Proteomics: Principles and Techniques
Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture No. # 23
Hybrid-MS/MS Configuration

Welcome to proteomics course, today we will talk about 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 advancement which have happened in this field, and to keep up the pace for these recent advancement.

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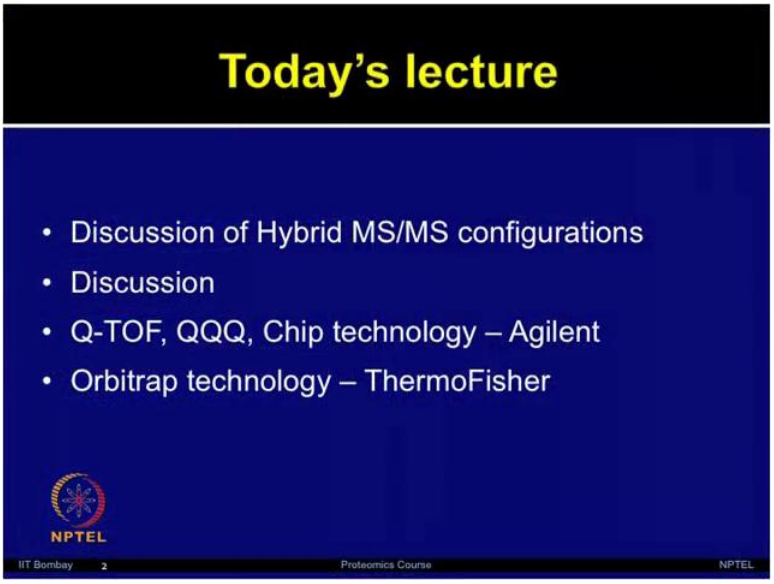

In today's lecture, I thought to involve a discussion on hybrid MS/MS configurations, and talk with two leading companies; one with the Agilent technologies for Q-TOF and triple quadrupole, as well as the chip technology, and then with thermo fisher about the orbitrap technology. So, we will discuss about quadrupole time of flight and triple quadruples, what are the advantages of using these types of hybrid configurations? What is the latest hip 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; Mister 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. Hello, it is my pleasure to introduce Mister Abhijit Kanungo, who is product specialist in the mass spectrometry division of Agilent technologies in India. So, welcome Abhijit for this brief discussion session on mass spectrometry and your experience in this area. So, first of all how long have you been working in the mass spectrometry division of Agilent?

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 Can you briefly tell us about your educational background, and what motivated you to work in the area of mass spectrometry?

I have done my graduation and post graduation from Mumbai University, and immediately after finishing of my post graduations I joined as a technical person in one of the leading instrument industry, and I was responsible for spectroscopic product line, but later on I got responsibility for the mass spectrometry, and at the same time the mass spectrometry was one of my favorite topic in subject in my college days. So, that was the same thing and because of that I got a flavor for the mass spectrometry, and because of that I entered in this field and after I keeps continuing with that.

So, can you mention what are the major applications of mass spectrometry, on both small molecules and large molecules such as proteomics? 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 applications. Small molecule applications involves many many application 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 biomolecule 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 mastering the printing, or a post transition 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 base solutions.

What is some of the major short comments you foresee in a mass spectrometry based applications which are currently being used? If you ask me, in principle mass spectrometry base proteomics particularly, liquid chromatography couple to electro spray ionization technique has provides very high throughput application, and vast identification and characterization of proteins in biological samples. Moreover, multiple techniques are available today, to monitor these changes in protein expression as post transition modification studies; however, people think that mass spectrometry suffers from limited dynamic range or a finite accusation 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.

So, what MS based instrumentation and technologies Agilent is able to provide in the field of mass spectrometry? 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 due to biomarker discovery and the quantitation studies. Protein analysis has many challenges, so Agilent has the 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 LCs 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 spectrometry 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 rate.

So, what is the strip base technology which you just mentioned? 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. Right. And to work with all these low volume; low concentration samples you have to work with the nano HPLC, and traditional or conventional nano LC's 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, tubing's, fittings, because of that you cannot detect the leakages.

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In fact, I am carrying a chip with me, if you look at conventional nanoLC; 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, 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 nanoflow pump comes and takes a sample to the nano columns, and in fact you have a ionization itself on the chip. So, it is completely integrated to avoid all the complications of conventional nanoLC, this chip technology has the best solution available today, and it is one of the best thing available for any of the proteomics lab.

So, now as mass spectrometry technology is very much (( )) 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 (( )), in the clinical hospitals?

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 simple, it gives you information in a minutes and saves times

money and more accurate results, the mass spectrometry is the basically a transformative technique, but the only questions is, how fast it will be adopted by the scientific community? That is the one thing, analysis 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.

So, as 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 base technologies in the hospitals and clinical sitting, 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 Yes

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. Yes, I am hundred percent agree with you, even I have seen most of the hospitals nowadays having a mass spectrometry for the clinical applications. Right. 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 base proteomics, they started using mass spectrometry.

Right, can you briefly describe about some of these technologies in which you mentioned as a triple quad and chip based technologies? 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.

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Whether you quantitate drug metabolites, measure herbicide levels in fluids or determinant contaminant levels in ground water, the triple quadrupole

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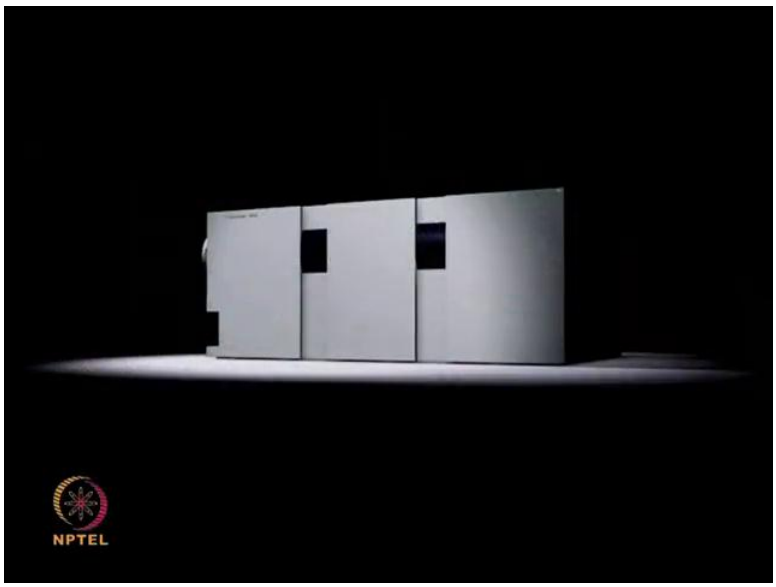

Mass spectrometer is unequal for quantitating trace organic compounds in complex matrices. The Agilent triple quadruple LC/MS delivers outstanding sensitivity, great ease of use and legendary Agilent reliability, all at a very attractive price. Great sensitivity starts with superior ionization technology.

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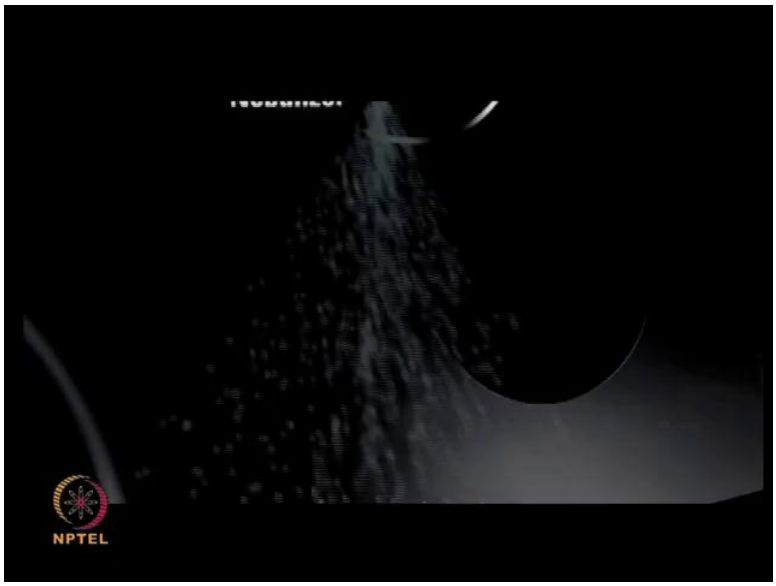

Agilent's LC/MS ion sources use are (( )) nebulization technology and high-volume counter-flow drying

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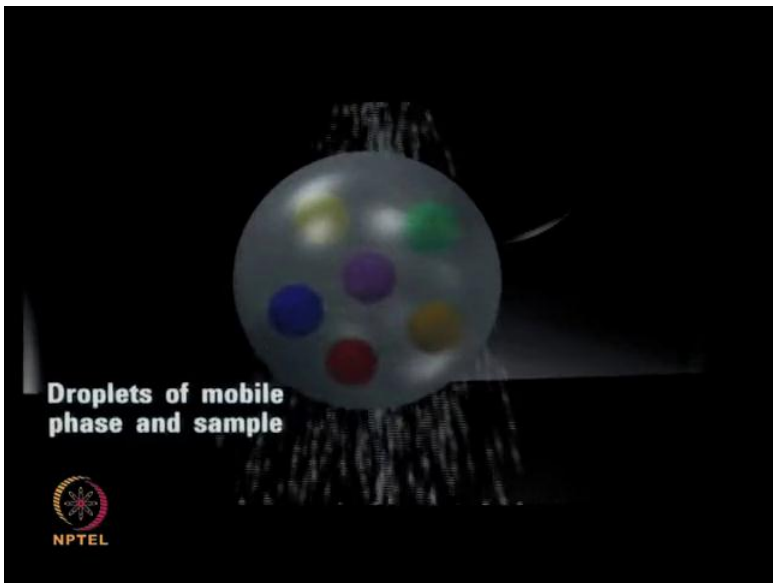

Gas, together they reduce noise related to incomplete drying solvent droplets,

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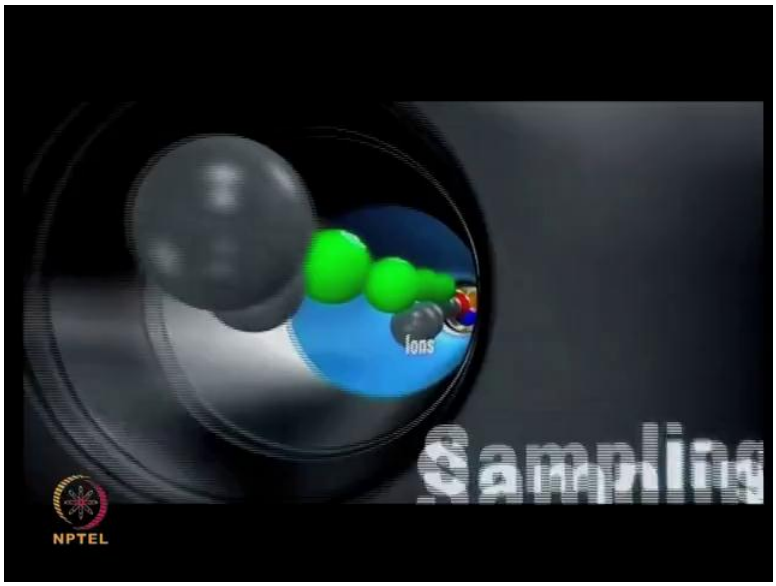

And keep the sampling capillary and ion optic cleaner for reduce maintenance. A thin skimmer aperture carefully matched whole size, and short capillary to skimmer distance reduced being broadening. An Octopole ion guide provides better ion transmission over a wider mass range, having the lenses enhance high mass ions transmission and increase sensitivity over a wider mass range, 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. In the hexapole collision cell precursor ions strike collusion gas molecules generating product ions and neutral fragments, linear axial acceleration and high collusion gas pressure simply operation, and then sure fast sensitive MS/MS without cross experiment memory effects.

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. In the detector conversion dynode operates at ten thousand volts to improve sensitivity, becomes the conversion dynode as of the main axis of the ion path, neutral molecules miss the dynode thus eliminating neutral noise. A secondary dynode helps to extend the use for life of the electron multiplier; the electron multiplier has a long life but it is also easily replaced.

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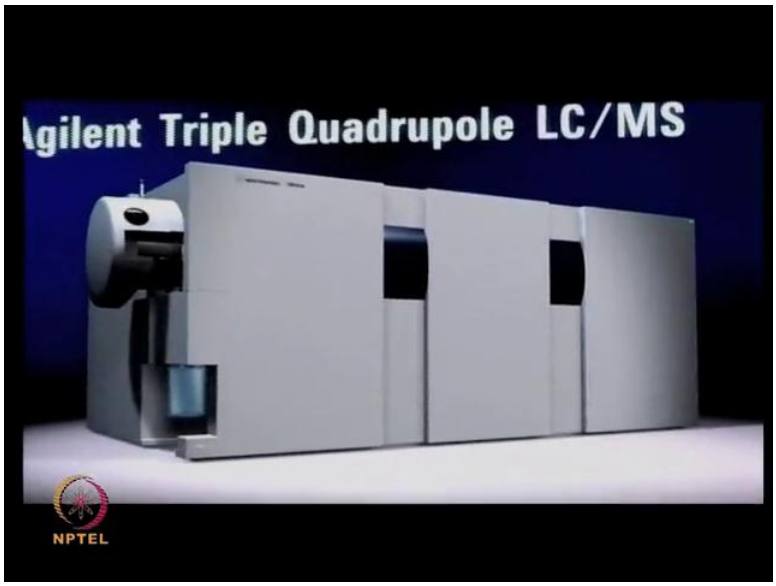

The Agilent 6410, triple quadrupole LC/MS establishes a new standard for value in a triple quadrupole mass spectrometer; it delivers outstanding sensitivity great ease of use, and imaginary Agilent reliability or at a very attractive price. Now, we have seen how the triple quadrupole is the best technique 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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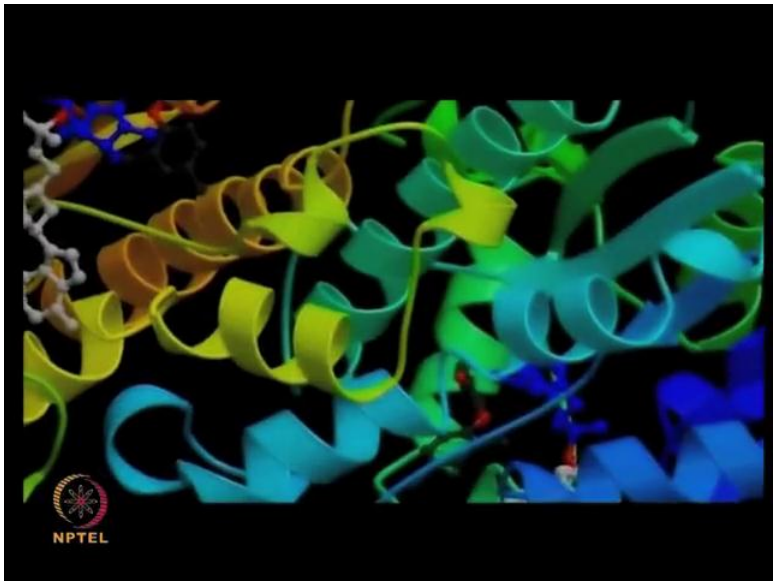

Whether you are identifying proteins and characterizing post-translation modification,

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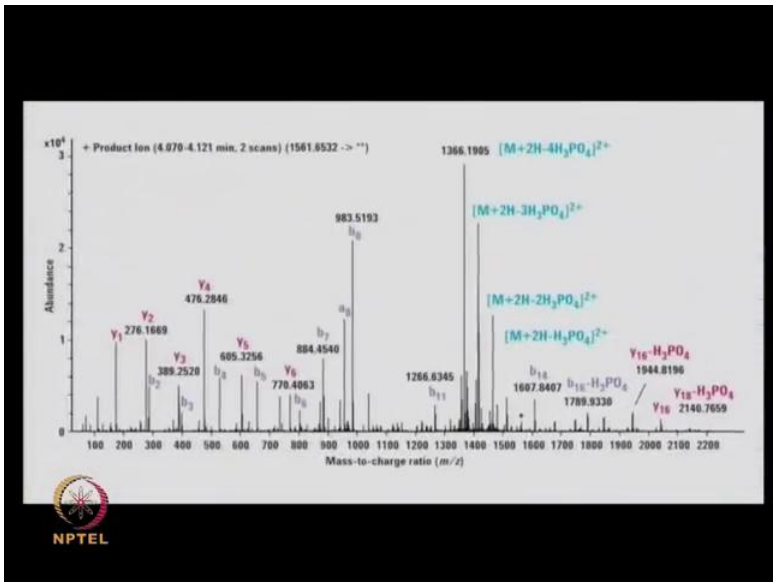

Searching for metabolite biomarkers or finding impurities in pharmaceuticals or fluid.

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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 surpassed Q-TOF sensitivity all in reliable and easy to use system. Great sensitivity starts with superior ionization technology; Agilent perpendicular nebulization technology produces final droplets and delivers more ions to the mass spectrometer.

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A second nebulizer, introduces reference mass solution that ensures continue mass access correction to the best possible mass accuracy, high-volume counter-flow drying gas reduces noise related to incomplete drying solvent droplets, and keeps the sampling capillary and ion optics cleaner for reduce maintenance.

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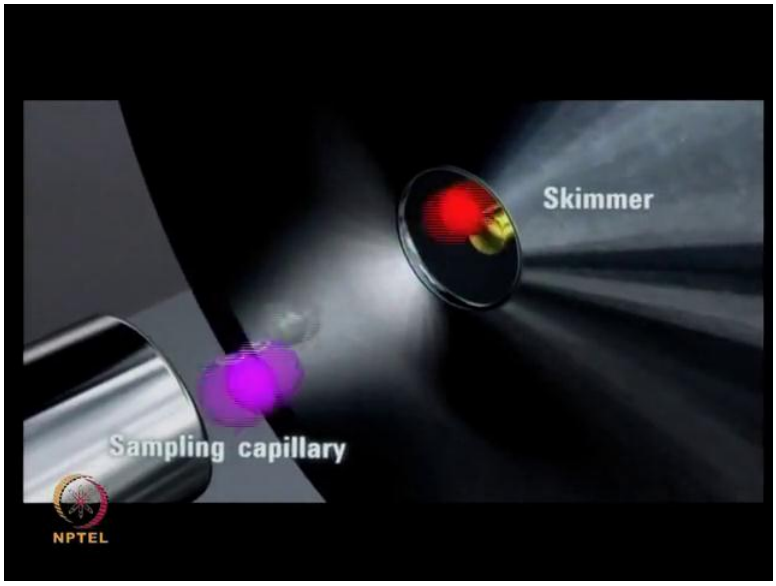

A thin skimmer aperture, carefully matched, whole size and short capillary to skimmer distance reduce being broadening.

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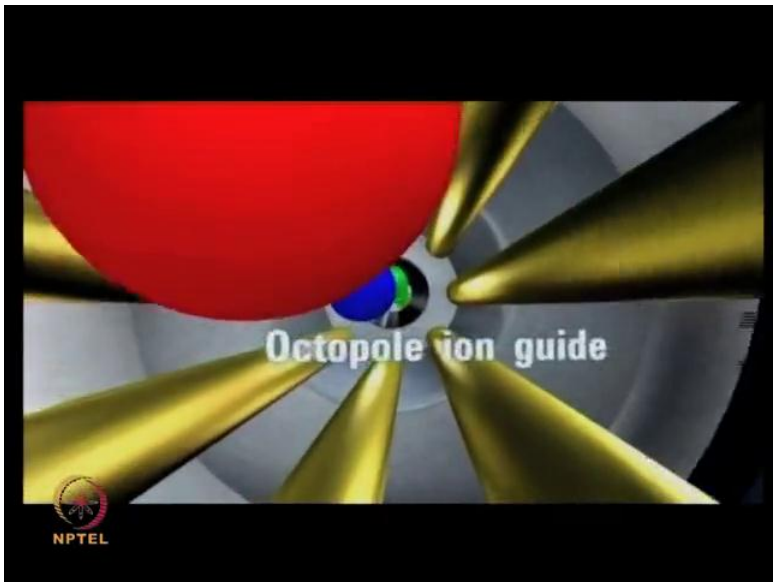

And on access octopole ion guide provides nearly one hundred percent ion transmission over a wider mass range. Having the lenses enhance high mass ion transmission and increase sensitivity over a wider mass range.

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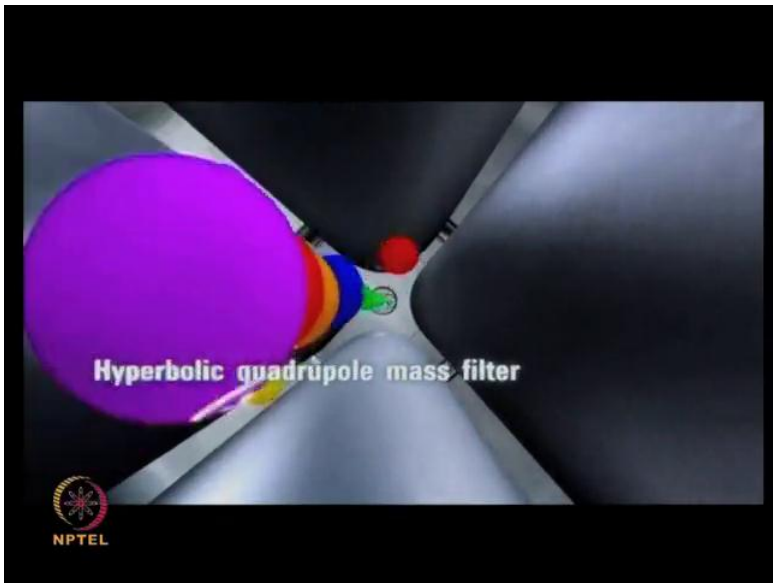

The first quadrupole mass filter allows only ions of the target mass to pass through, the hyperbolic shape of the rods enhances ion transmission and spectral resolution.

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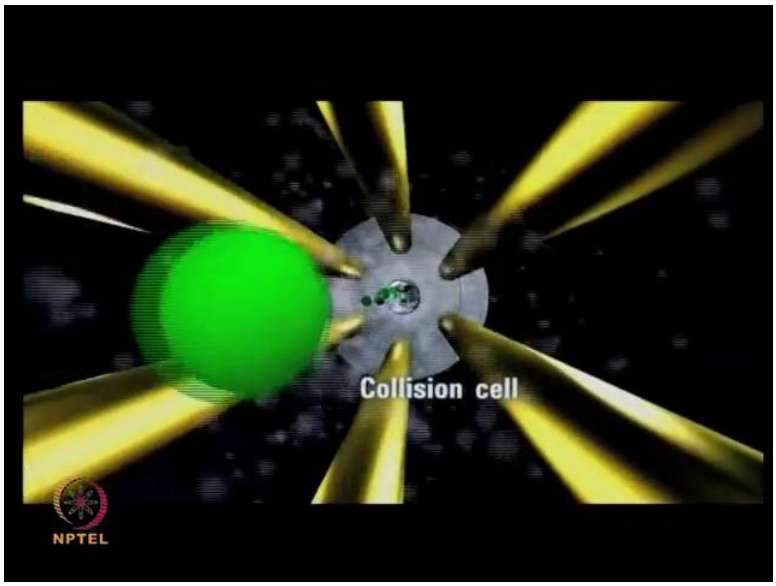

In the hexapole collision cell, precursor ion is dry collision gas molecules generating product ions and neutral fragments, linear axial acceleration and high collision gas pressure ensures that all ions exit the collision cell with nearly identical energy. This allows the same mass calibration factors to be applied to MS and MS/MS ions, result is better than 5 ppm mass accuracy for MS /S ions.

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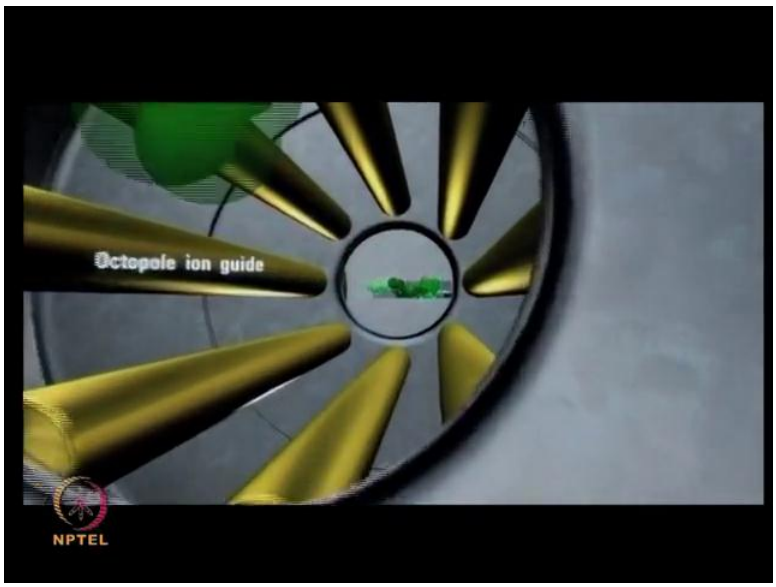

Another Octopole ion guide keeps the ions together while allowing excess collision gas to be pumped away, a quadrupole ion guide flattens the stream of ions for better transmission through the slicer;

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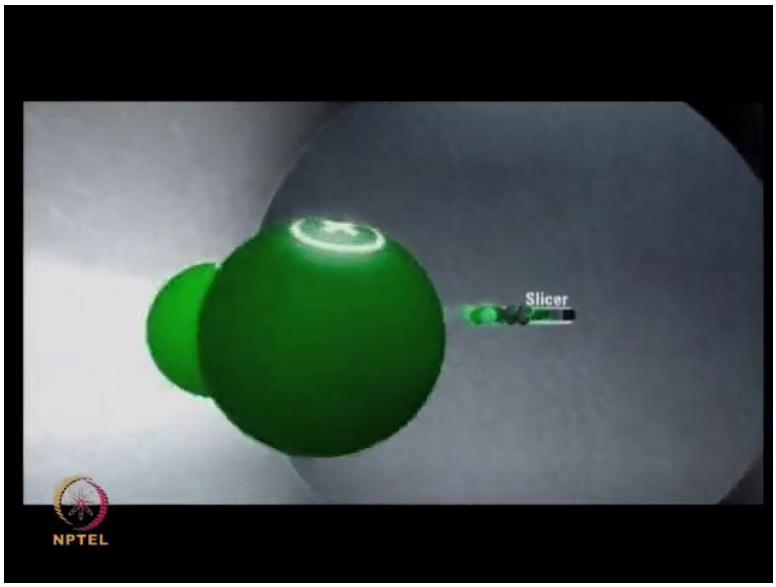

The slicer reduces variations in the vertical momentum of the ions. Ions with too much vertical momentum do not reach pulser; this improves mass accuracy for all ions. The flight tube is constructed a special materials with very low collision of the thermal expansion, so it is less sensitive to temperature changes.

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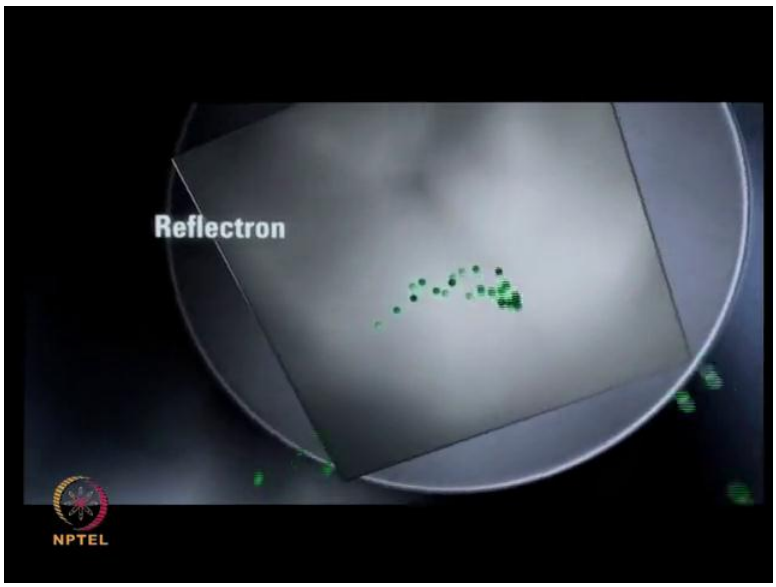

The Reflectron compensates for minor velocity differences improving the resolving power of the 6510.

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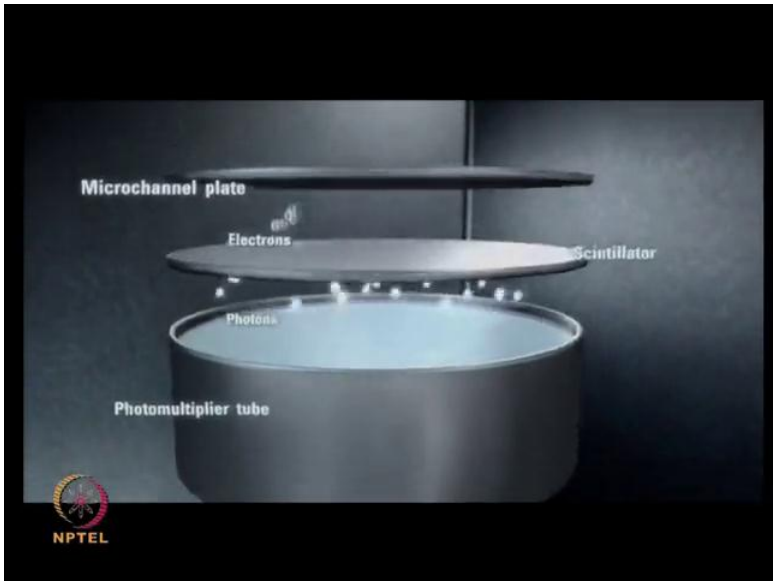

The microchannel plate detector converts the ions signal from the electrons to photons and back to electrons. This electrically isolates the high voltage flight tube and front of the detector from a signal pass to the electronics;

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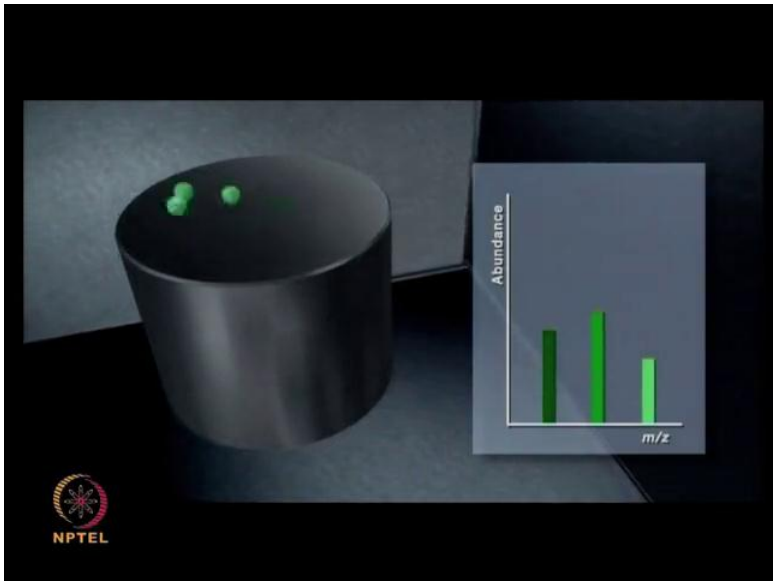

ADC digitizer electronics provide extremely high mass accuracy over a broader dynamic range. The 6510 offers outstanding in-spectrum dynamic range for a time of flight instrument.

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When you need the ultimate in LC MS/MS power and versatility, the Agilent 6510 Q-TOF provides it. An ease of use and reliability, many found in a research great mass spectrometer. Is it not 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 explains you how chromatography technique; the conventional chromatography technique, and how chip technology has an advantageous feature, look at the video also.

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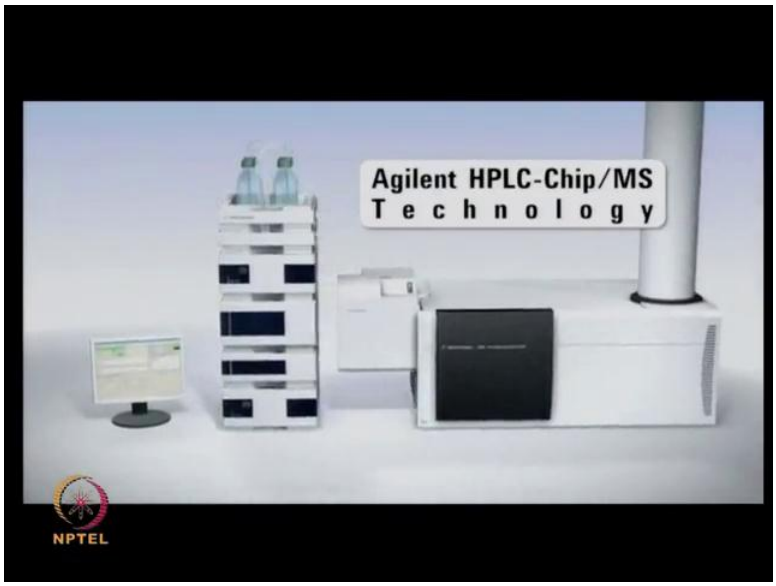

It is time to prepare for a new generation LC/MS technology from Agilent; HPLC chip/MS. The Agilent 1200 series HPLC chip/MS platform takes you to a new level of nanoflow LC/MS performance by combining microfluidics, is an easy to use plug and play interface that leads you focus on your results. The Agilent HPLC chip/MS platform; is based on the Agilent HPLC chip and Agilent HPLC chip/MS interface, that is design for use with all Agilent 6000 series mass spectrometers.

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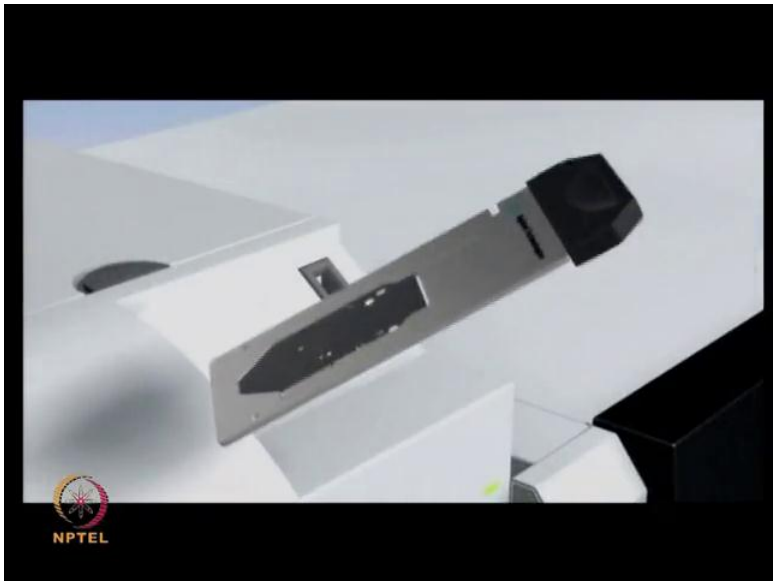

The Agilent HPLC chip, integrates enrichment and analytical columns, microvalve connections and metal coated nanoelectrospray tip on an inert multilayer polymer film, and it is smaller than a credit card. The compact architecture of the Agilent HPLC chip reduces peak dispersion, and combines all steps from sample loading through compound ionization for seedless operation.

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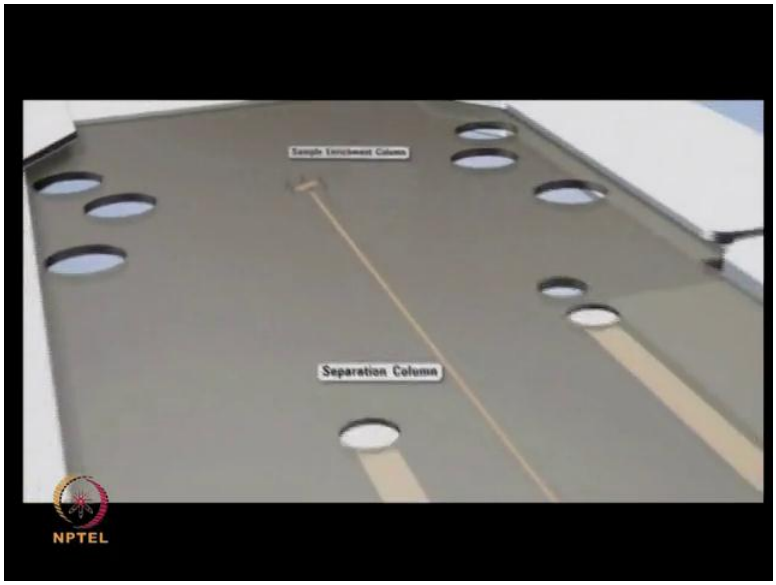

A closer look at the HPLC chip reveals that sample enrichment, and separation columns of a nanoflow LC system are integrated with interconnection and nanoelectrospray tip for compound ionization in mass spectrometry. This eliminates 50 percent of the traditional fittings and connection typically required in nanoflow LC/MS system, which dramatically reduces the possibility of leaks and dead volumes, and significantly improves ease of use sensitivity, productivity and reliability. The HPLC chip also incorporates all electrical contacts for the nanoelectrospray tip, and features and embedded radiofrequency id tag; attracts the usage and operating parameters of the chip.

The HPLC chip are housed in 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 microvalve switching. 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 autosampler and loading pump connected directly to the chip cube. An HPLC chip is loaded and leak tight fluid connections are established automatically, by sandwiching the chip between the rotor and the stator of the built-in multiport microvalve. The rotor and stator ducked on to the chip, and establish of flow path from the nano LC to the ports on a chip surface, fast moment of the rotor ensures reliable switching between sample loading and sample analysis positions on the HPLC chip. Replacement of the HPLC chip is simple and can be completed in a few seconds.

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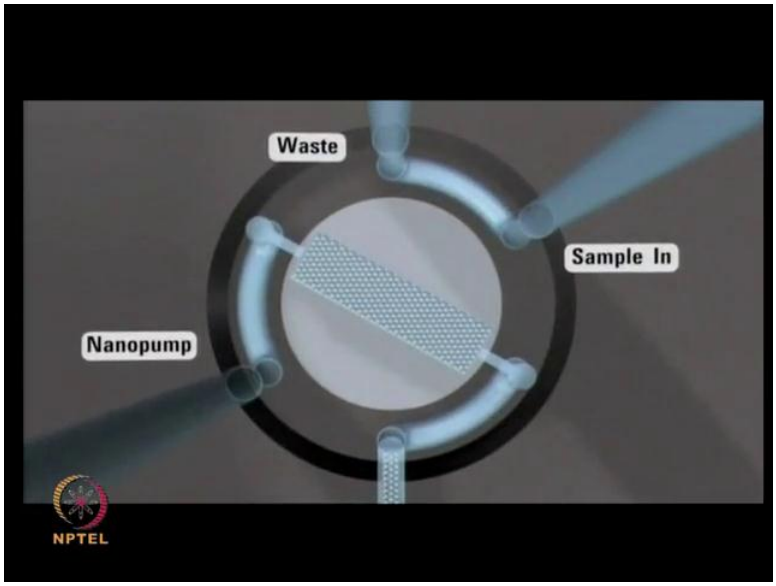

Let us look at how the Agilent 1200 series HPLC chip/MS system can be applied to a typical protein identification analysis, the Agilent microwell plate autosampler loads the digested proteins, a solvent flow moves the peptides into the trapping column, the microwell changes the flow path, the gradient flow from the nanoflow pump takes the enriched sample from the trapping column to the separation column, the peptides are separated just like on a conventional nanoflow column, reduced peak dispersion yields better separation efficiency and sensitivity.

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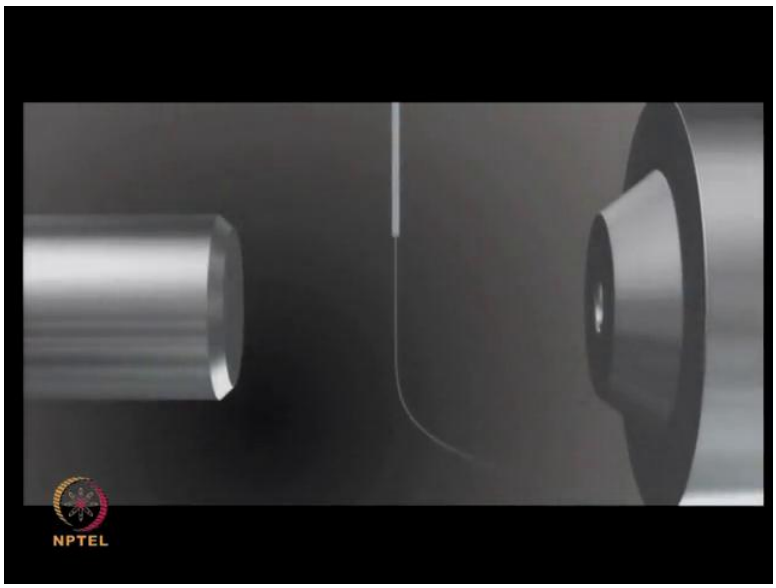

The integrated nanospray tip ensures reproducible nebulization of the effluent, right over for optimum ionization of compounds and best results, proven and nanoflow LC/MS technology, and the new and exciting capabilities of microfluidics, combined to form a system, that is; easy to set up and easy to maintain, scientists can now get more results faster.

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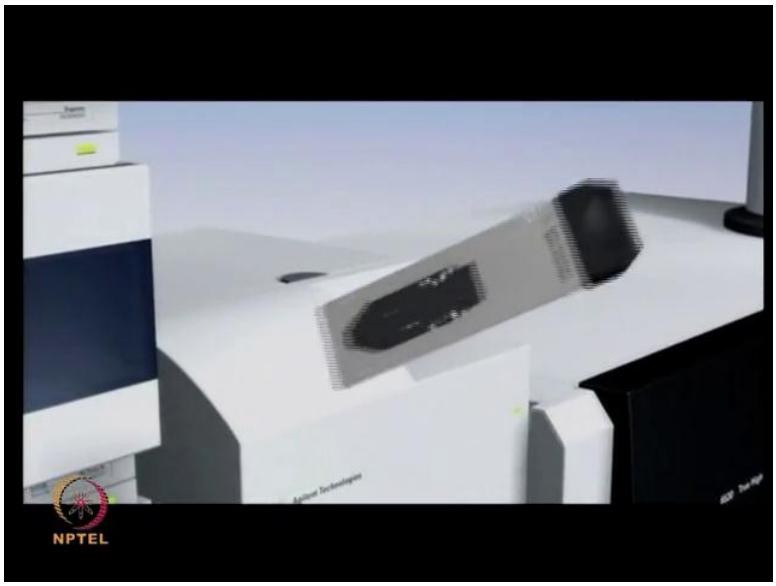

The flexibility of the HPLC chip design, and the HPLC chip/MS interface microwell technology in integrating additional chemistries and separation strategies, opens up a wide range of potential solutions for many research challenges. On chip multidimensional nano LC is one of many possible new applications.

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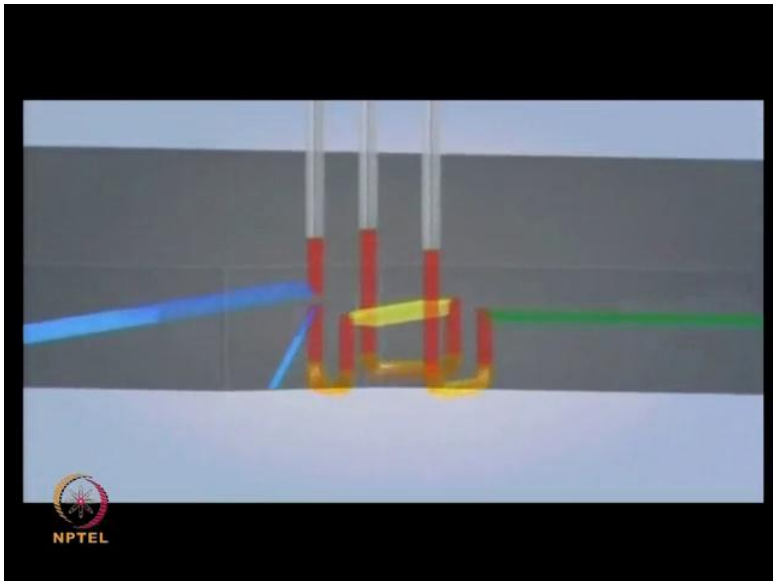

By adding more layers to the HPLC chip additional capabilities, such as two dimensional HPLC; the affinity chromatography and on chip chemistries such as on chip protein digestion are possible. These new applications, and many others such as chips with

different columns lengths, and packing materials are part of Agilent exciting custom HPLC chip portfolio and packing materials are part of Agilent exciting custom HPLC chip portfolio.

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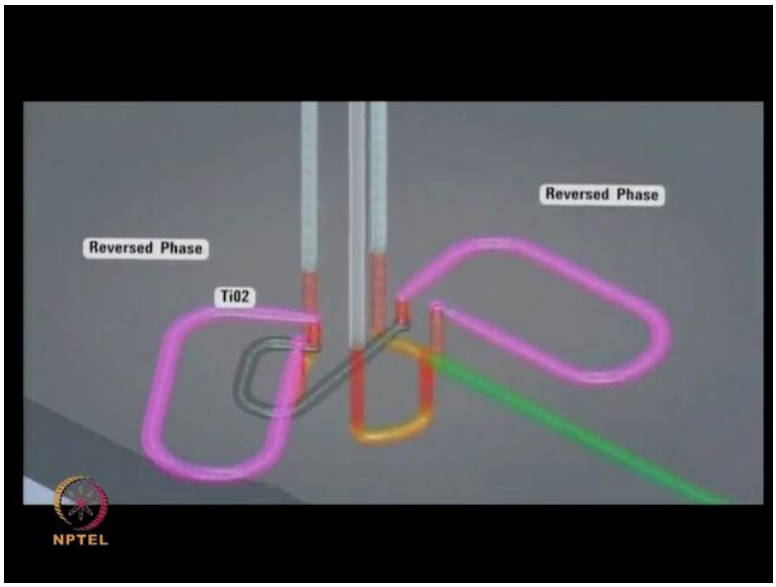

Moving beyond protein identification, the new phosphor chip with sandwiched reversed phase titanium dioxide trapping column; provides researches working on post-translation modification with a convenient tool targeted at phosphorylated peptides.

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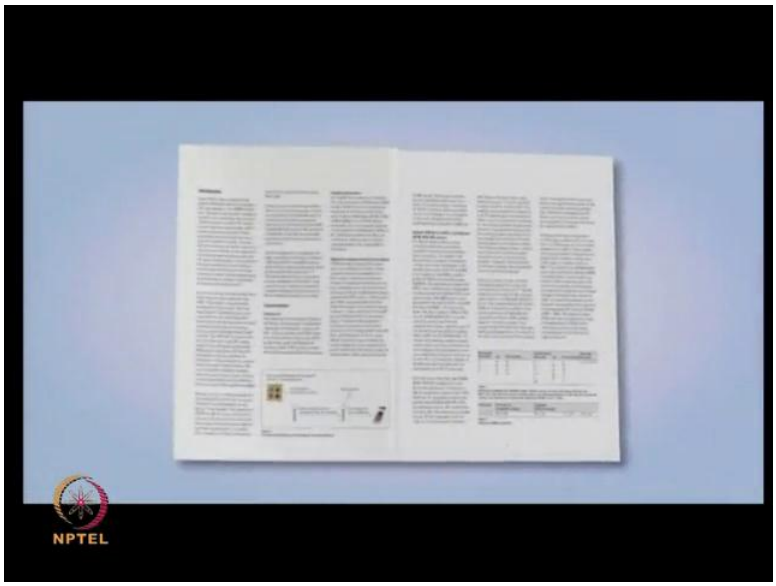

Pushing beyond proteomics, the new ultrahigh capacity chip with a 500 nanoliter trapping column facilitate analysis of pharmaceuticals; such as drug metabolism pharmacal kinetics with better sensitivity and much lower sample requirement, this will be extremely attractive when the single animal testing model is implemented in pharmaceuticals analysis.

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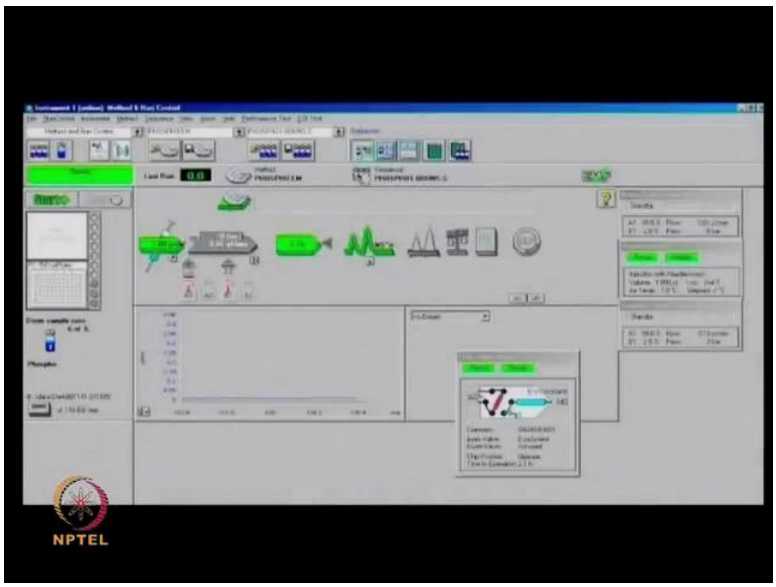

The HPLC chip/MS interface, is stranded module within the Agilent 1200 series LC portfolio, and is fully controllable through the Agilent chemstation or Agilent mass under software.

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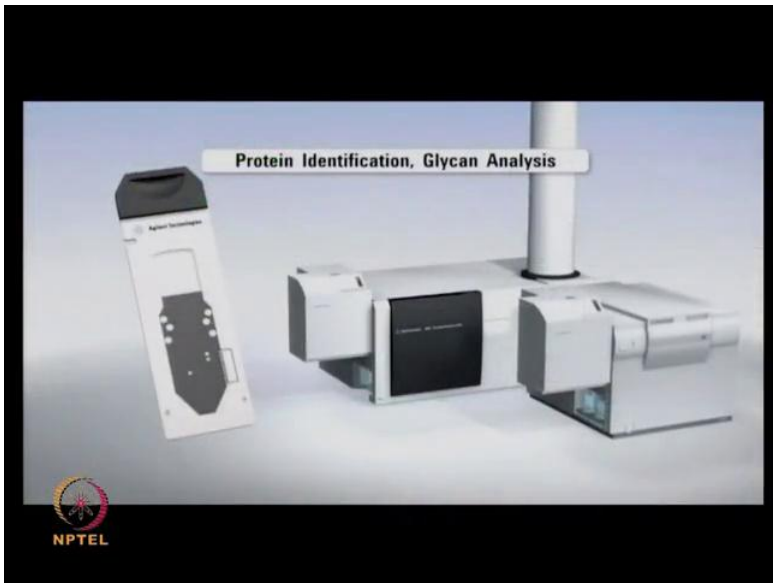

Step by step, chip by chip; Agilent facilitate new applications in life science, pharmaceutical and chemical analysis. HPLC chip MS, a growing trend in LC/MS technology. So, it was very good to see the latest advancement in this field of mass spectrometry, and what new technologies Agilent is offering. My final question to you is what will be your advice to our students or users, and how do you like to conclude this session for the mass spectrometry. That is a very good question actually, mass spectrometry has a tremendous potential in the field of analytical as well as research application, but one should we need to explore the possibilities what all we can do, it is all in our hand instrument generates a data, but how actually we can utilize this information, and how we can use this information for our purpose, we need to explore the possibilities. And I should say this is one of the best technology available today in this world, and one proteomics lab; any proteomics lab should have the mass based solutions for their applications. Thank you very much.

So, thank you very much Abhijit for very good discussion, and sharing some of the latest advancement in this technology from our students.

Thank you.

Thank you very much

Thanks

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 the 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 Mister Sangram Pattanaik from thermo fisher.

This is my pleasure to introduce Mister Sangram Pattanaik, the product manager LC MS division of thermo fisher scientific, he is working in the mass spectrometry area. Hello Sangram,

Hello, doctor Srivastava.

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.

For last 6 year I am looking at the mass spectrometry divisions of different platforms, in thermo fisher for last I am there in handling the orbitrap technologies, and we have own ion trap and triplet code systems. Early prior to that, I am for last 4 and 5 years handling the Q-TOF systems as well as triplet codes from other (( )). Now, altogether I am there for last 15 years in analytical industry, so these are the experience I carried.

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 areas?.

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 talk about comprehensive proteomics, and in targeted people are more talking about biomarker validations. In comprehensive proteomics, people's talks about the identifier, 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.

So, I think you rightly mentioned that emphasize 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.

Yes

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 discover.

Absolutely, it is as you are mentioned correctly the biologically matters itself is very complex in nature, when you have different kind of approach or workflow of applications; you look at the analytical solutions which can fulfill that remark.

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 record, those are the are areas people are now looking at.

So, proteomics is quite competitive and challenging field, and right now lot of emphasize from all the companies are in this field for the mass spectrometry, how to provide good solutions for analyzing the complex proteome? So, what type of major mass spectrometry instrumentations are available from thermo fisher currently?

As a thermo fisher, we have different technology standing 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. If you do not mind, can you just give some overview about the orbitrap technology currently available?

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

Please go ahead

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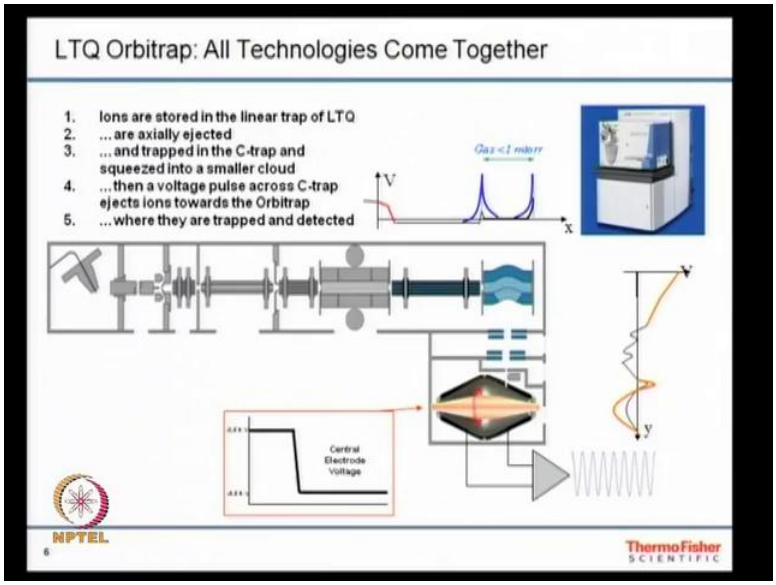

This is how the systems looks like, I if you look at on the ionization source, and there is two mass specs are in combinations to each other we called it is a hybrid technology. The first part is a linear ion trap, and the second part is the orbitrap. In the firstly, ionization and generated from the source it goes to the linear ion trap and it has been trapped there, once it has been trapped axially ejected and then goes into the c-trap, the c-trap squeezes the ion it packet and eject that ions into the orbitrap, once the ions get ejected into the orbitrap.

So, probably you are showing those trajectories in the PPT. So, this ions wants to get ejected into the orbitrap, 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 to charge ratio. If you look at there is a 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. I think you mentioned, that you want to show some more detail for the path motion. Path motion yes, I have a small video which we can also see to that.

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Just introducing the newest technology that is LTQ orbitrap elite, in the LTQ orbitrap elite if you look at this hybrid systems which has two mass spectrometer, the mass spectrometer which is sitting front as a linear trap, so LTQ orbitrap velos, and then the back and which is there is the high field orbitrap or called the orbitrap elite.

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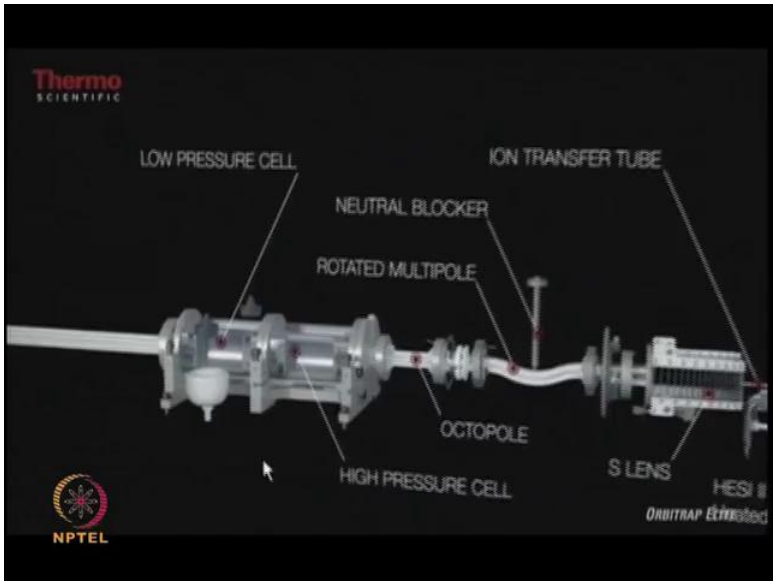

If you look at the schematic of the system and the front part of the system, it is the ionization source which sits on the front. Then you have the transfer optics, and the transfer optics we have the s lens which is the newest generations transfer optics, and

after the transfer optics we have the transfer tubes, and we have the neutral blockers which blocks the neutral which is coming from the source, then we have the Octopole, and then the ion trap. In the ion trap we have two regions; one is the high pressure cell, low pressure cell. Then on the radial there is two detectors; detector one and detector two, when you work with the linear trap detector can use for the detections of ion from the linear trap. From the linear trap it goes to the transfer optics, then we have the c-trap which dynamically squeezes the ion into packet, and allows that ions to go into the high field orbitrap then you have the (( )).

So, coming back to the ionization source, the ions generated from the source and goes into the transfer optics, and after going ions from the transfer optics it goes to the linear trap. And the linear trap in a full scan modes the ions are stored into the high pressure cell, once this ions are stored under high pressure cells in the full scan mode the ions then sends into the c-trap through the ion transfer optics, then it goes under the c-trap. This c-trap does cooling the ion and makes it into the packet of ion, so that the packet of ion injected into the orbitrap, because the orbitrap itself is an a static field, so you have to eject that ion and it tangent to the field.

So, once the ions goes into the orbitrap, it moves in into the orbit and the frequency is recorded which gives you in term the mass spectra. If you wants to work the linear trap and the orbitrap at the same time, you can run both the same at the same time. 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 on 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.

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. It is a very difficult to answer, but in a simplest way I can tell you it is all dependence on the on the applications area. People if you look at the selection of any instruments dependence 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.

It is ocean of thing, if you can have instrument you can generate a lot of data, but it all dependence on interpretation of the those data's, and it have has to be application oriented. 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.

Mass spec 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 Mister Sangram for sharing your orbitrap and different experience of mass spectrometer with us. Thank you. Thank you thank you very much

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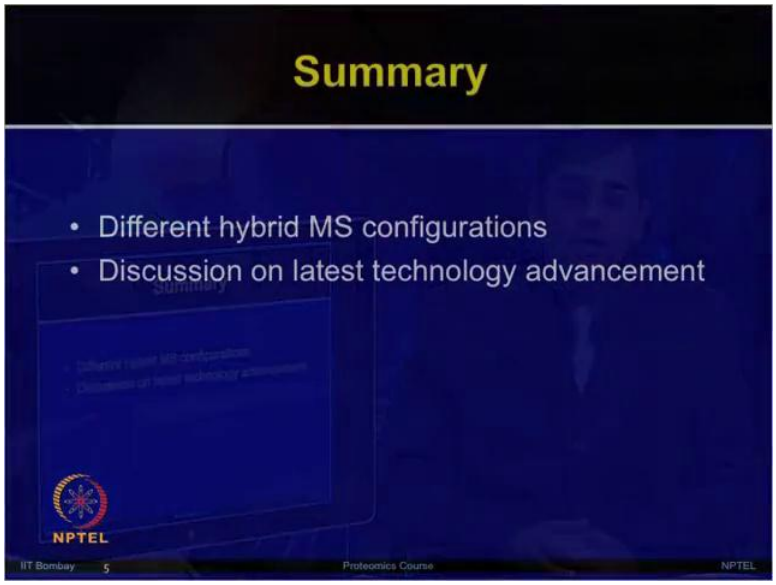

So, as you have seen the two interviews and discussion on different type of hybrid MS configuration, we talked about quadrupole time-of-light, triple quad and orbitrap technologies. You also seen the latest advancement in the field, the introduction of chip based technology, these are just few examples, there are many other good a configuration available from various manufacturers in the field. These interviews were mainly intended to showcase different type of hybrid MS configuration 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 two examples to showcase different type of advancement. Now, we will continue our

discussion on different type of mass spectrometry based application in the next lecture. thank you.