest configuration; the Orbitrap, which has very much similarity with the ion trap, but it is one of the very latest addition to this proteomics of workflow, where people are applying this for various type of biomarker discovery and different other applications.

So, I have invited one of the application experts from Thermo Fisher to discuss about, what is the Orbitrap technology, its principle, and how it can be applied for different type of applications? So, I will have a discussion with Mr. Sangram Pattanaik from Thermo Fisher.

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(Refer Slide Time 27:15)

Professor Expert conversation start

Professor: This is my pleasure to introduce Mr. Sangram Pattanaik, the Product Manager LC MS division of Thermo Fisher Scientific.

(Refer Slide Time 27:20)

Professor: He is working in the mass spectrometry area. Hello Sangram.

Expert: Hello, Doctor Srivastava.

Professor: So, very good to see you in this short conversation about mass spectrometry, and some of the latest developments which are happening, I thought it will be good idea to talk about some of the latest advancement happening in this mass spectrometry field, and I

thought I will invite you and seek your expertise about the Orbitrap and new mass spectrometry applications available.

Before I start this conversation, I would like to know little bit about your educational background, as may be about your experience in this mass spectrometry field.

Expert: Yeah, for last 6 years I am looking at the mass spectrometry divisions of different platforms. In Thermo Fisher for last one year I am there handling the Orbitrap technologies, and we have our ion trap and triplet quad systems.

Early, prior to that, I am, for last 4 and 5 years, I am handling the Q-TOF systems as well as triplet quads from other company vendors. Altogether I am there for last 15 years in analytical industry.

Professor: Ok, so…

Expert: These are the experience I carry.

Professor: Great, so you have a long interest in this field it seems, and you have seen different type of advancement in the field. So, with that experience, can you share what type major applications of mass spectrometry are currently being used in the proteomics area?

Expert: Yes, in proteomics area, mostly there are two basic applications area that people are looking at is, the discovery and the targeted quantification. In the biologically discovery, people are more talking about comprehensive proteomics, and in targeted, people are more talking about biomarker validations.

In comprehensive proteomics, peoples talks about the identifier one wants to quantify, all in the same sample. As well as, they look at what is the post-translation modifications in the whole proteome. So, these are the new areas of applications which is going on currently, these are the trends which going on currently.

Professor: Right. So, I think you rightly mentioned that emphasis is moving more towards the quantitation of those proteins, rather than just only identifying them and leaving at only the abundance level, which was the case earlier.

Expert: Yes

Professor: So, in that light, can you brief us about some of the shortcomings of currently available mass spectrometers, and what challenges we have to overcome to have a really comprehensive coverage of proteome, and to really do various applications including the PTM, including targeted quantification and different type of application in the biomarker discovery?

Expert: Yeah, absolutely. As you are mentioned correctly the biologically matrix itself is very complex in nature. When you have different kind of approach or workflow applications; you look at the analytical solutions which can fulfill that demand.

Professor: Right

Expert: So, most of the areas, applications, people are more talking about the sensitivity or resolution, mass accuracy, these are the areas. Now the new area coming out, what kind of different fragmentation patterns or fragmenting capabilities are required, those are the areas people are now looking at.

Professor: So, proteomics is quite competitive and challenging field, and right now lot of emphasis from all the companies are in this field for the mass spectrometry, how to provide good solutions for analyzing the complex proteome. So, what types of major mass spectrometry instrumentations are available from Thermo Fisher currently?

Expert: As a Thermo Fisher, we have different technology starting from Ion trap to Orbitrap, but for proteomics platform we normally try to providing them Orbitrap with different workflow solutions. So, Orbitrap is the main choice of scientists now, if we look at globally as well as in India.

Professor: If you do not mind, can you just give some overview about the Orbitrap technology currently available?

Expert: Right, the current what Orbitrap we have, we have three different platform of Orbitraps starting from the low end to the highest end. If you look at Orbitrap, the Orbitrap is nothing but one kind of an ion trap, where the ions move in the orbit. So, to give you a brief idea, it is better to have a small presentation what I can show you.

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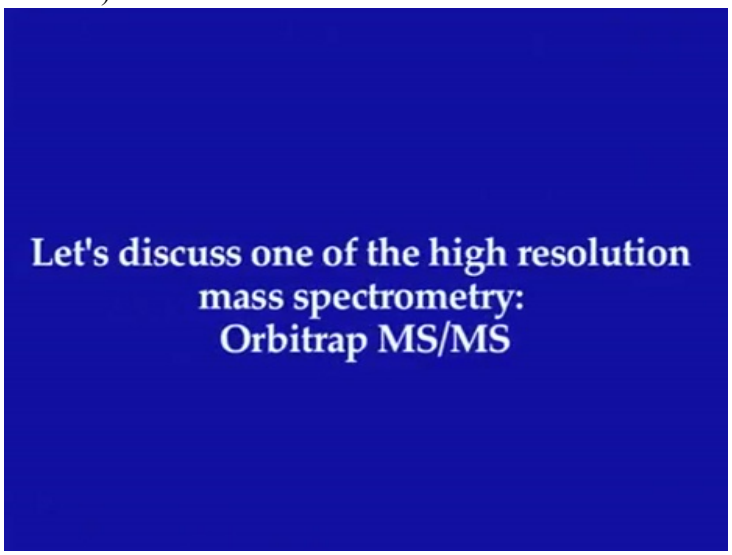

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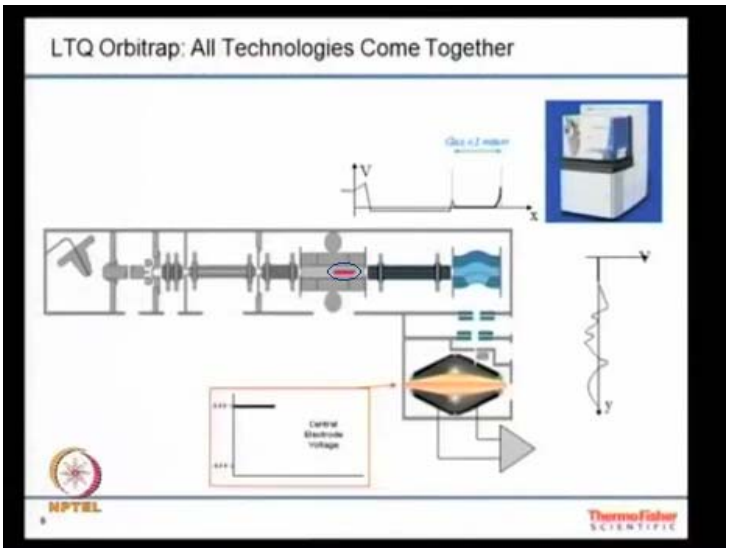
Presentation start



Expert: This is how the system looks like. If you look at the front end, it is the ionization source, and there is two mass specs are in combinations to each other we called it is a hybrid technology.

The first part is a linear ion trap, and the second part is the Orbitrap. In the firstly, ionization, ions generated from the source it goes to the linear ion trap and it has been trapped there.

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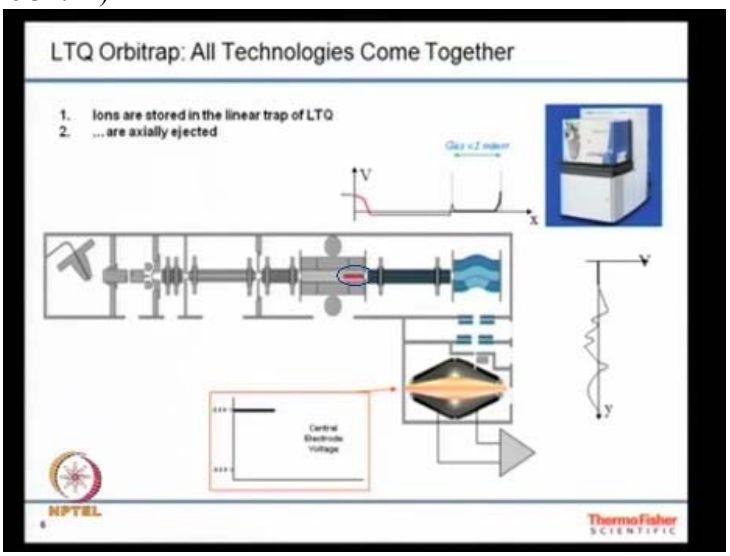

Expert: once it has been trapped, axially ejected and then goes into the c-trap.

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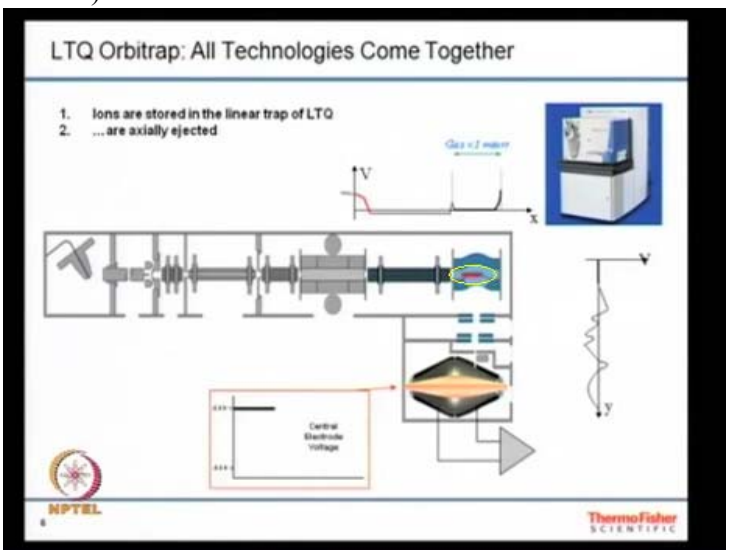

The c-trap squeezes the ion it packet and eject that ions into Orbitrap.

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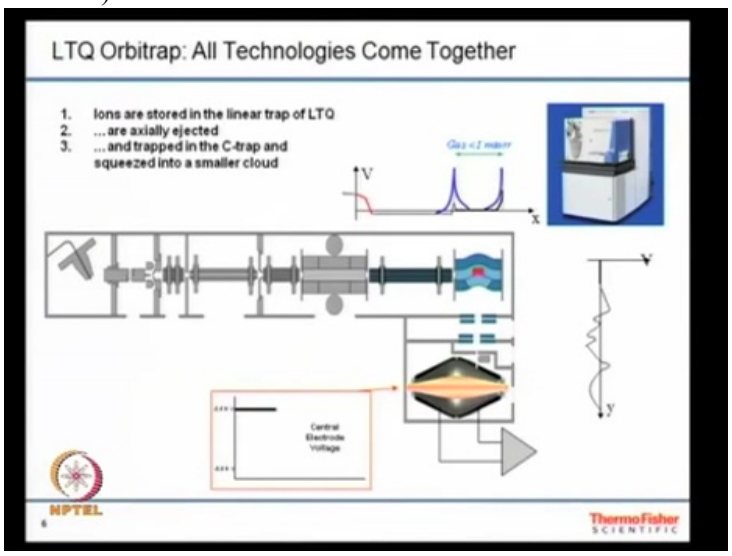

Expert: Once the ions get ejected into the Orbitrap....

Professor Expert conversation start

Professor: So, probably you are showing those trajectories in the PPT, right?

Expert: Yes, PPT

Professor: Ok

Professor Expert conversation end

Expert: So this ions once injected into the Orbitrap….

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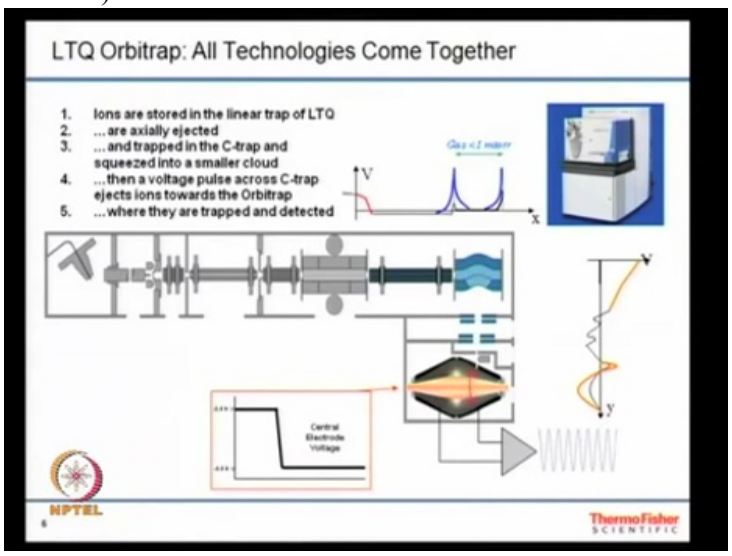

Expert: … it goes into axial motion as well as in radial motion. We measure the axial frequency of the ions; the frequency which is being measured is transferred into mass by charge ratio. If you look at there is an end electrode, which has been connected to the frequency domain which measures the frequency.

Simplest way you can say, it gives you the MS current which has been measured. So, this how the Orbitrap works.

Presentation end

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Professor Expert conversation start

Professor: I think you mentioned, that you want to show some more detail for the path motion.

Expert: Path motion yes, I have a small video which we can also see to that.

Professor: Ok

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Video start

Expert: Just introducing the newest technology that is LTQ Orbitrap Elite. The LTQ Orbitrap Elite if you look at this hybrid systems which has two mass spectrometer;

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Expert: the mass spectrometer which is sitting in front as a linear trap, Velos, LTQ Orbitrap Velos…

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Expert: … and then the back end which is there is the high field Orbitrap or called the Orbitrap Elite. If you look at the schematic of the system,

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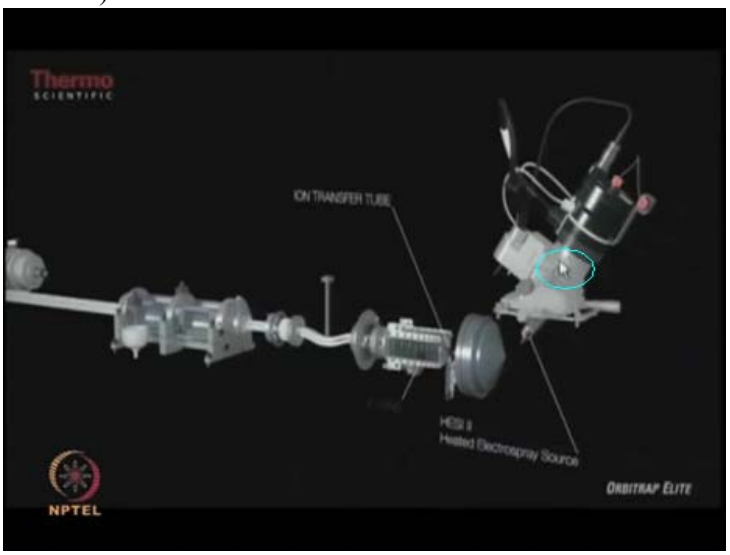

Expert: in the front part of the system, it is the ionization source which sits on the front.

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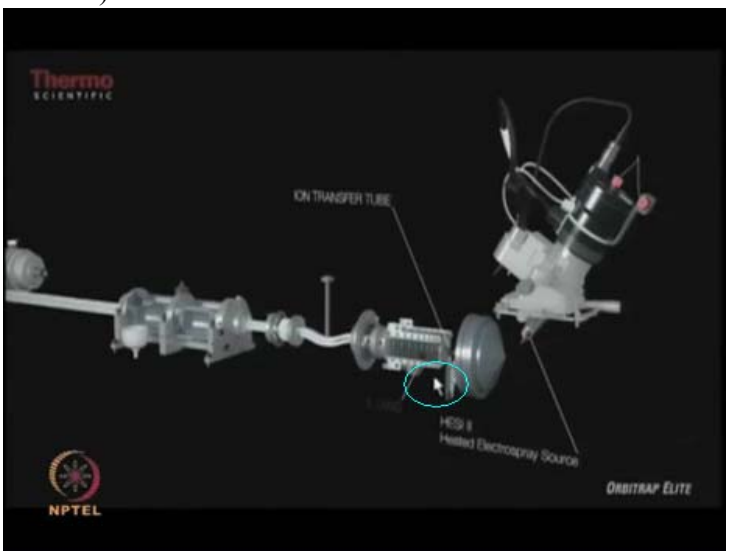

Expert: Then you have the transfer optics, in the transfer optics we have

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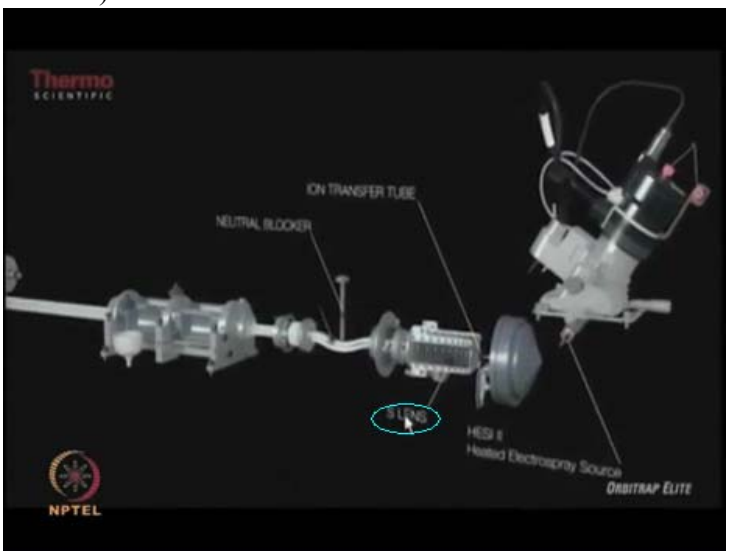

Expert: the S Lens which is the newest generations transfer optics, and after the transfer optics we have

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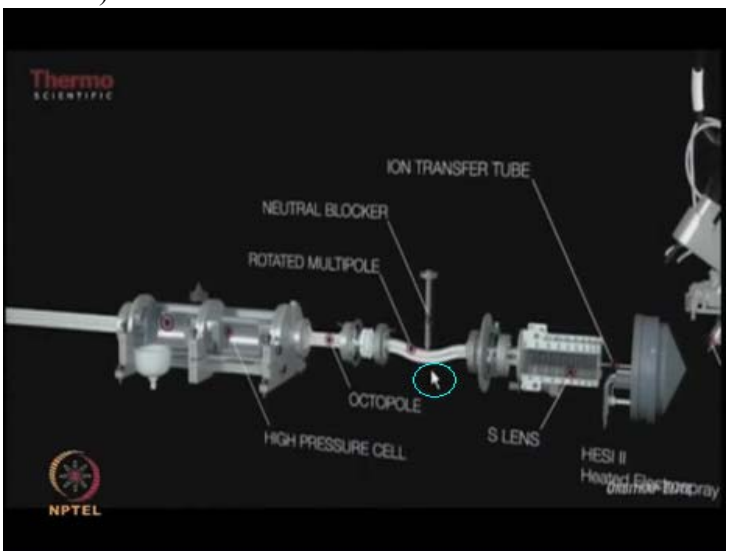

Expert: the transfer tubes, and

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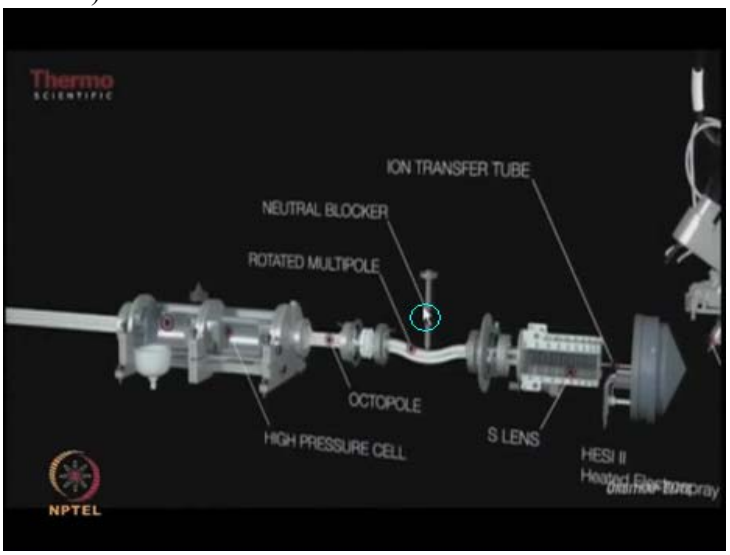

Expert: we have the neutral blockers which blocks the neutral which is coming from the source,

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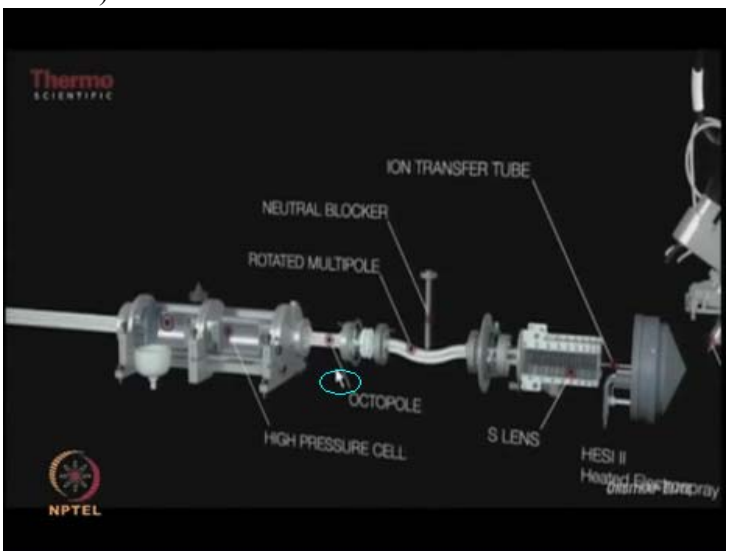

Expert: then we have the Octopole, and

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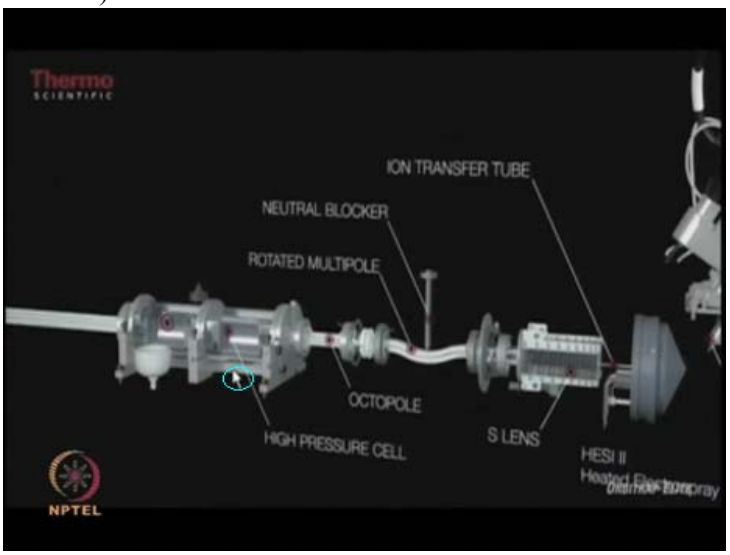

Expert: then the ion trap. In the ion trap we have two regions;

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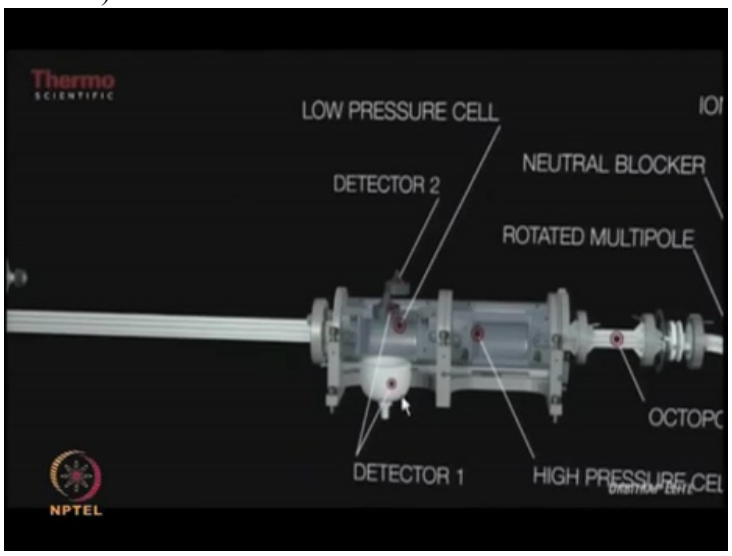

Expert: one is the high pressure cell, low pressure cell.

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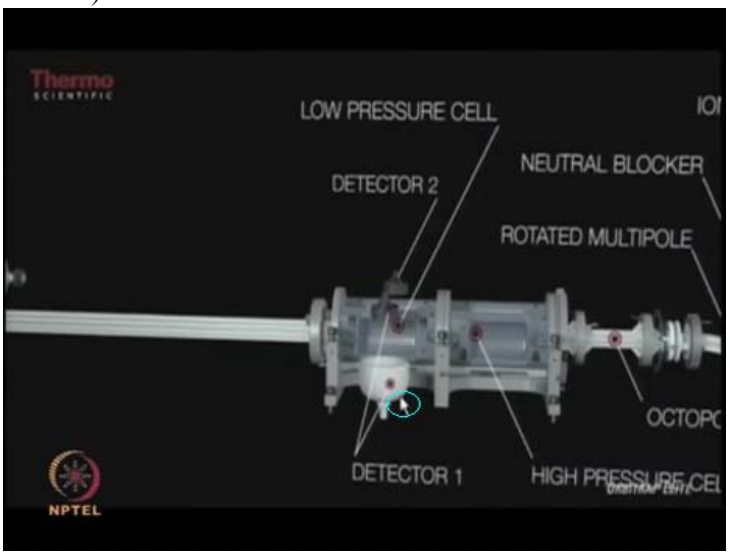

Expert: Then on the radial there is two detectors; detector 1

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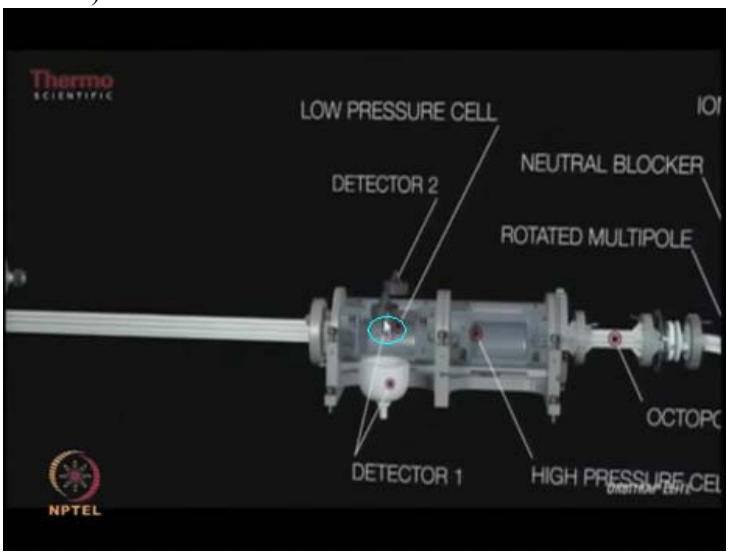

Expert: and detector 2, when you work with the linear trap. Detector can use for the detections of ion from the linear trap.

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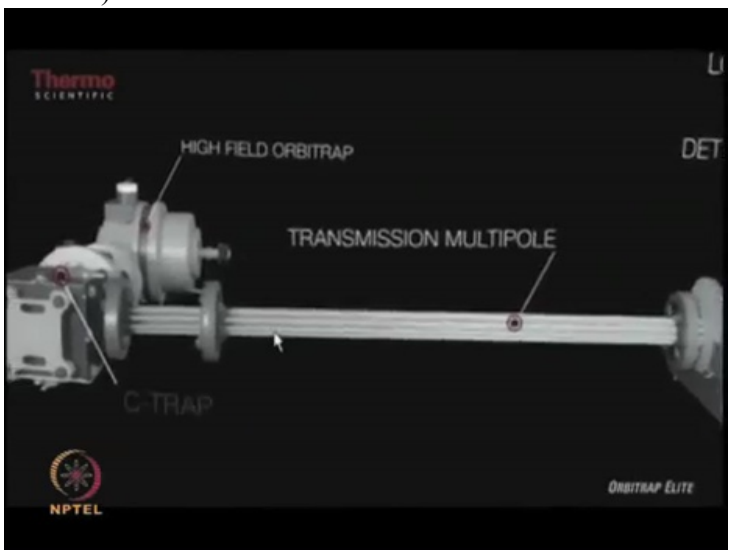

Expert: From the linear trap it goes to the transfer optics, then we have

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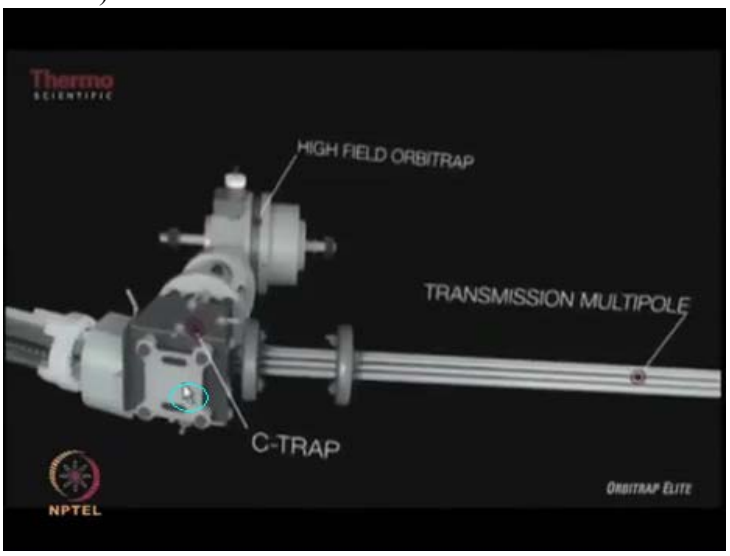

Expert: the c-trap which dynamically squeezes the ion into packet, and allows that ions to go

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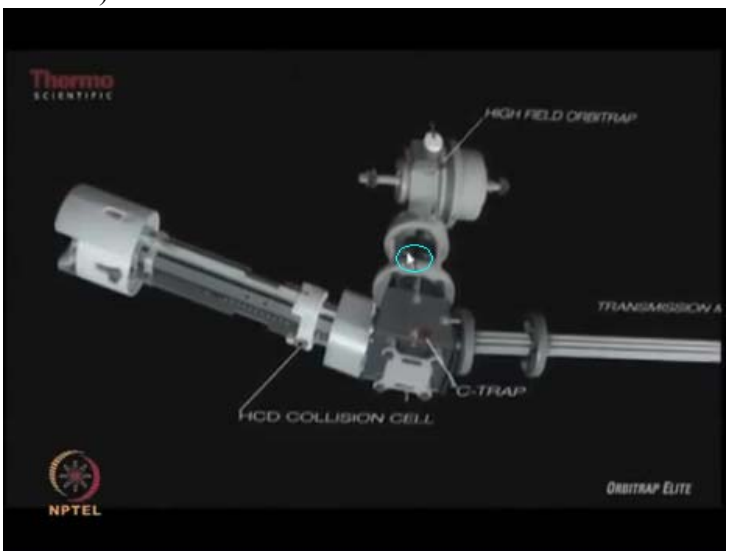

Expert: into the high field Orbitrap.

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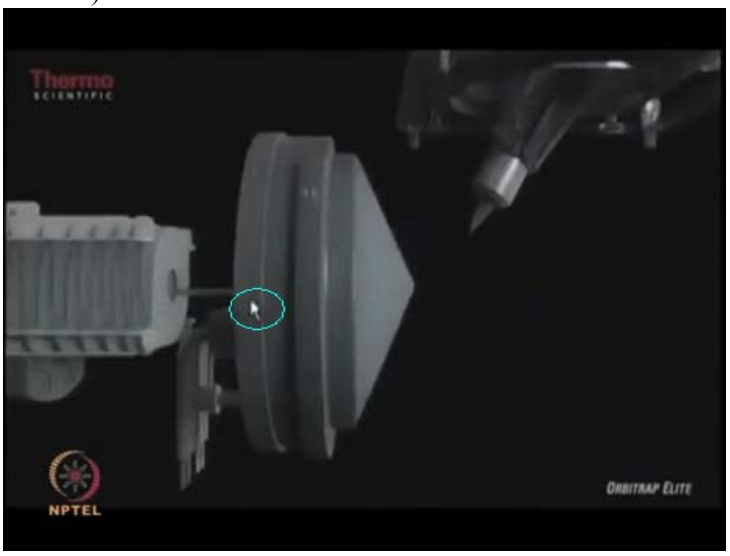

Expert: Then you have the HCD cell.

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Expert: Coming back to the ionization source, the ions generated from the source

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Expert: and goes into the transfer optics, and after going from the transfer optics

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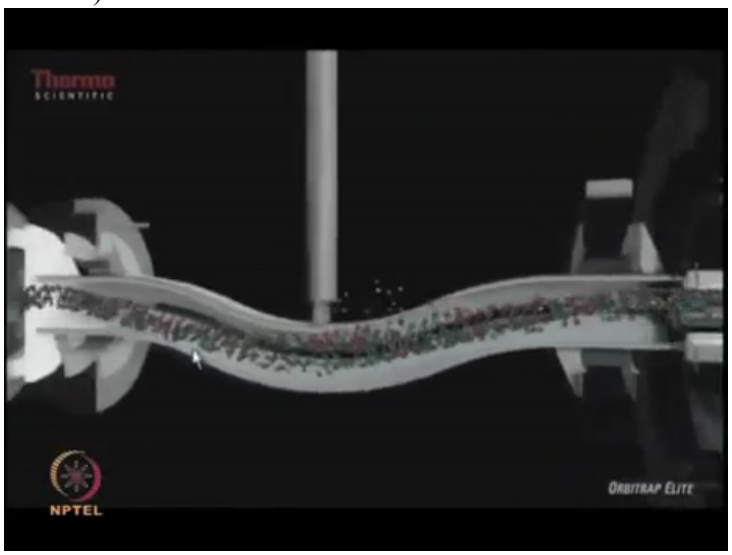

Expert: it goes to the linear trap.

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Expert: And the linear trap in a full scan modes the ions are stored into the

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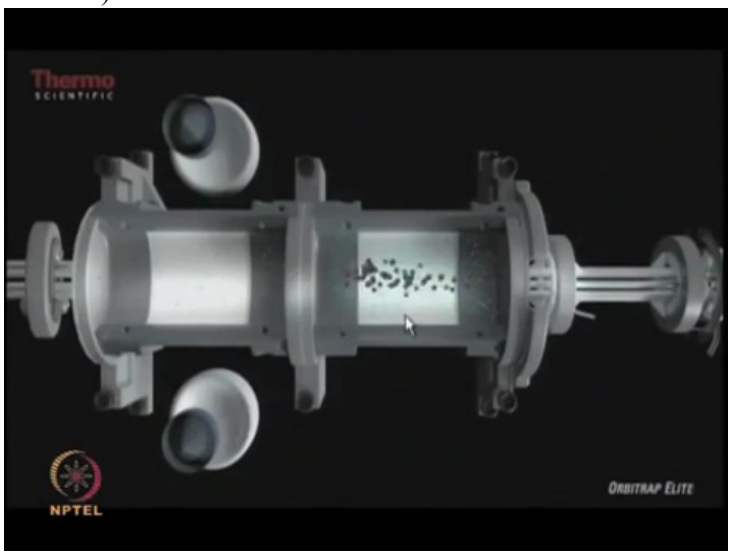

Expert: high pressure cell, once this ions are stored under high pressure cells in the full scan mode, the ions then sent into

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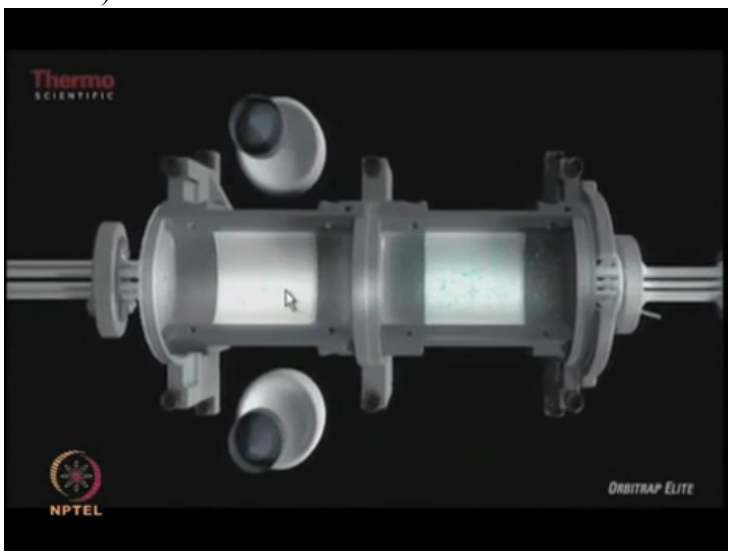

Expert: the c-trap

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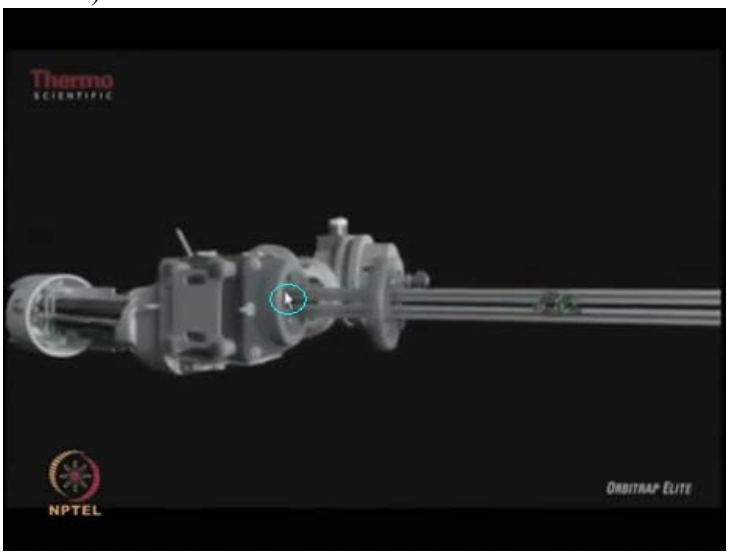

Expert: through the ion transfer optics, then it goes under the c-trap.

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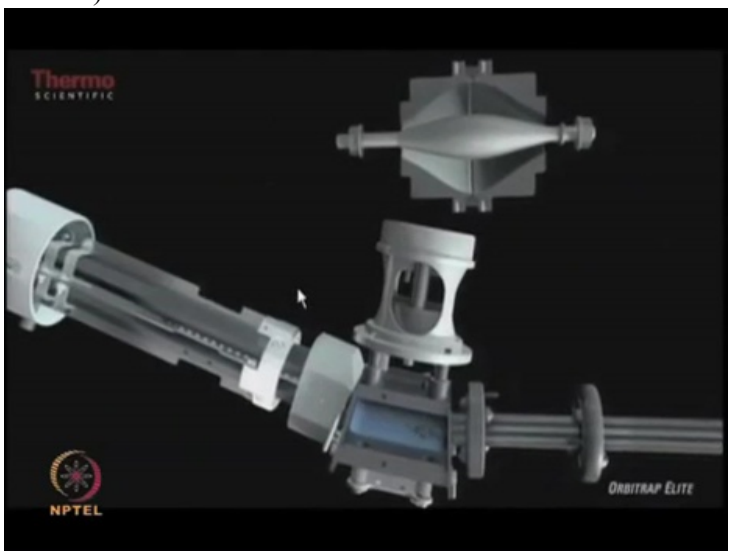

Expert: The c-trap does cooling the ion and makes it into the packet of ion, so that the packet of ion injected

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Expert: into the Orbitrap. Because the Orbitrap itself is in

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Expert: …a static field, so you have to

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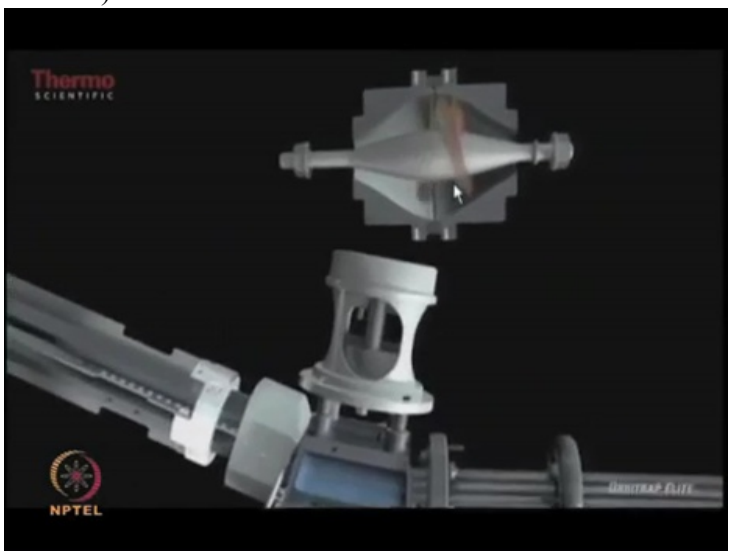

Expert: inject that ion and tangent to the field. So, once the ions go into the Orbitrap,

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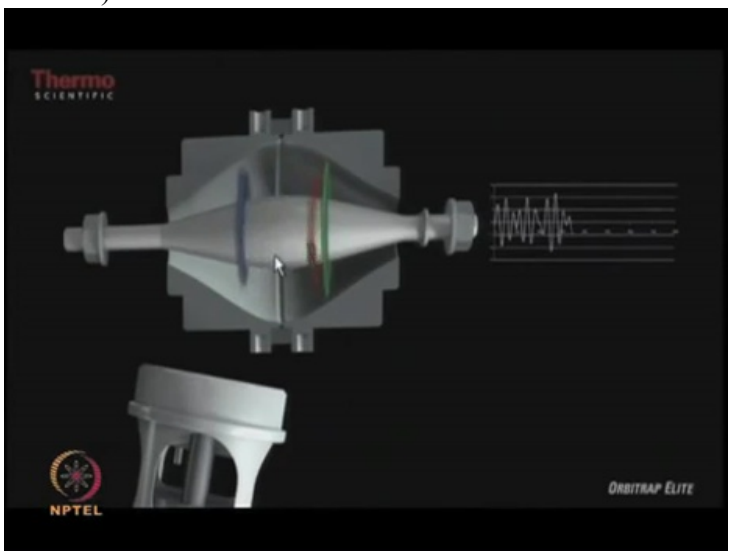

Expert: it moves in into the orbit and the frequency is recorded which gives you in turn,

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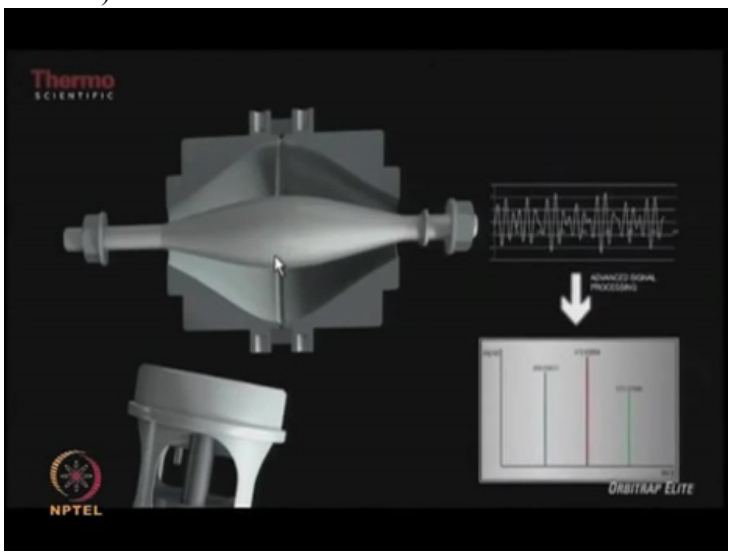

Expert: the mass spectra.

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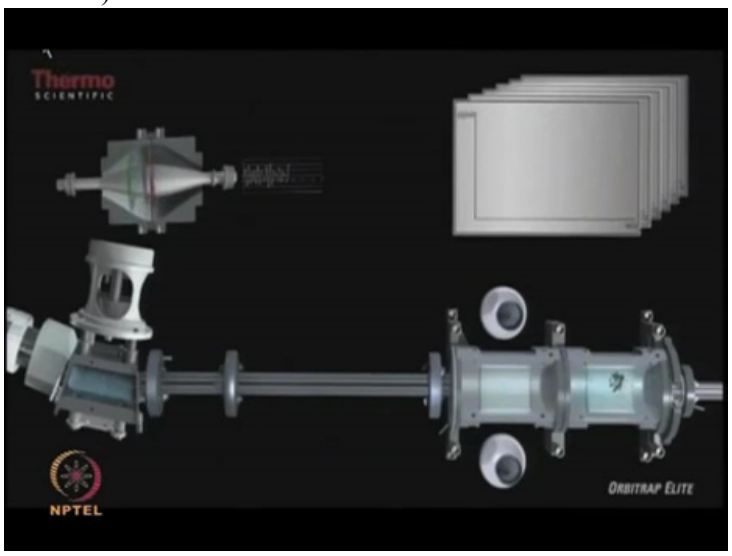

Expert: If you want to work the linear trap and the Orbitrap at the same time, you can run both the same at the same time.

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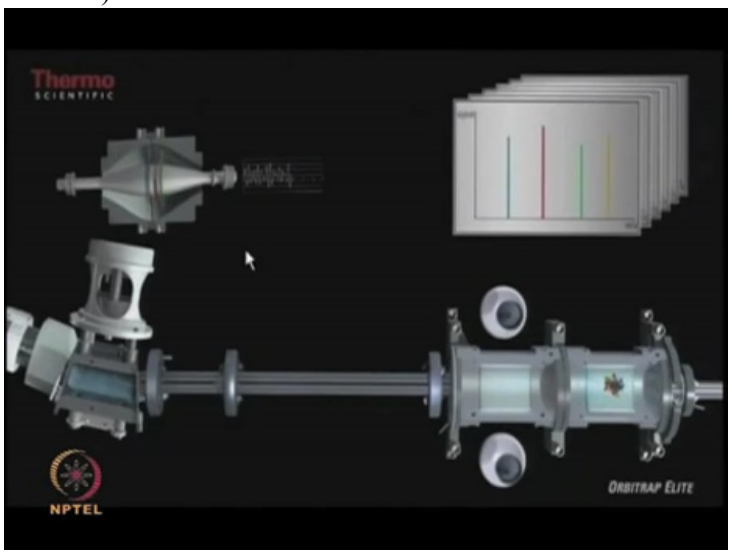

Expert: In the linear trap, the ions get stored and fragmented and then it sends into the Orbitrap. So, you can have the full scan MS as well as full scan MS/MS at the same time.

Apart from that, you can, in addition to the full scan MS and MS/MS, you can have also low resolution scan from the linear trap. So, this is what you can obtain from the Orbitrap.

Video end

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Professor Expert conversation start

Professor: So, it is very good to learn about the Orbitrap technology and how it works. So, finally, I would like to ask you what is your recommendation for mass spectrometry user. what are different challenges occurring this field; and may be your final message to the users of mass spectrometer?

Expert: It is a very difficult to answer, but in a simplest way I can tell you it is all depends on the on the applications area. People, if you look at the selection of any instruments, depends on what kind of applications people are looking at. It is more of a thing, which people should more look at more global scenario, how people are going into doing applications in those area.

Professor: Right

Expert: It is ocean of thing, if you can have instrument you can generate a lot of data, but it all depends on interpretation of the… those datas, and it have has to be application oriented.

Professor: So, you mentioned very rightly that mass spectrometry has infinite possibilities. It is tremendous potential and depending upon your application, one can explore seeming less possibilities taking out from these mass spec.

Expert: Mass spec

Professor: And how one can interpret those data is actually becoming more challenging, and people are coming up with very creative ways of analyzing the data for different application. So, with that I conclude this interview, and I will again thank Mr. Sangram for sharing your Orbitrap and different experience of mass spectrometer with us. Thank you.

Expert: Thank you, thank you very much

Professor Expert conversation end

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Alright, as you have seen the 2 interviews and discussion on different type of hybrid MS configurations we talked about, Quadrupole Time Of Flight, Triple Quad, and Orbitrap technologies, you have also seen the latest advancement in the field, the introduction of chip-based technology.

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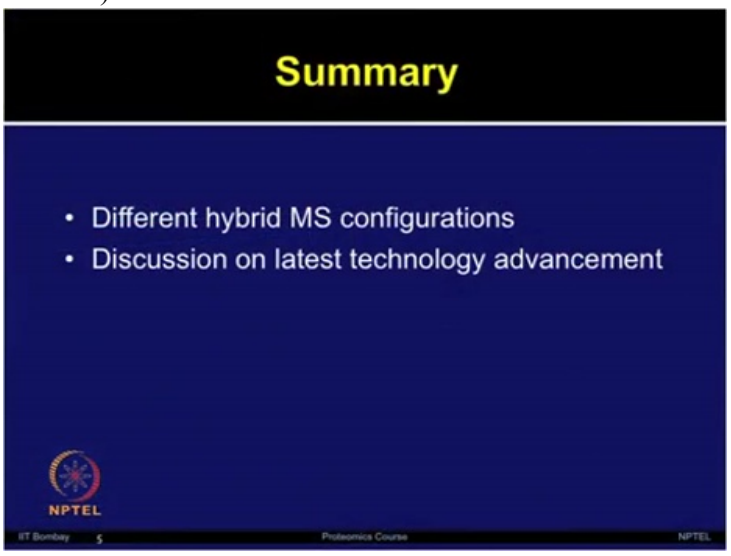

These are just few examples. There are many other good configurations available from various manufacturers in the field.

These 2 interviews were mainly intended to showcase different type of hybrid MS configurations available and more latest technologies which are trying to overcome the HPLC based methods and their limitations.

There are many other good manufacturers available and these two are just 2 examples to showcase different type of advancement.

Now I will continue our discussion on different type of Mass spectrometry-based applications in the next lecture, thank you.

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