Mass Spectrometry Based Proteomics
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Mod 01 Introductory Lecture

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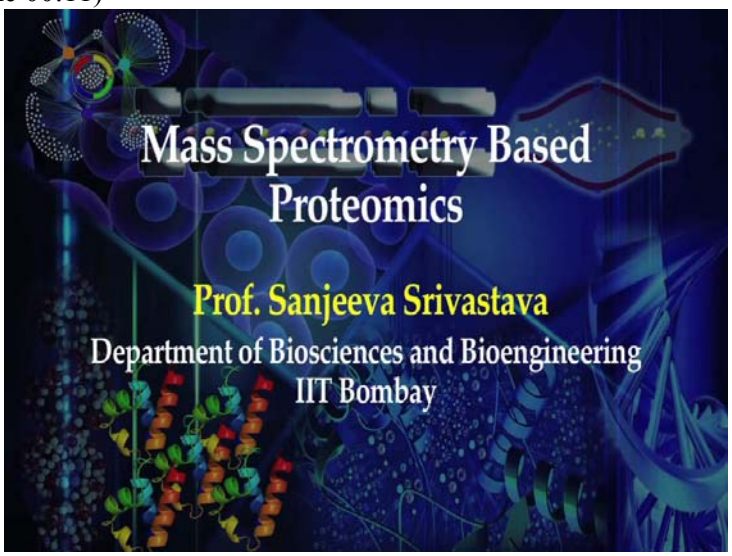

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Welcome to the MOOCs NPTEL Course on Mass Spectrometry based Proteomics.

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The proteome describes the protein compliment expressed by a genome. The extent of diversity and complexity due to alternatively splicing and post translational modification is tremendous. Therefore, the studying proteins and proteome becomes very important.

In recent years, mass spectrometry has played a major role in Proteomics level investigation. And in 2014, 2 human proteome reference maps were published using high resolution mass spectrometry.

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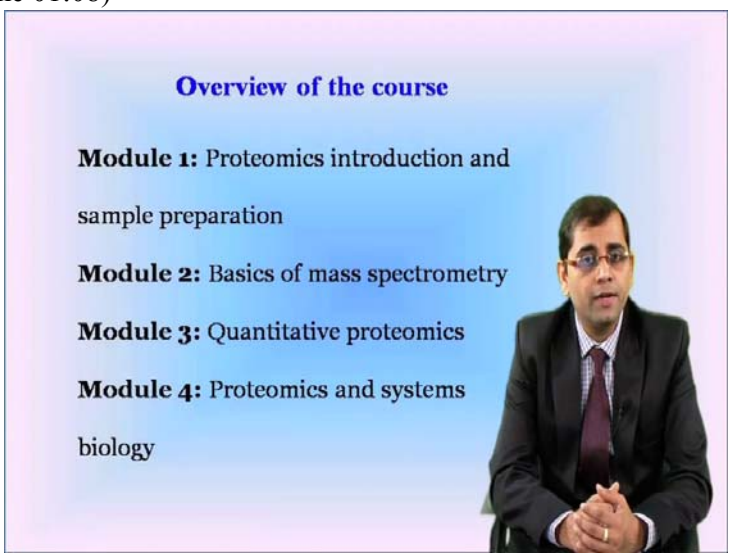

This course is divided into 4 modules. Each module, we will be finishing in 1 week. There will be 20 lectures of around 30 minutes duration which will cover the key concepts, experiments and laboratory demonstration to explain the concept effectively to the students.

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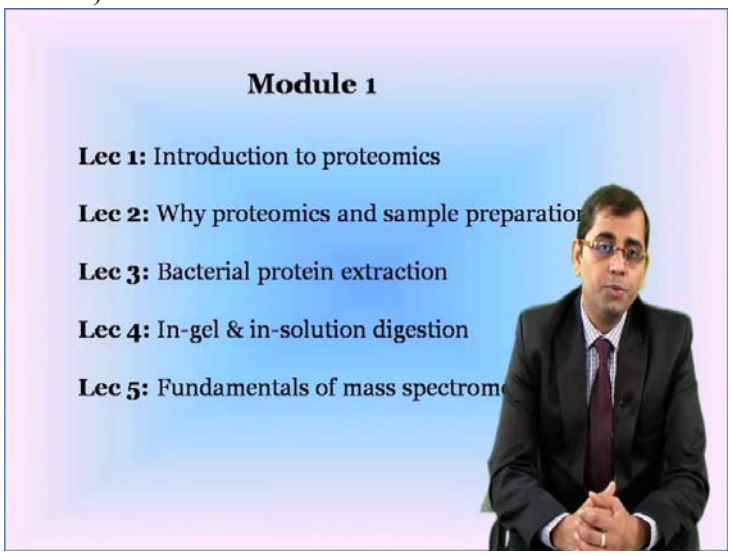

In first module, we will discuss about basic concepts of proteomics and the importance of sample preparation. Further we will discuss the fundamentals of mass spectrometry and the advancements in technology.

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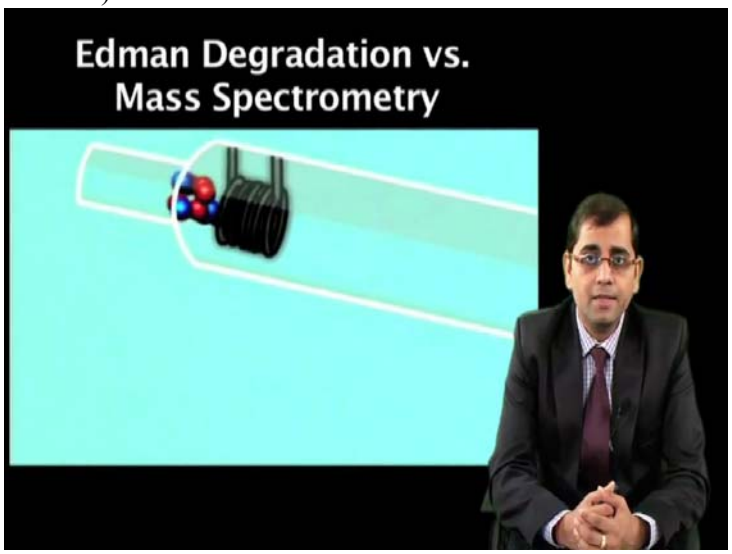

Lecture 1 will focus on basic understanding of proteomics, the technological advancements….

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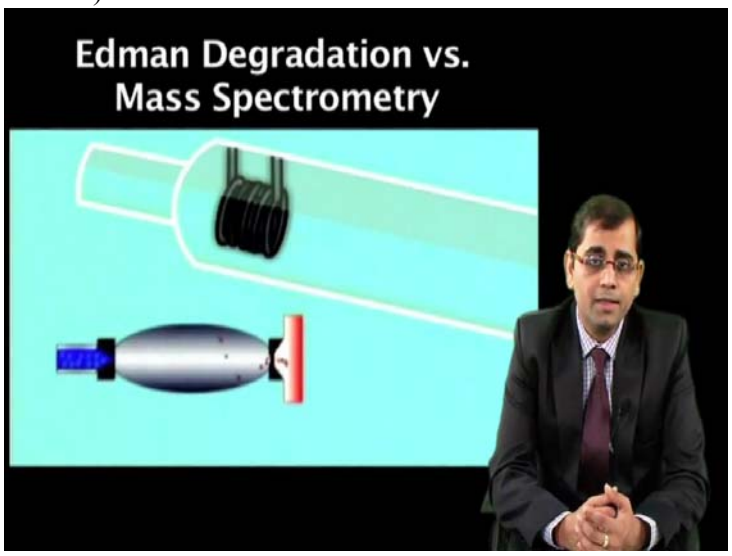

…in analytical techniques with increased sensitivity…

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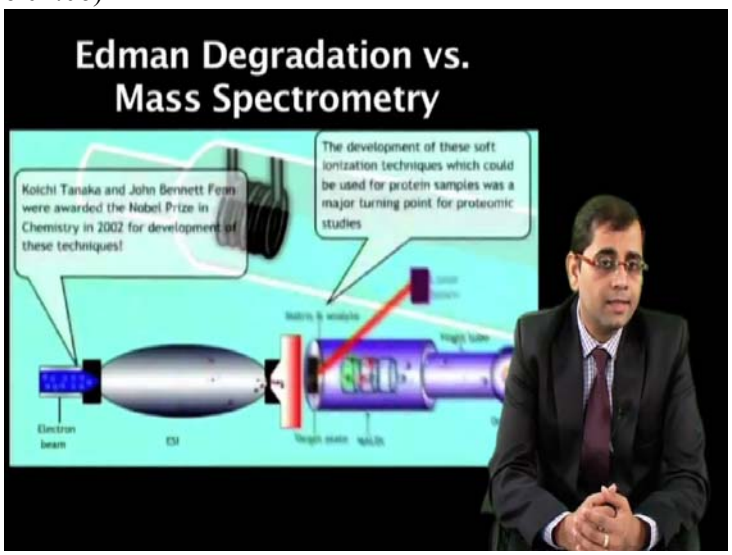

… resolution …

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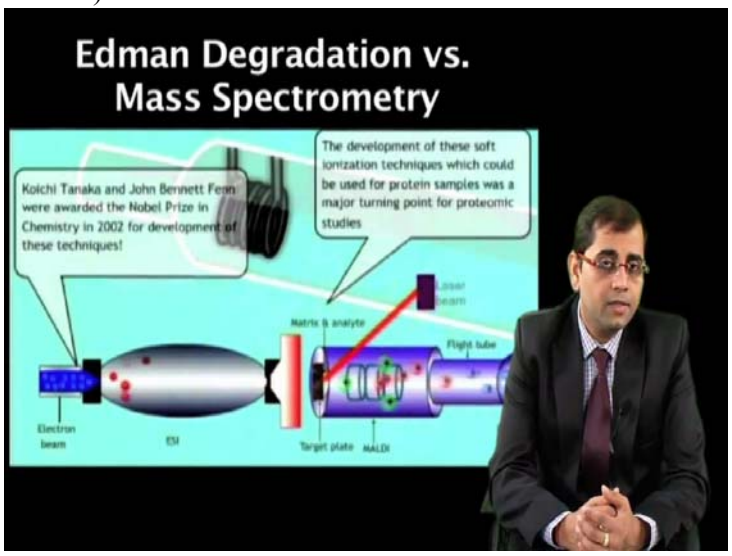

...and capability to carry out high throughput studies…

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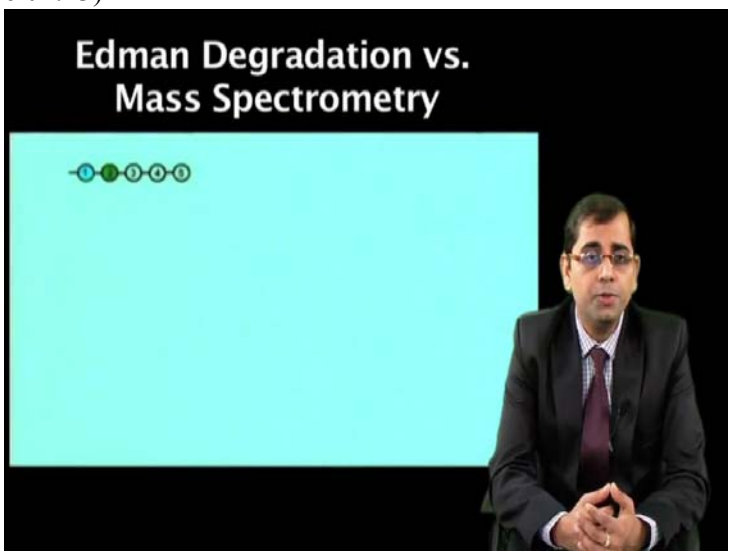

…. have led to the transition from…

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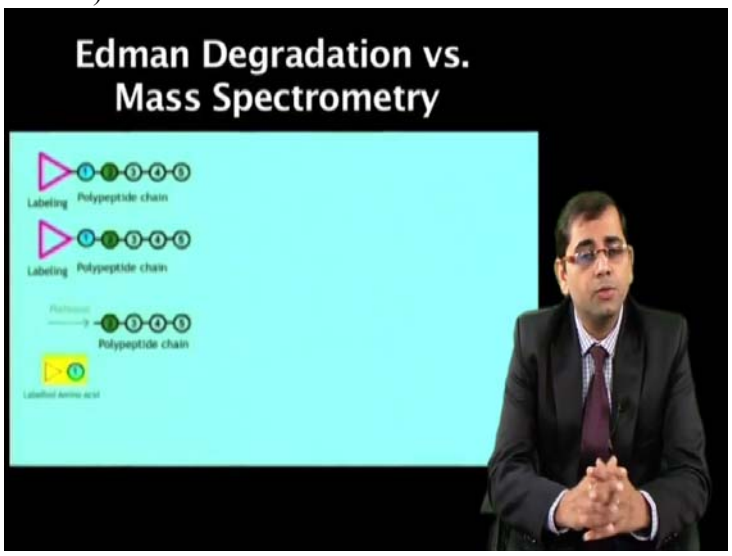

… protein chemistry…

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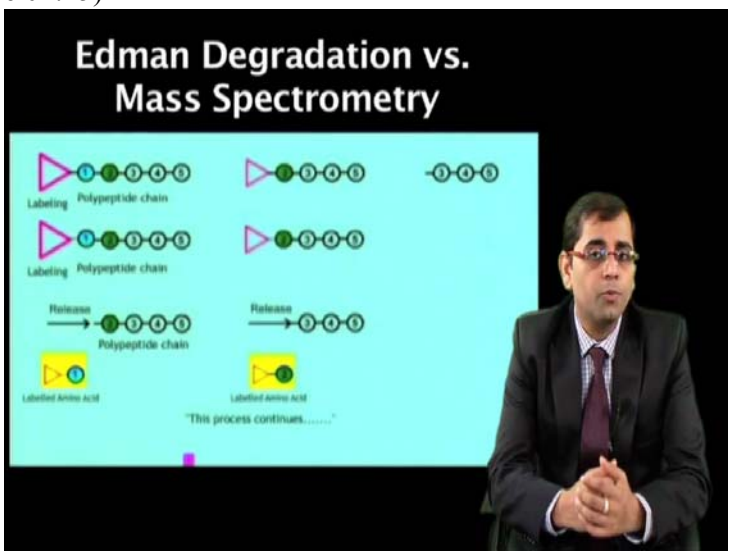

… to the new field of proteomics. How limitations of mass spectrometry for protein analysis …

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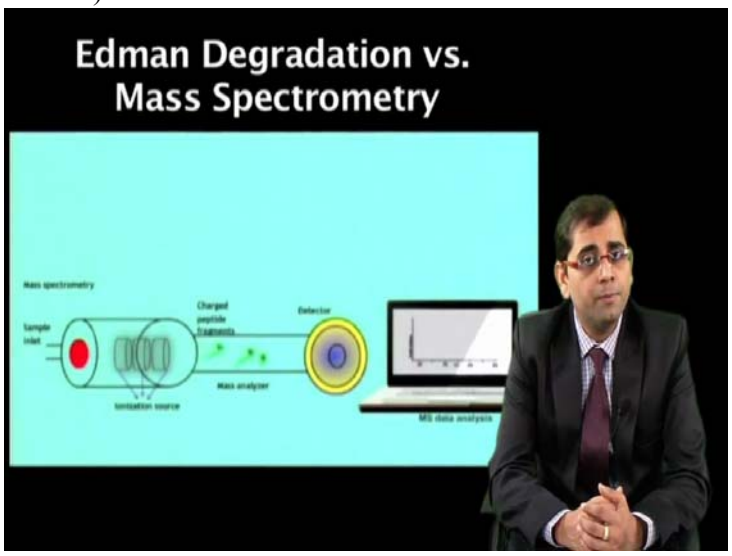

…. was overcome by development of soft ionization techniques…

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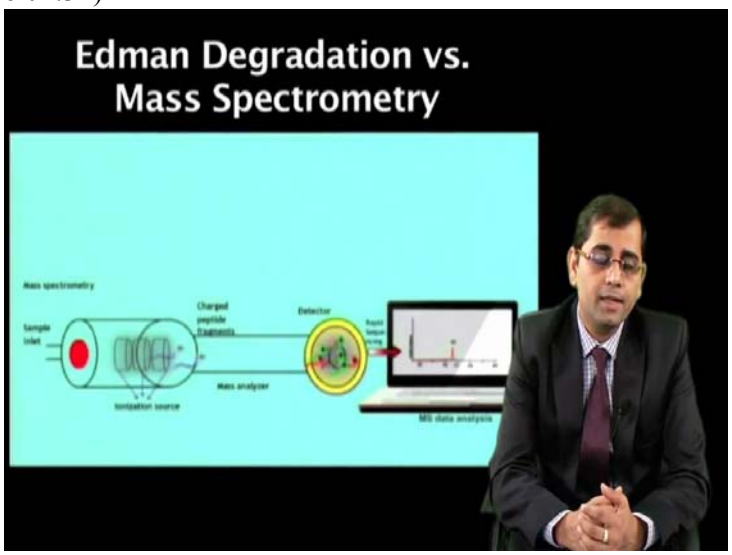

…such as MALDI and ESI will be discussed in this lecture

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In lecture 2, we will talk about sample preparations for proteomics application. Protein extraction is crucial step for any proteomic investigation. The protein extraction method involves cell lysis, prevention of proteolysis during lysis, different types of protein precipitation methods…

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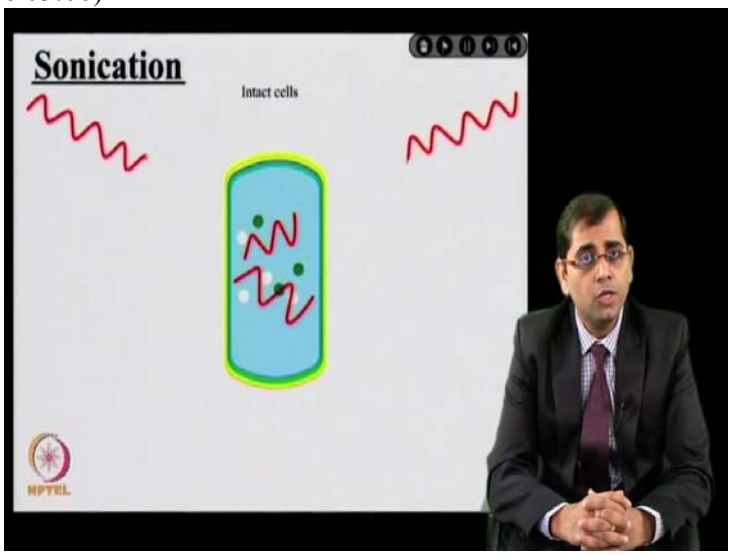

…protein solubilization…

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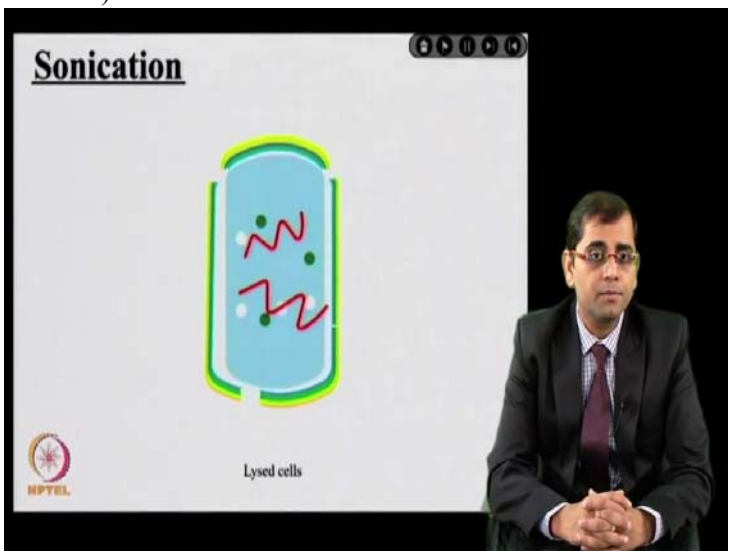

…and removal of …

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…various interfering components

The protocol standardization becomes a major challenge in proteomics …

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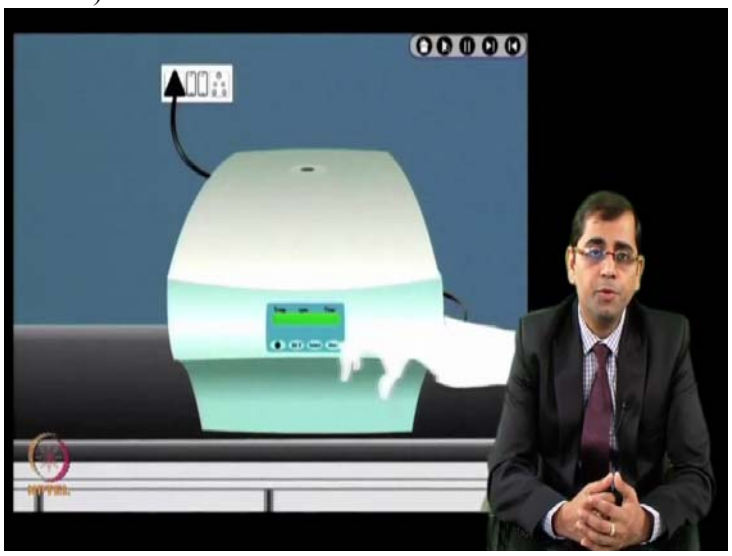

…and there is no generic protocol…

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…which exists in literature …

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….which can be used …

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… for every sample type. Therefore we will discuss few sample preparation strategies…

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…and how the good sample…

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…can be prepared …

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….for the proteomic analysis. We will continue the protocol standardization…

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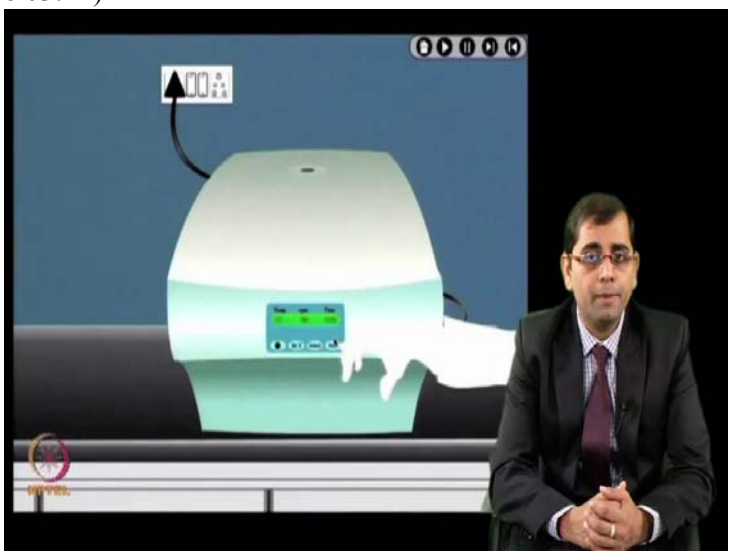

… using bacteria and discuss about cell lysis…

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…. protein precipitation and quantification and discuss about trizol extraction protocol.

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In lecture 4, we will talk about in-gel digestion or in-solution digestion of the protein which is essential prior to the mass spectrometry analysis.

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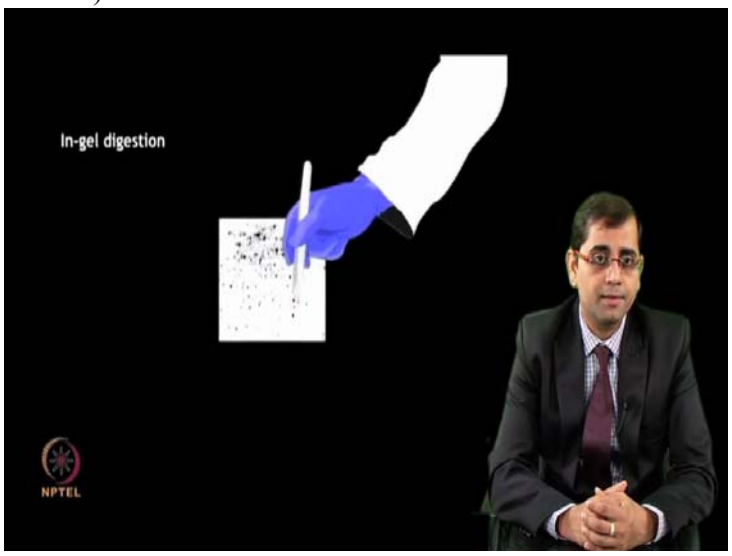

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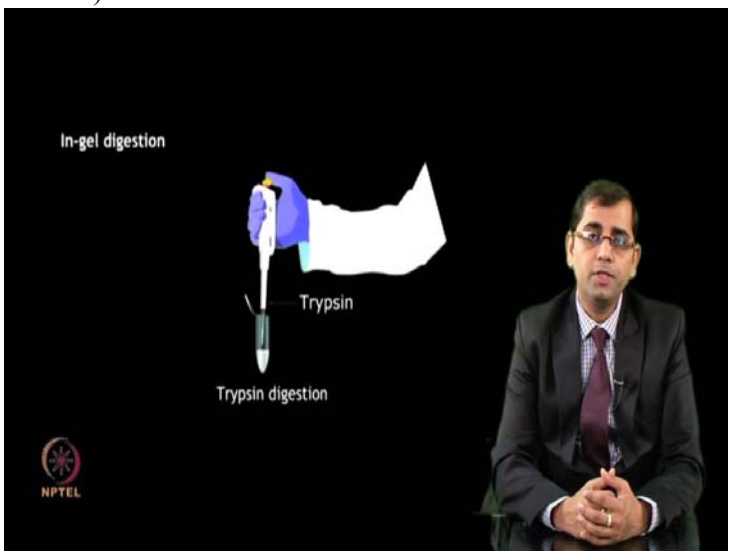

Often the in-gel digestion is used to extract proteins…

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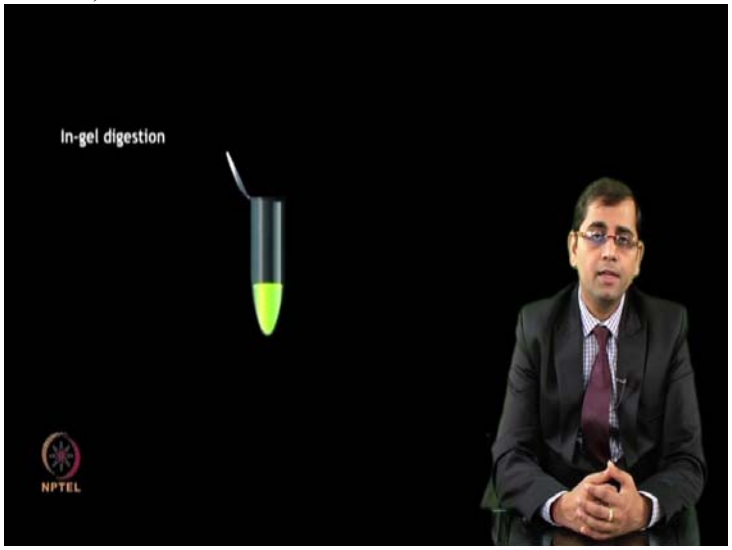

or peptides…

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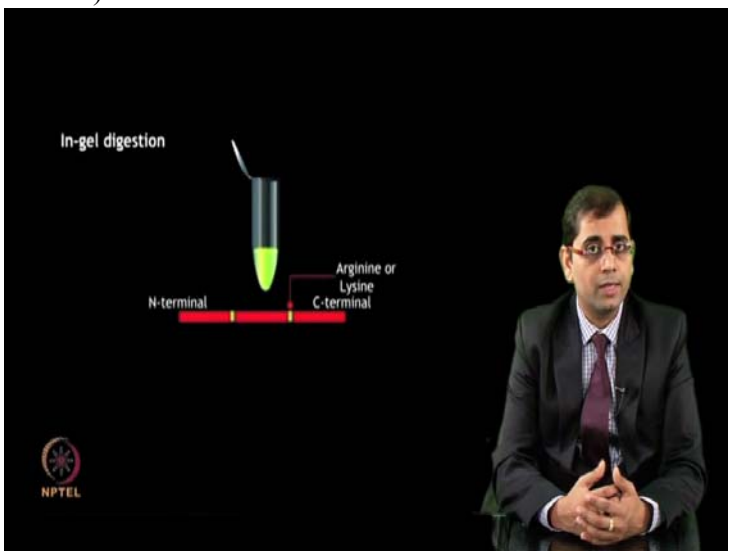

… separated on the gel electrophoresis. In gel proteolytic digestion

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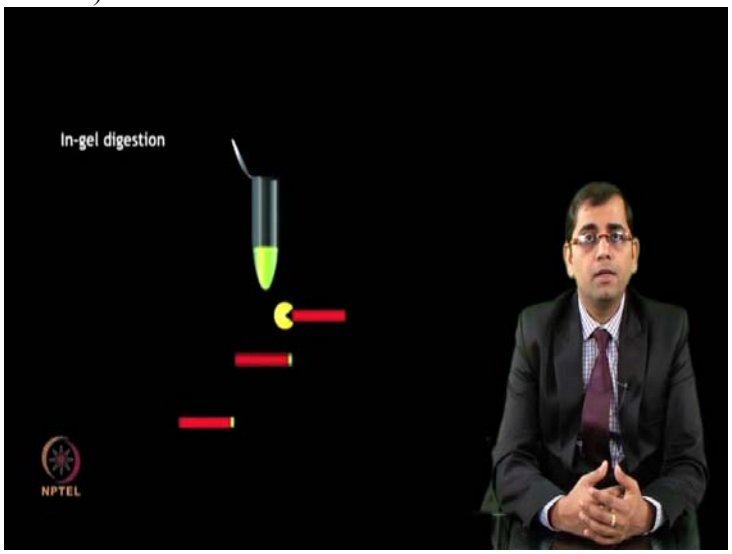

the in-gel proteolytic digestion is performed …

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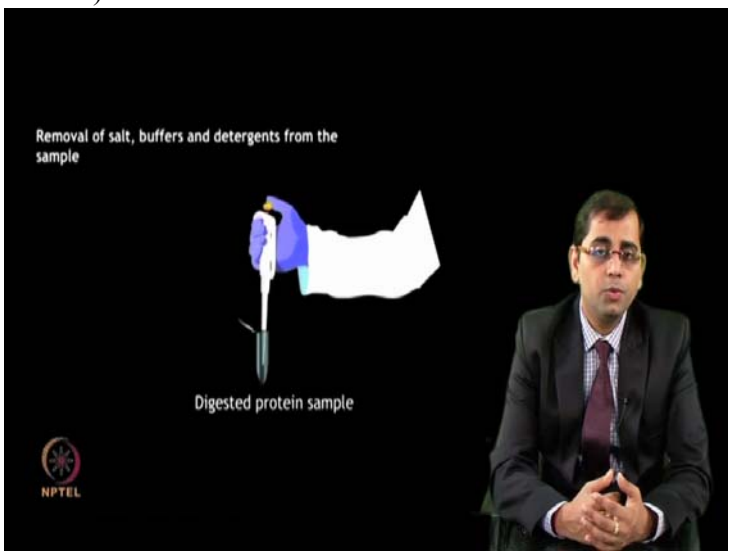

…. to cleave the protein of interest present within the polyacrylamide matrix

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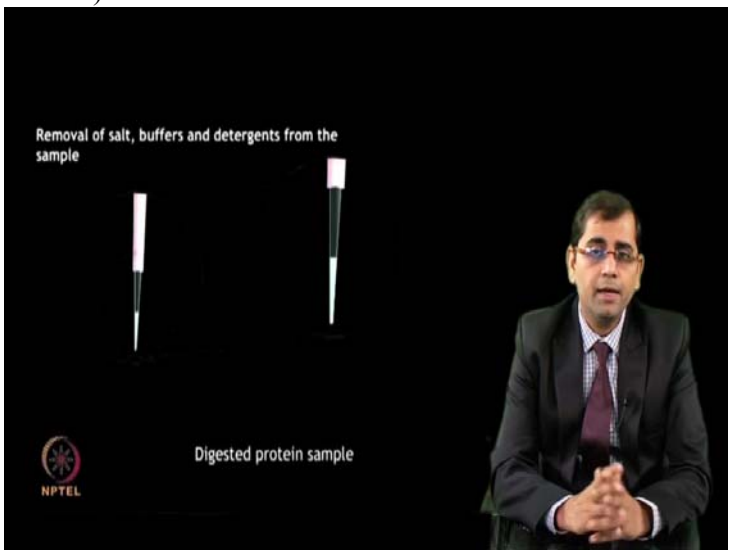

Mass spectrometric identification of the target protein greatly depends on…

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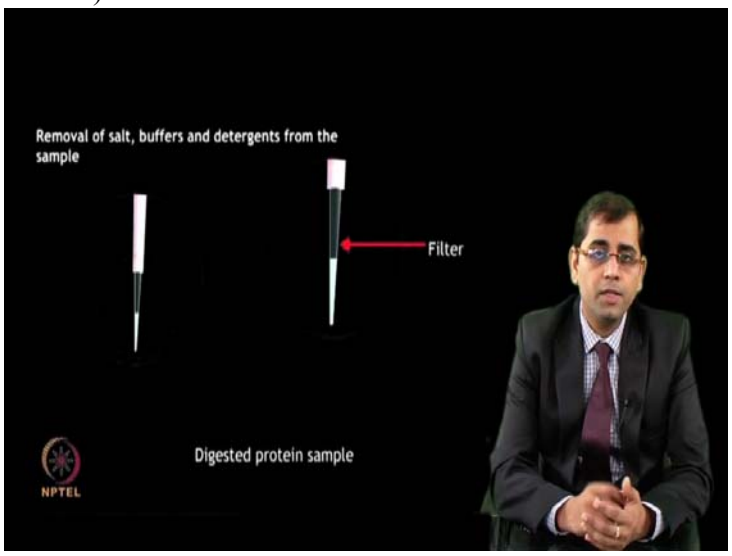

… the efficacy of the in-gel digestion protocol which generates mixture of peptides from the target protein through proteolytic digestion.

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In lecture 5…

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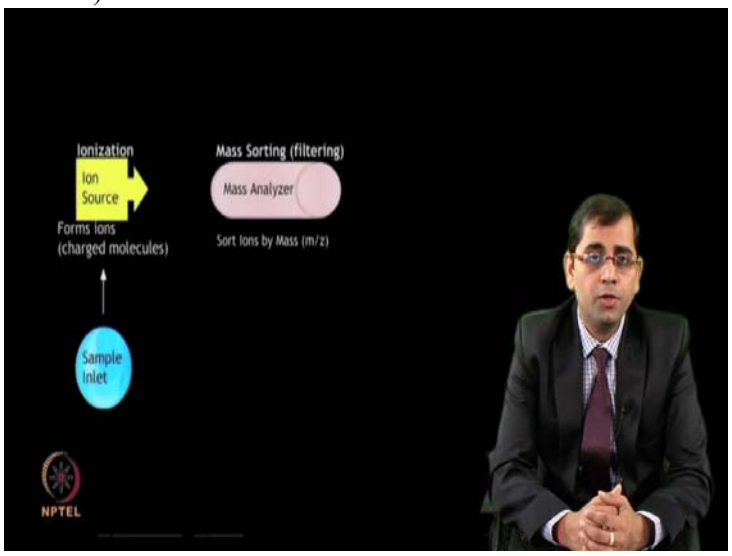

…we will talk …

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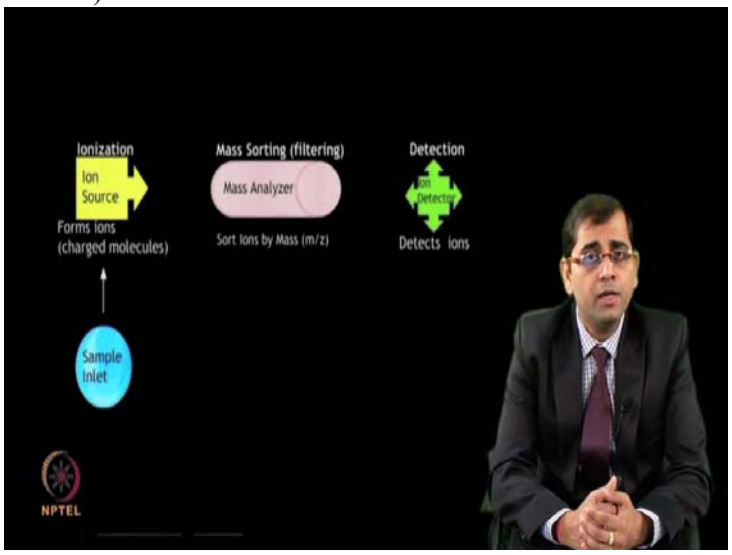

… about …

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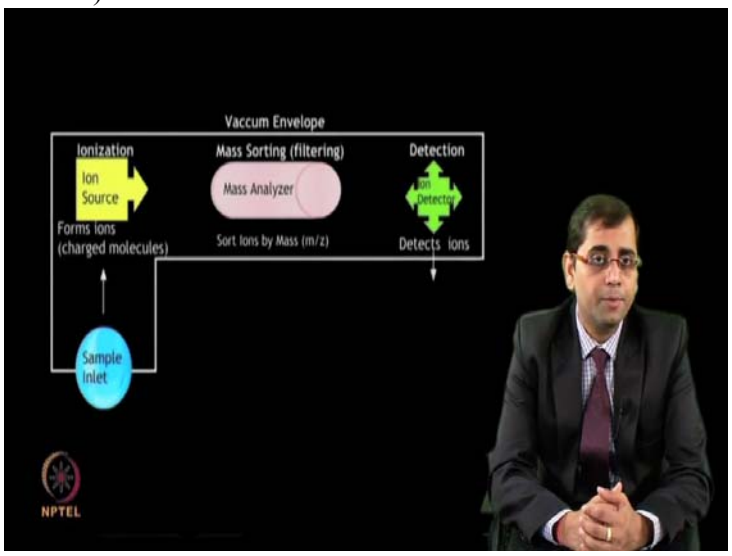

…the fundamentals of mass spectrometry

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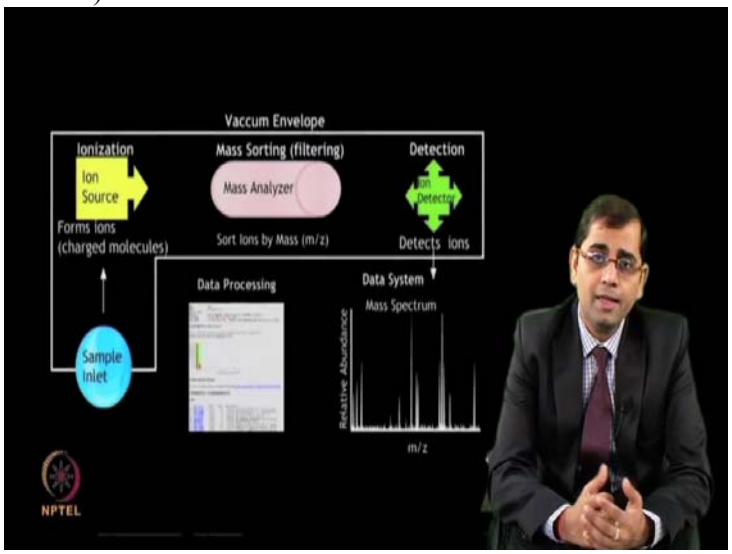

Mass spec is highly sensitive, balanced to measure the mass of the molecule in vacuum based of mass to charge ratio. It consists of an ionization source to ionize the molecule, a mass analyzer resolve the analyzed molecules in vacuum, and detector to read the signals coming from mass analyzer.

There are different ionization sources and mass analyzers being used for the proteomic analysis. If you know the principle of each one of these, it becomes much easier to select which configuration to use and when for your proteomic investigations.

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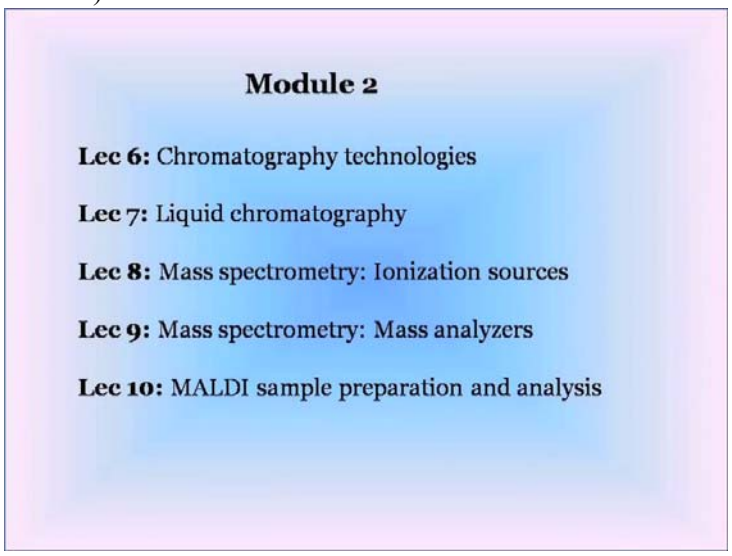

In second week, we will discuss in detail about different types of chromatographic techniques…

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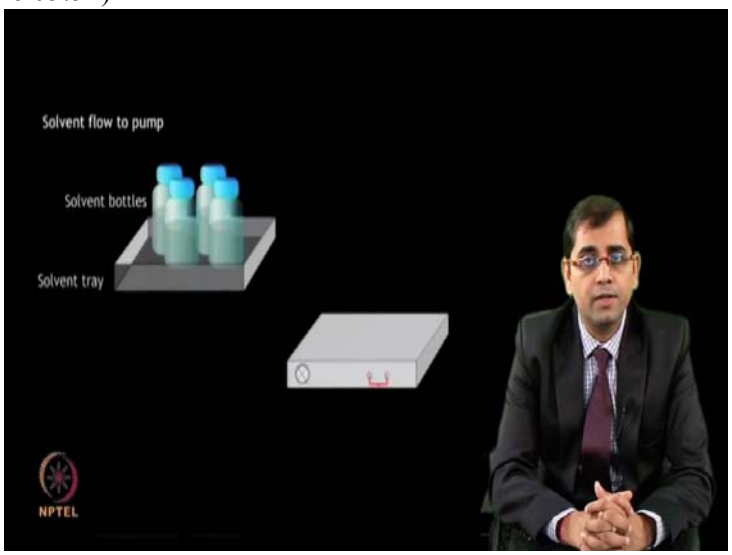

… like gel filtration, ion exchange chromatography…

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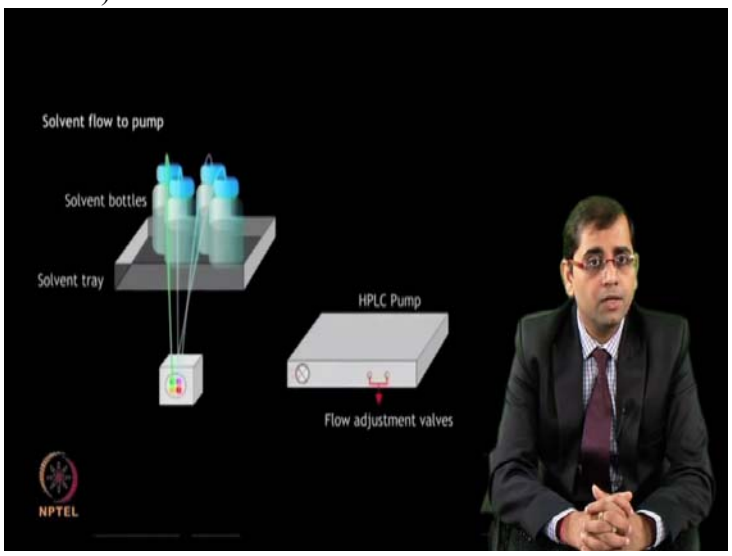

…affinity chromatography, SCX or Strong cation exchange…

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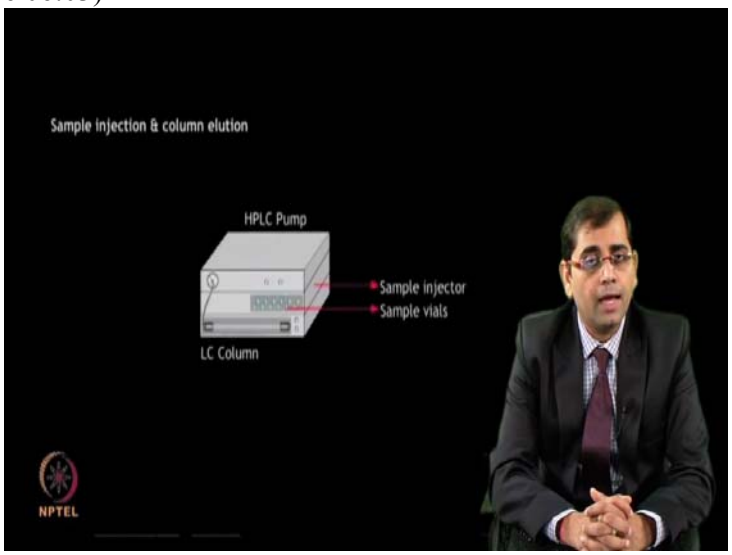

…and reverse phase chromatography.

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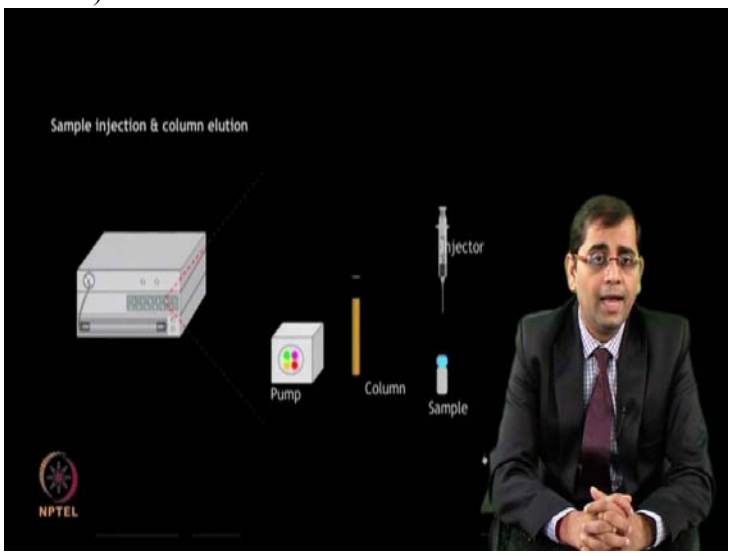

As we will progress…

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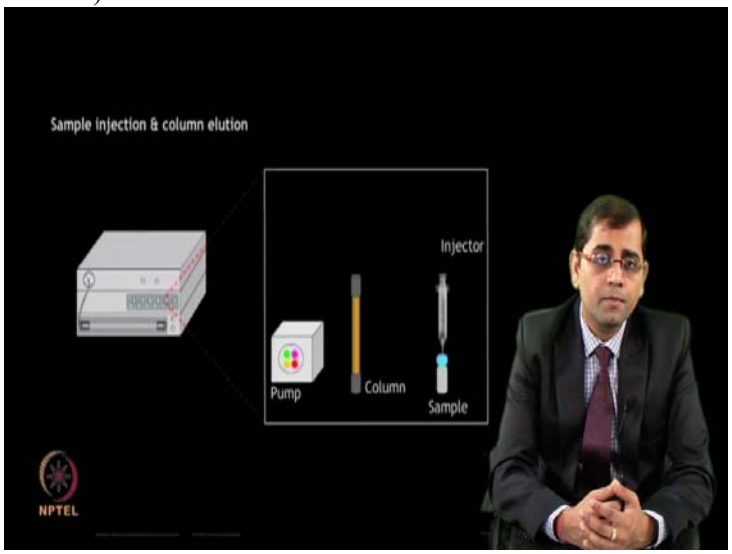

… through lectures 6 and 7, we will discuss…

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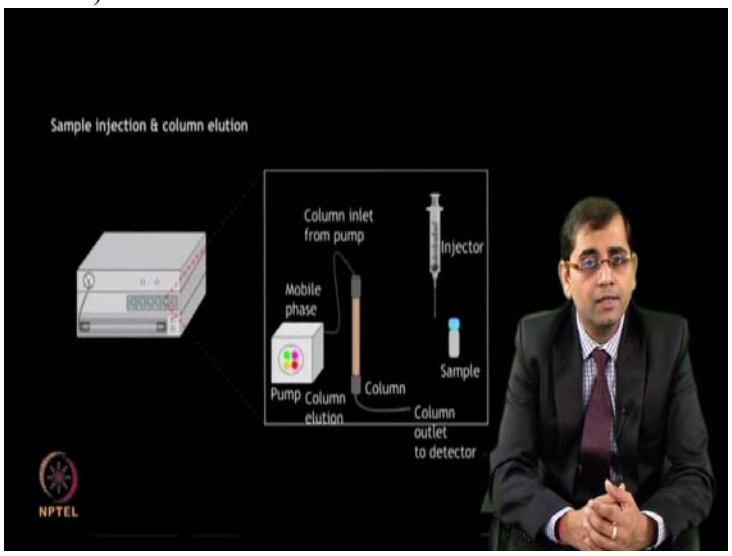

… how different chromatographic techniques work based on different principles…

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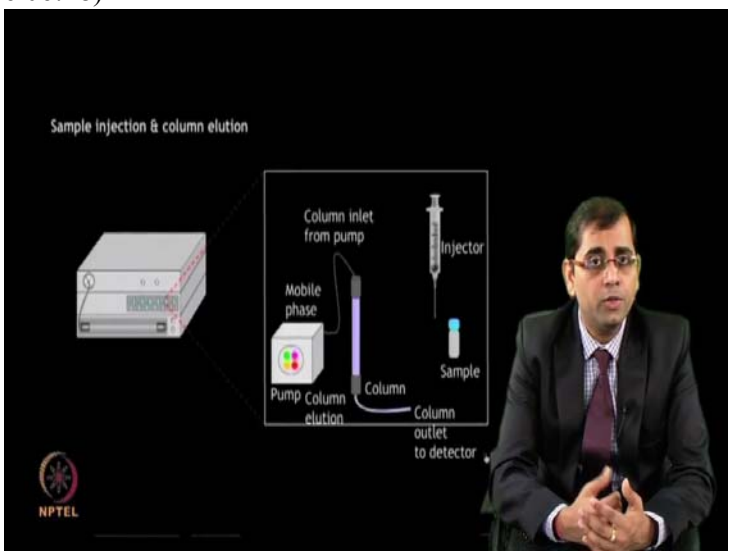

…and how one could employ those …

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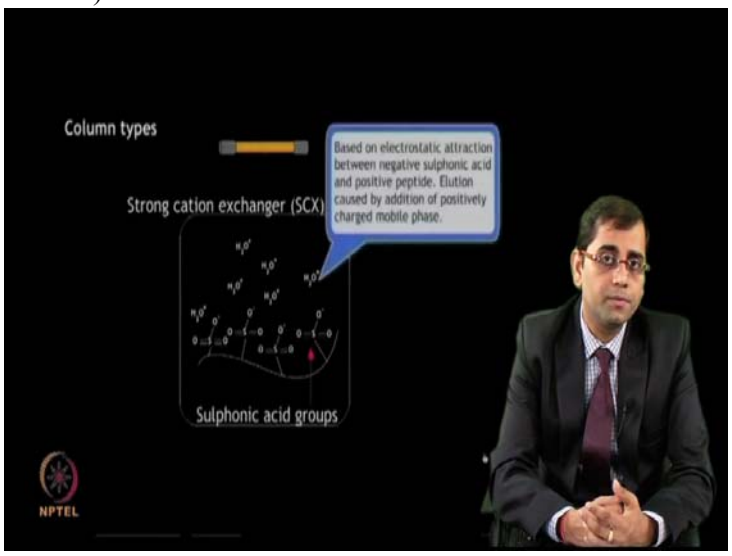

…in proteomic workflow

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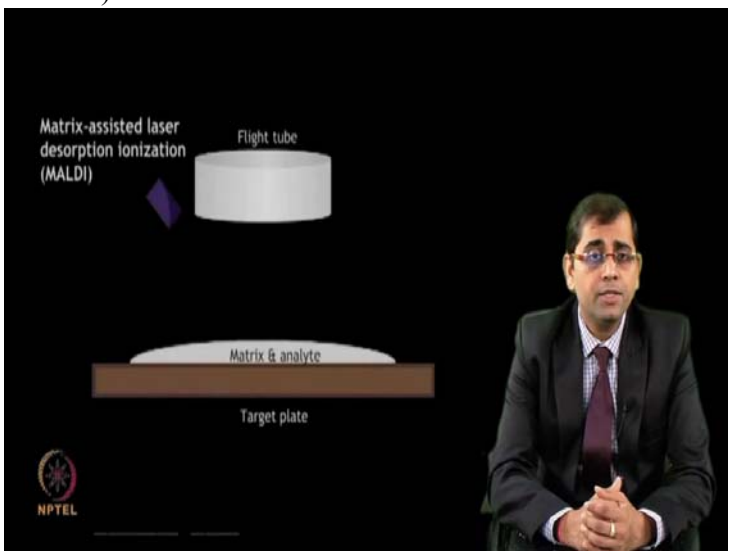

Lecture 8 will focus on….

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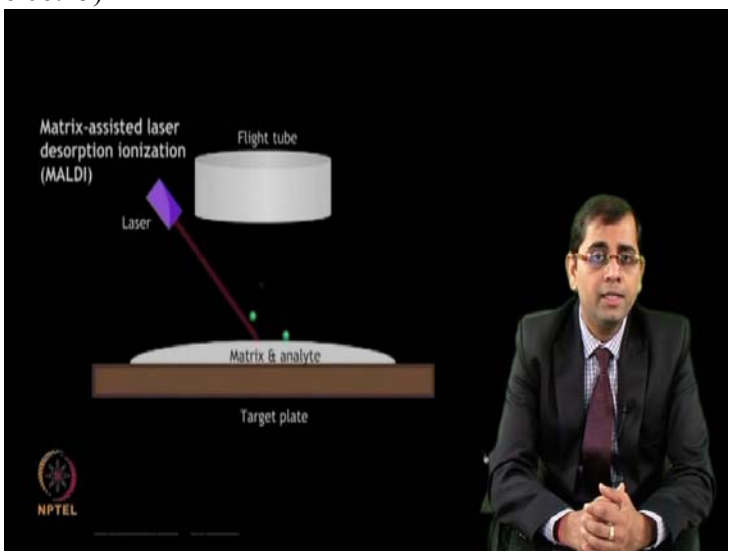

…ionization sources

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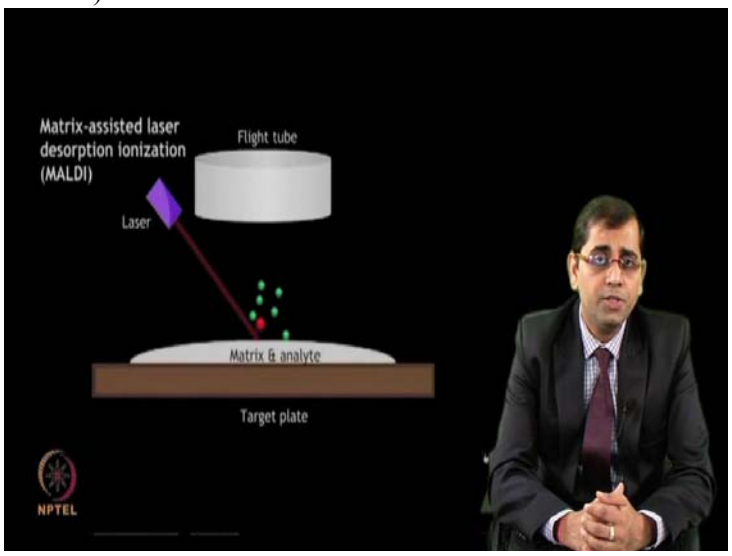

The ionization sources are responsible for

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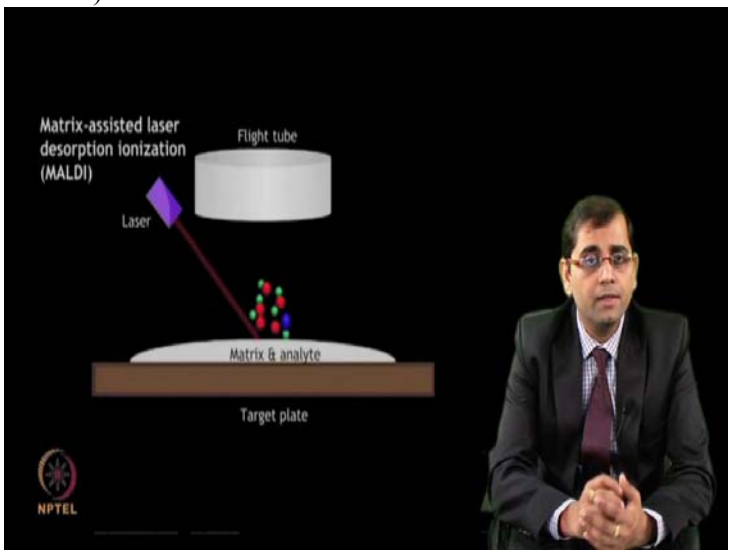

…converting the analyzed molecule into …

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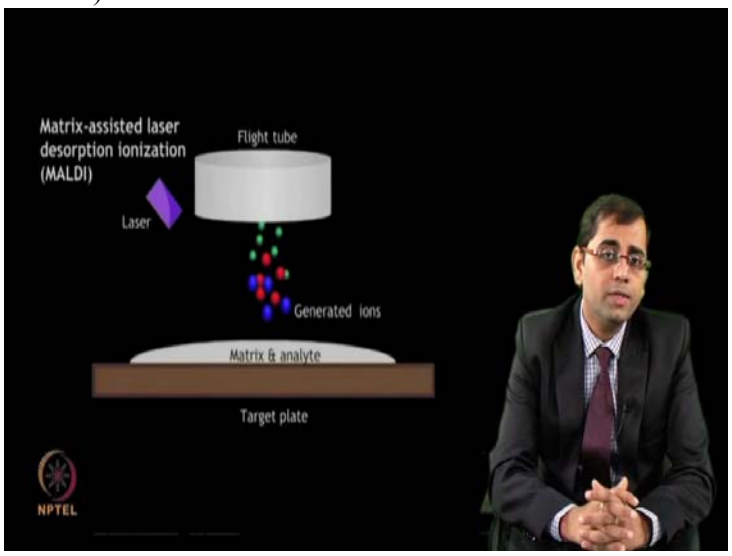

…gas cations into vacuum. The ions generated by the ionization source …

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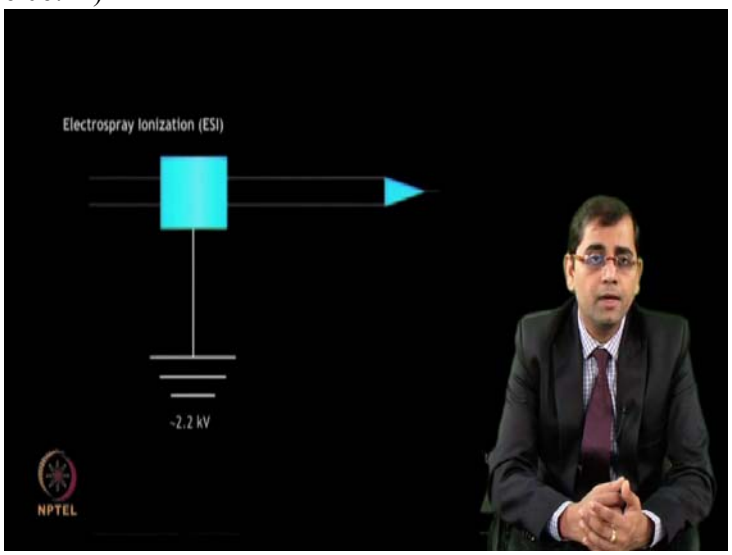

…are then integrated with the mass analyzer …

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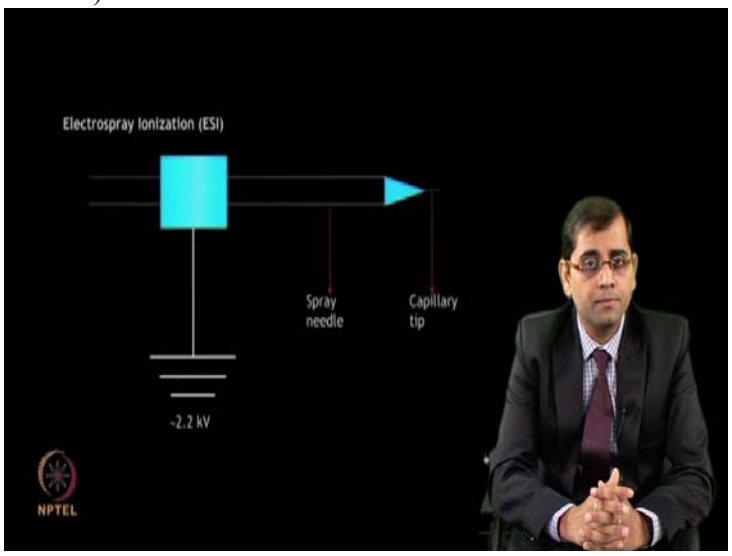

. The commonly used …

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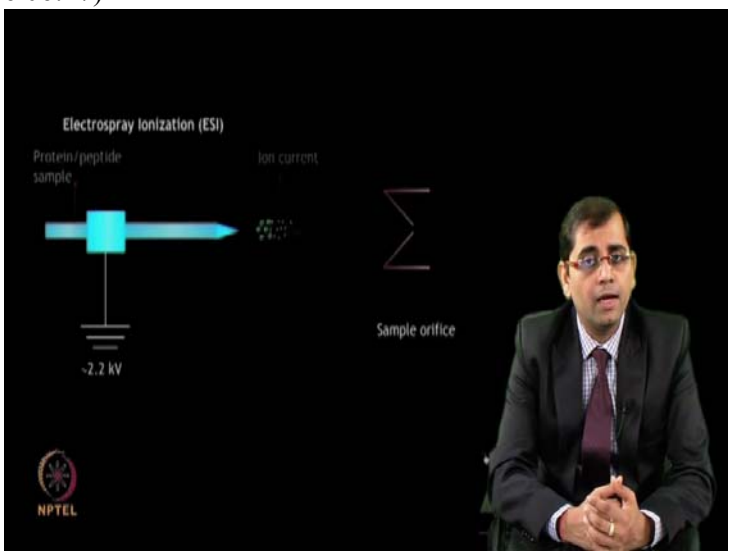

…. ionization sources are MALDI …

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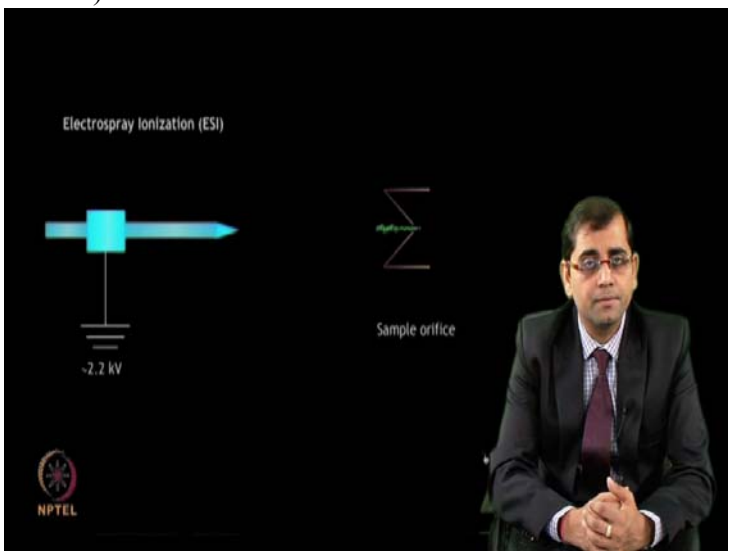

…and ESI

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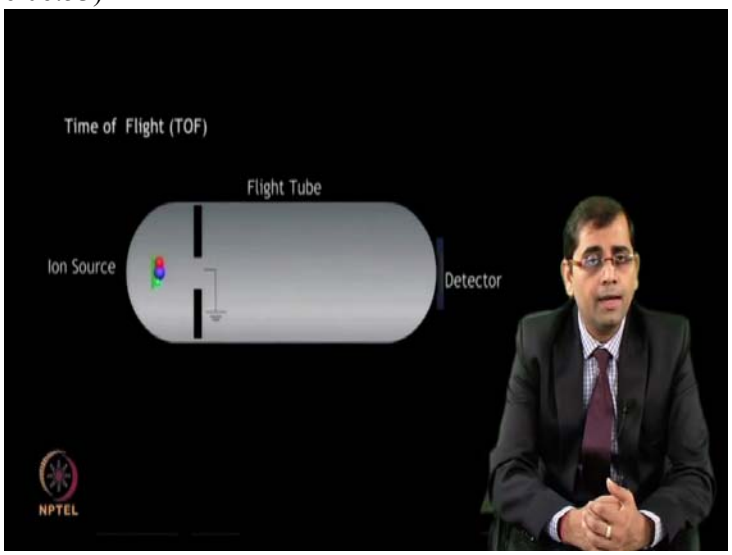

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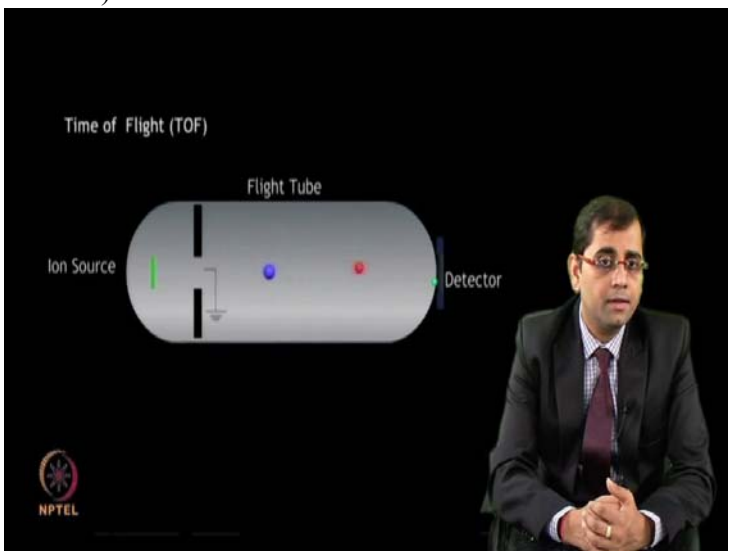

In lecture 9,

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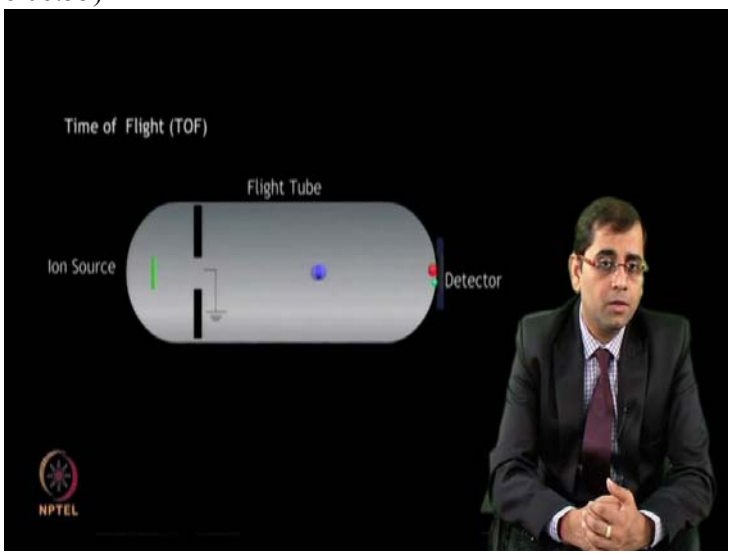

…we will talk about mass analyzers. The mass analyzers….

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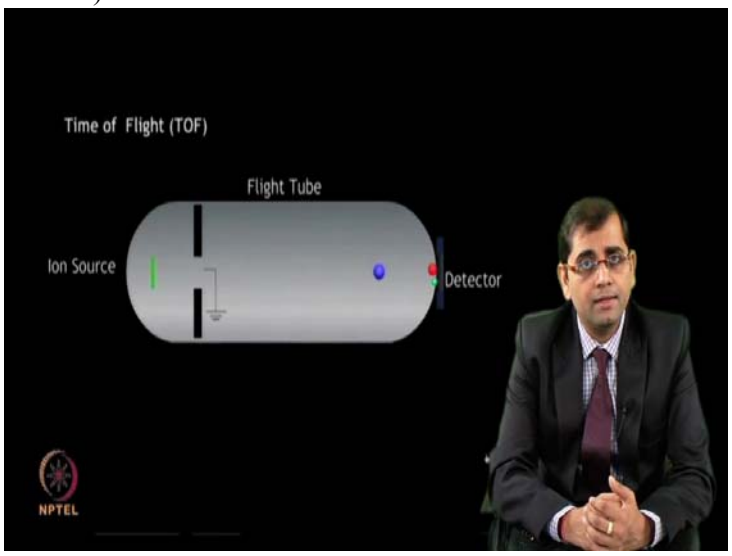
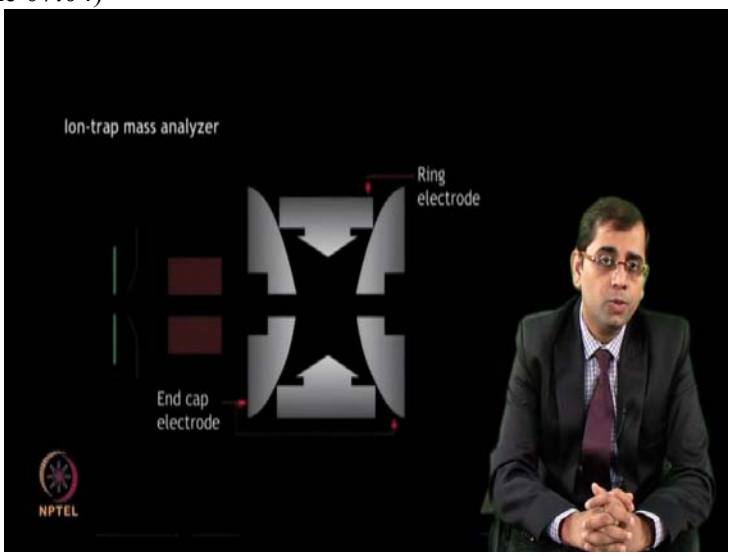


…resolve the ions produced by ionization source …

(Refer Slide Time 07:04)



…on the basis of …

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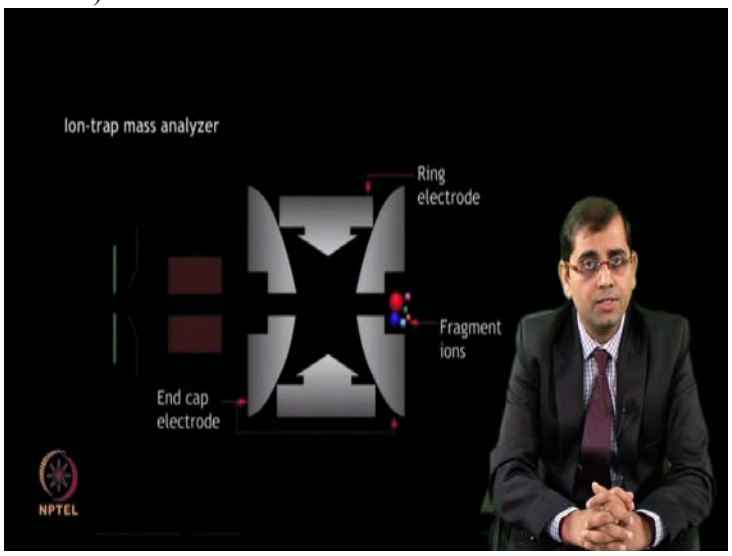

… their mass to charge ratio. Various characteristics such as resolving power….

(Refer Slide Time 07:15)

…accuracy, mass range and speed determine the…

(Refer Slide Time 07:20)

…efficiency of the analyzers

(Refer Slide Time 07:22)

The commonly used mass analyzers are …

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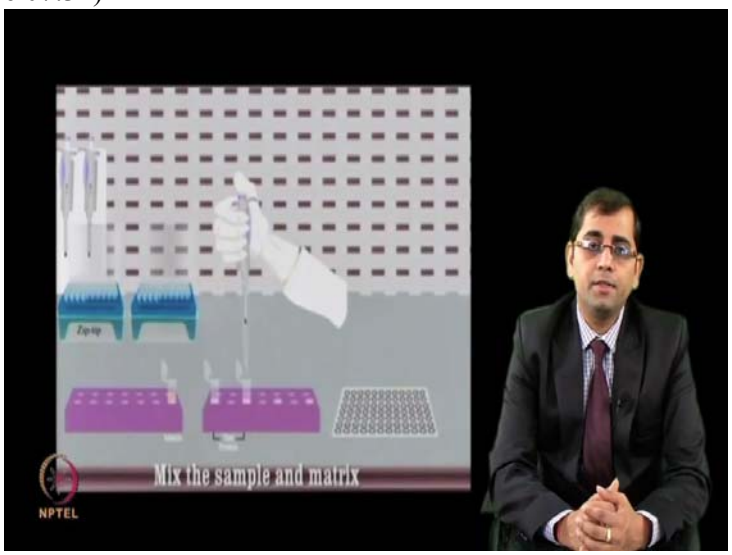
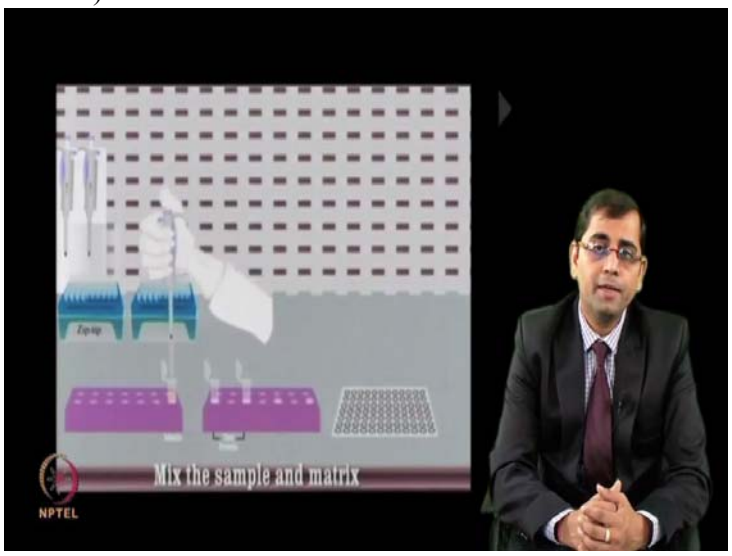
…Time of Flight TOF, Quadrupoles and Ion Traps

(Refer Slide Time 07:34)



The sample preparation strategy prior to mass spec analysis …

(Refer Slide Time 07:36)



…will be discussed…

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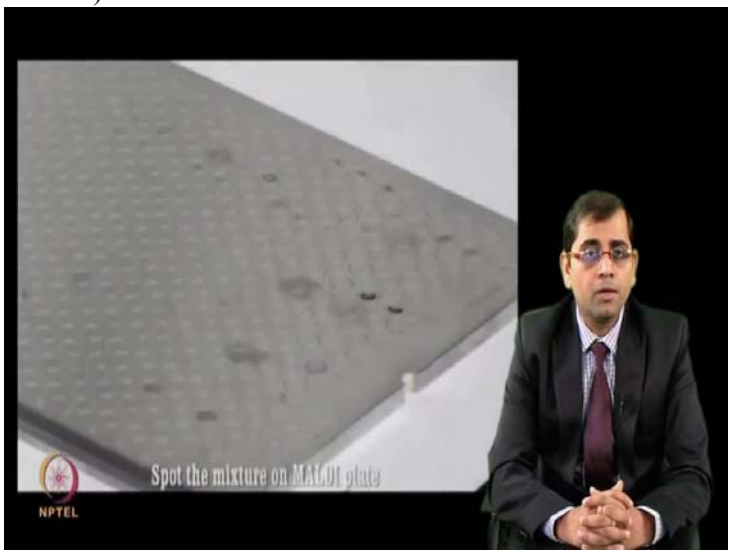
…in lecture 10

(Refer Slide Time 07:40)

Once…

(Refer Slide Time 07:41)



…the protein sample has been digested, all the salt, buffers and any detergent …

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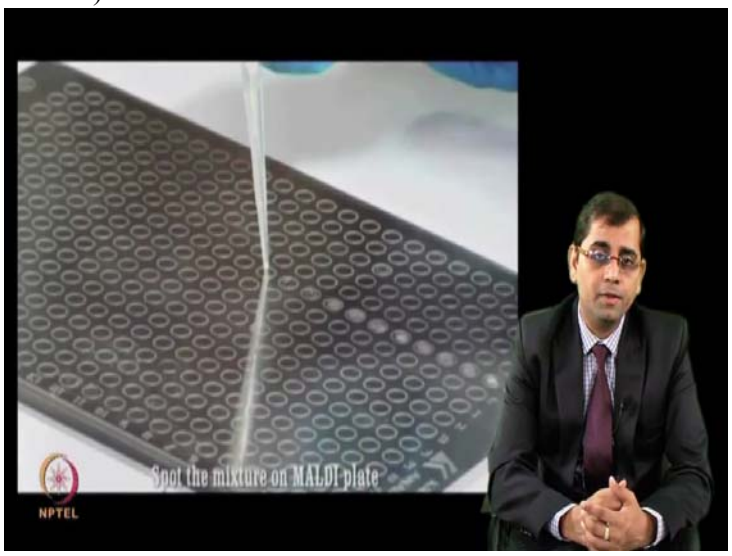

.. must be removed from the sample which can be effectively performed …

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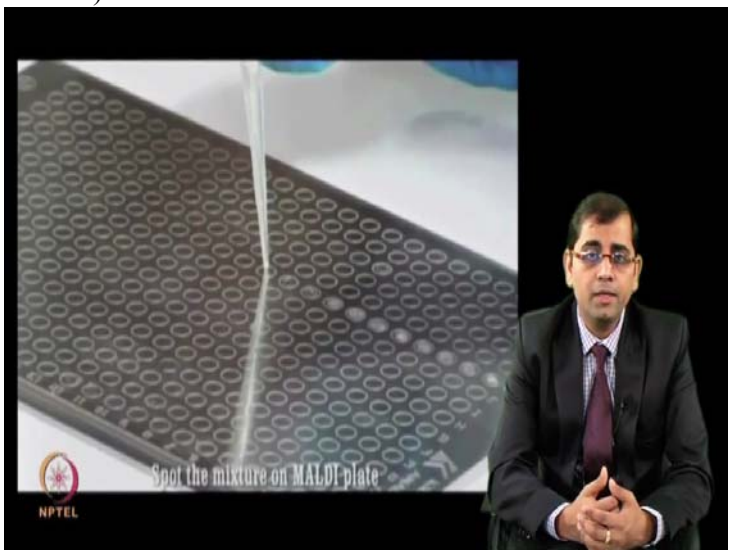


…by using some filters …

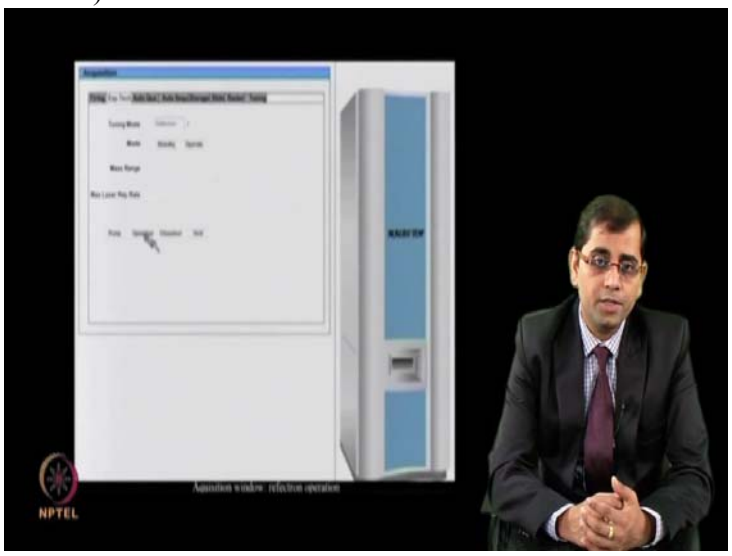
(Refer Slide Time 07:56)

… such as ZipTips. It offers several ….

(Refer Slide Time 07:59)

… advantages such as pick purification….

(Refer Slide Time 08:04)



… sample enrichment and ensures there is no contamination.

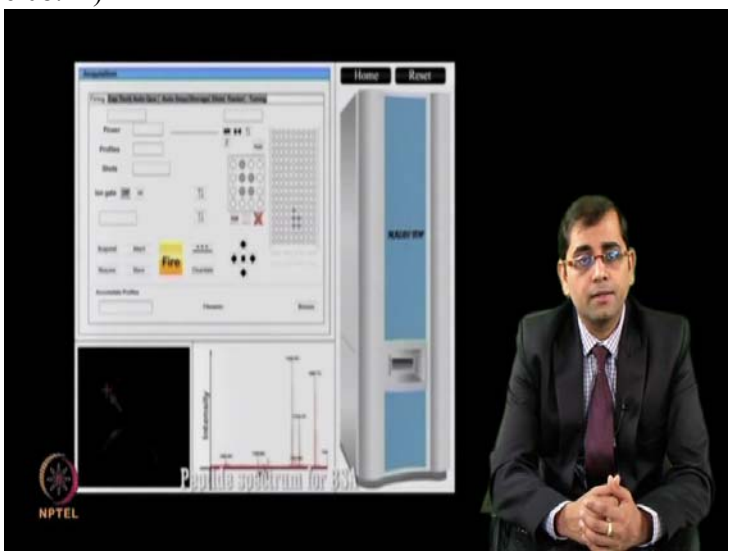
(Refer Slide Time 08:14)

However it can purify only a limited volume of the sample and …

(Refer Slide Time 08:17)

…also adsorbs some amount of protein sample …

(Refer Slide Time 08:22)



….thereby leading to losses

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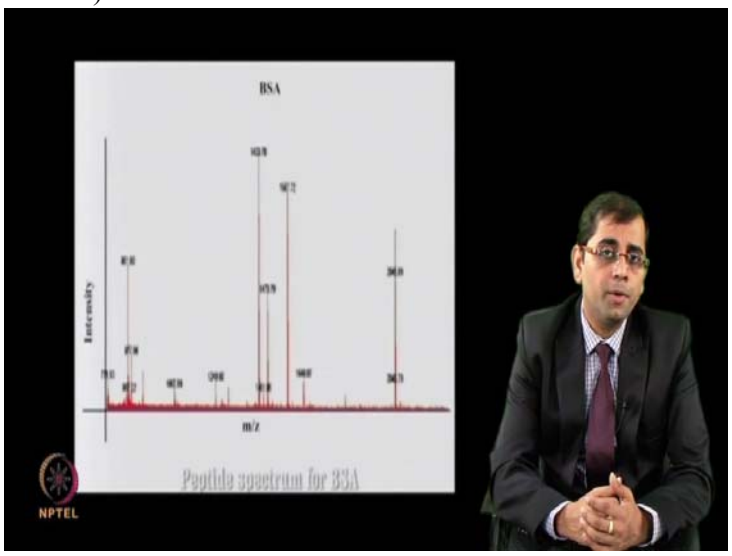

Further we will talk about how to use MALDI to analyze your samples in high throughput manner.

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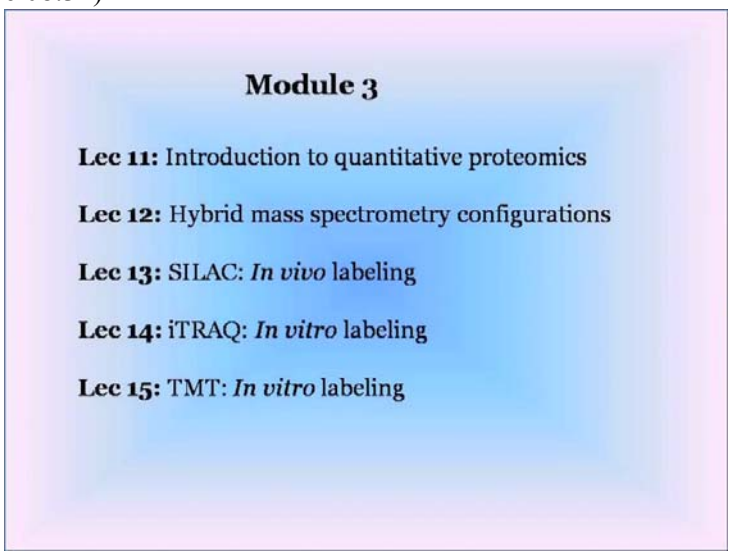


The third module will cover introduction to quantitative proteomics and hybrid mass spec configurations

(Refer Slide Time 08:45)

. The complexity and dynamic nature of proteomes presents major technological challenges. Mass spectrometry advancements have improved in high throughput identification and quantification of proteins and now offer an opportunity to understand human diseases and discovery by mass specs.

(Refer Slide Time 09:08)

The lecture 11 and 12 will cover hybrid and MS/MS configurations…

(Refer Slide Time 09:12)

…as well as …

(Refer Slide Time 09:14)

…discussion on two latest hybrid MS technologies …

(Refer Slide Time 09:20)

... Q-TOF and Orbitrap

(Refer Slide Time 09:22)

(Refer Slide Time 09:25)

The basics of quantitative proteomic analysis …

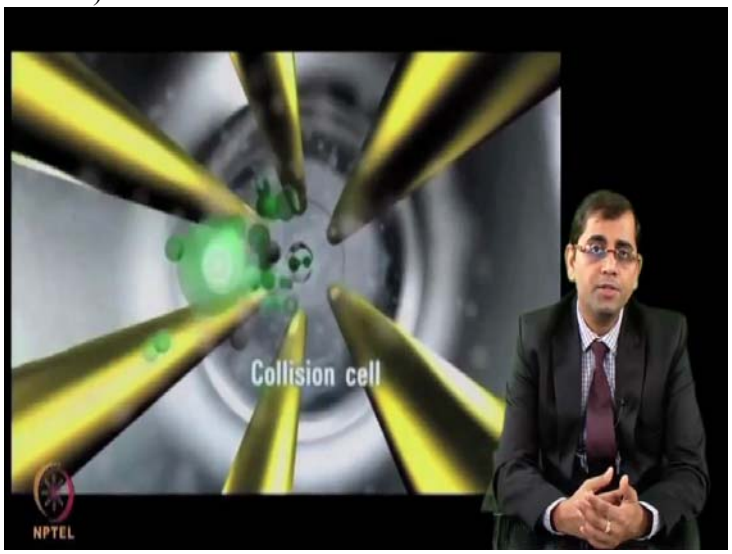
(Refer Slide Time 09:27)

and what are the different types of ….

(Refer Slide Time 09:29)

…quantitative methods exist….

(Refer Slide Time 09:30)



… in literature using mass spectrometry will be discussed.

In lecture 13, we will talk about quantitative proteomic analysis using Stable Isotope Labeling by Amino acids in Cell culture or SILAC.

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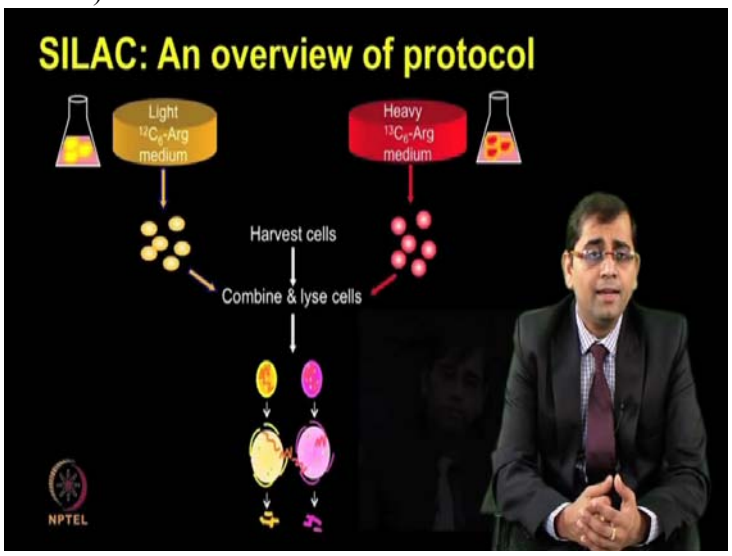

SILAC is an in vivo labeling method; the labels can be introduced in vivo by growing an organism in the media enriched with specific isotopes.

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There are different ways of in vivo labeling such as enrichment of 15 Nitrogen media, Culture Derived Isotope Tags known as CDIT or SILAC.

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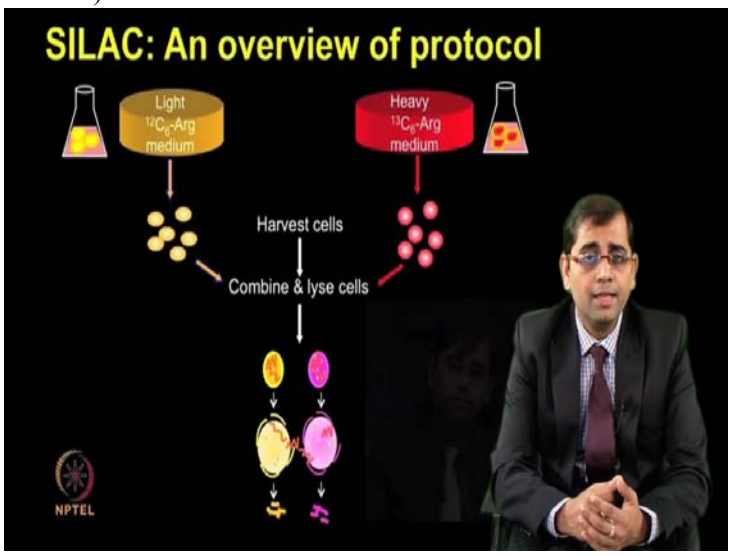

In this lecture, the major emphasis will be on the SILAC method for quantitative proteomic analysis.

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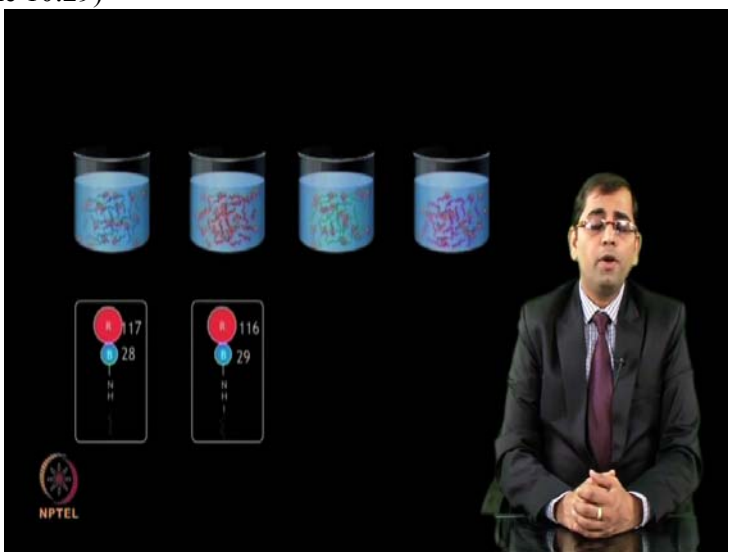

The next lecture, we will talk about quantitative proteomic using iTRAQ technique. iTRAQ involves identification and quantification of ….

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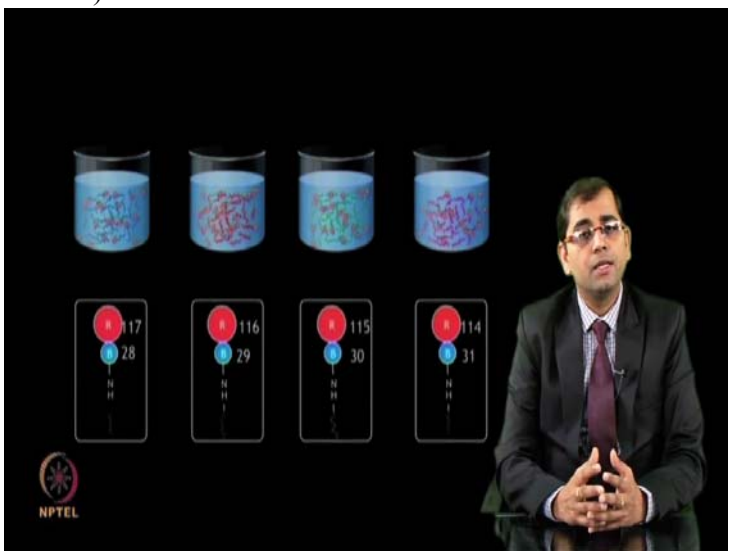

… complex protein mixtures by MS based quantitative proteomic techniques

The iTRAQ reagent consists of amine specific stable isotope reagent which can label peptides of up to 4 or different biological samples. The iTRAQ method provides multiplexing capabilities of 4 or 8 sample analysis which is not possible …

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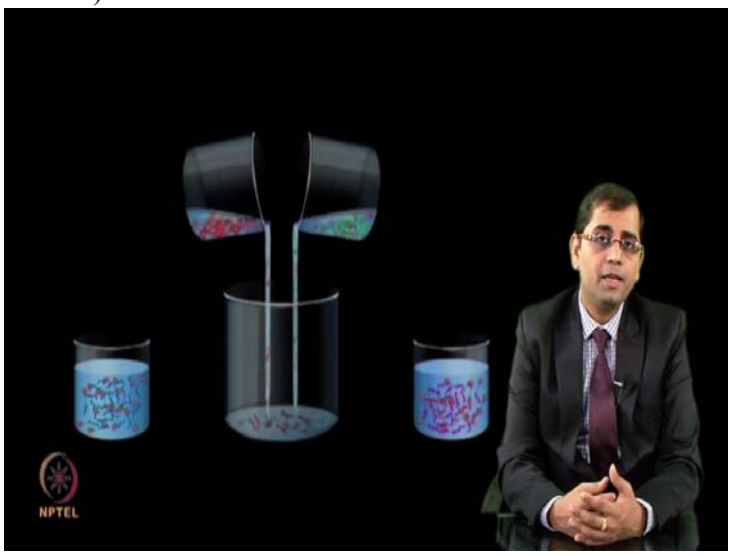
…. using iCAT where only 2 samples

(Refer Slide Time 11:14)



can be labeled and analyzed.

(Refer Slide Time 11:19)

In ITRAQ, 4 plates reagent sets…

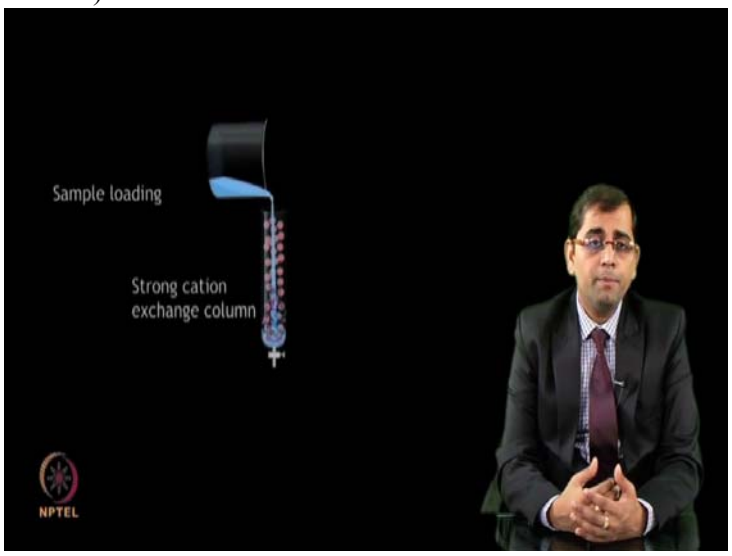
(Refer Slide Time 11:24)

…the anodizing value ranging ….

(Refer Slide Time 11:26)

…from 114 to 117,…

(Refer Slide Time 11:29)



…there is a balance group…

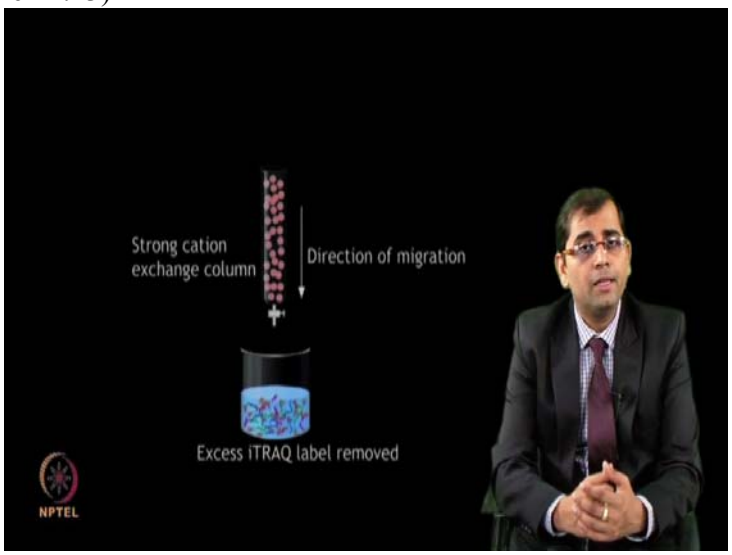
(Refer Slide Time 11:32)

…of mass 28 to 31 Dalton, therefore the overall mass of the reporter and balancer component …

(Refer Slide Time 11:39)

… remains constant.

(Refer Slide Time 11:43)



During the MS/MS fragmentation, the reporter ion gives the peak at 114, 15, 16, and 17…

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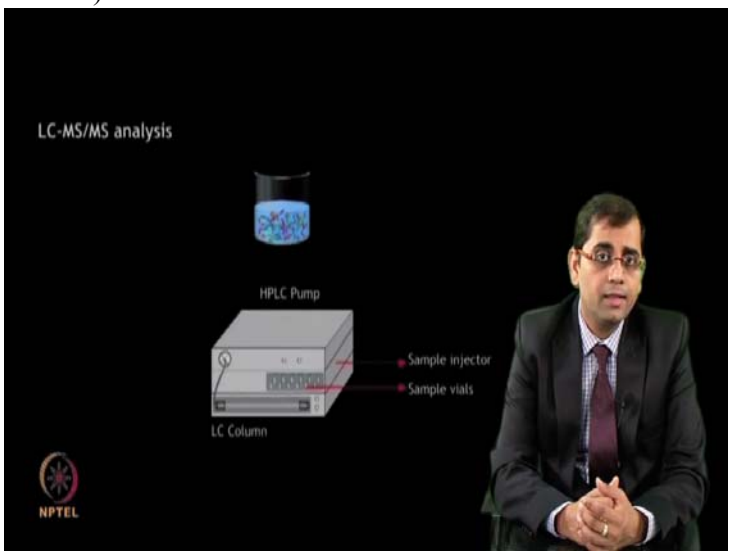

….which provides the information about …

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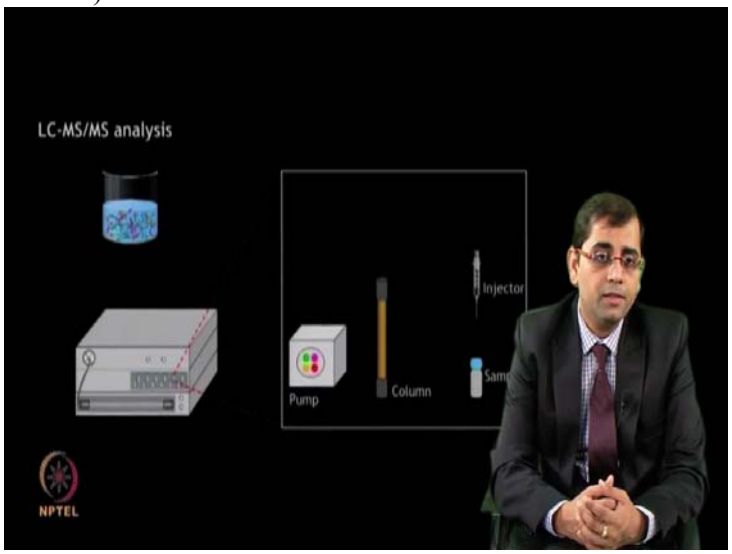

… the peptide or protein abundance.

The next lecture will focus on another quantitative proteomic technique based on Tandem Mass Tags known as TMT.

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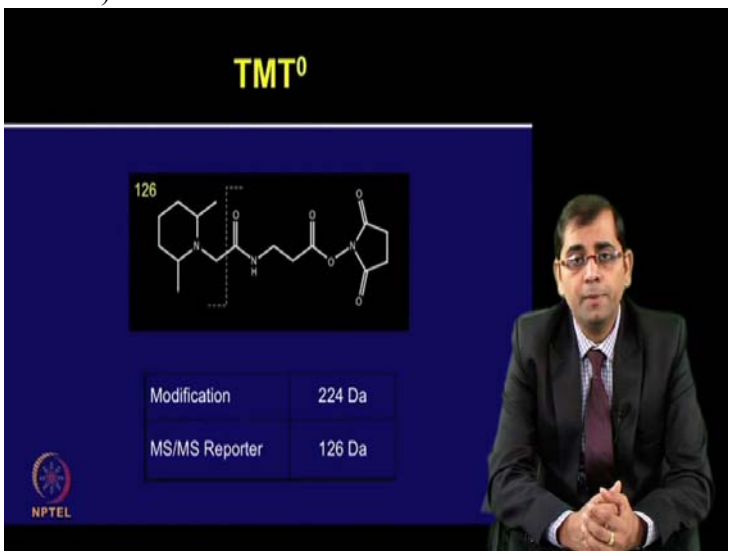

TMT is also in vitro labeling method which is similar to iTRAQ method.

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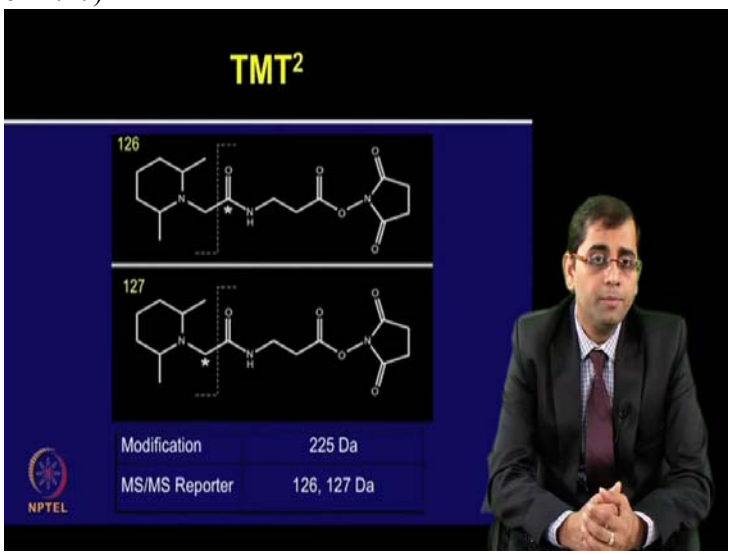

TMT is MS/MS based quantitative technique which uses the isotope labeled model referred as Tandem Mass Tags.

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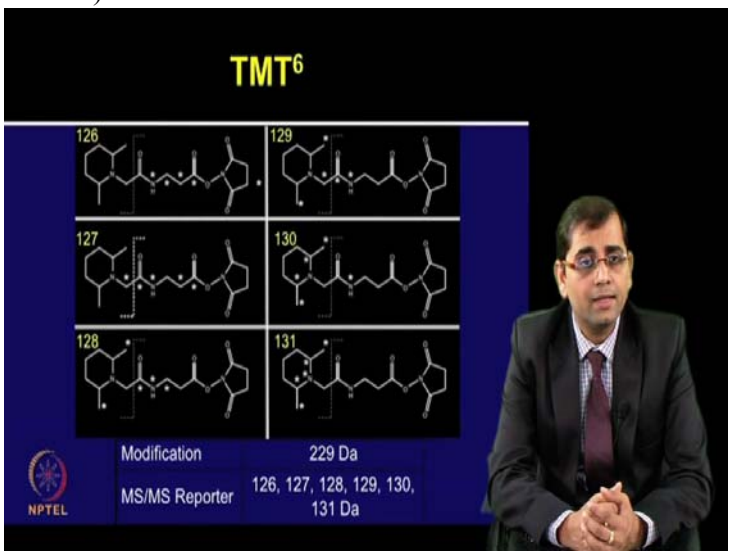

It provides the accurate quantification of peptides and proteins. By using different types of TMT tags, one could perform multiplexing experiments of 2, 4, 6 or 10 plex.

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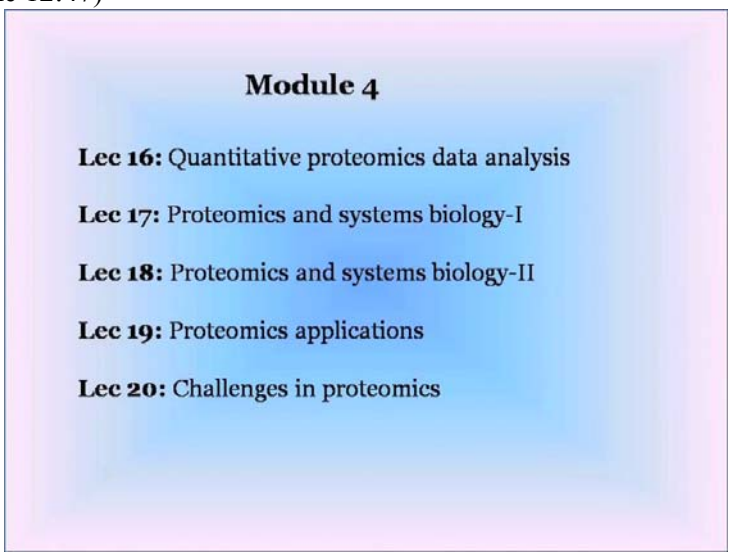

In the last module we will talk about quantitative protein data analysis and some aspects of System Biology applications.

The quantitative proteomic technology aims to identify the differentially expressed protein in a biological sample. The differential expression of proteins can be caused by a disease state or various external factors like stress, drugs or different experimental conditions.

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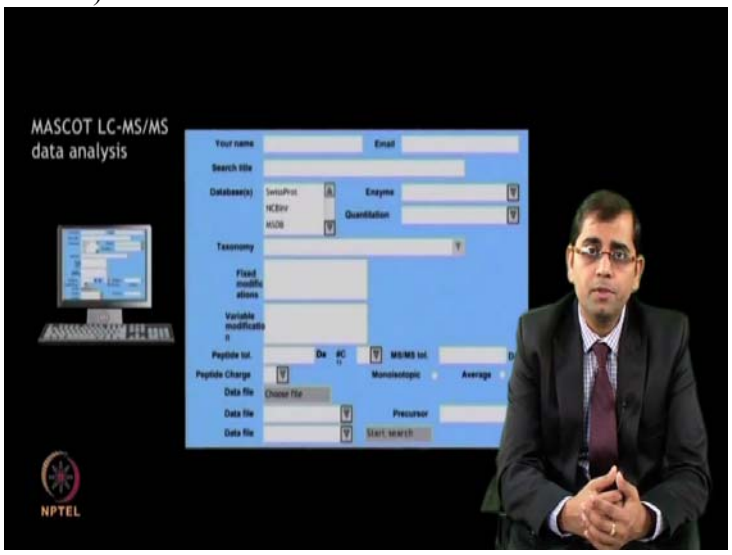
The data analysis is …

(Refer Slide Time 13:25)



an important step for protein identification and quantification in proteomics workflow. The accurate quantification of protein abundance becomes very important for the quantitative protein analysis. This lecture will focus on data analysis for the protein identification using

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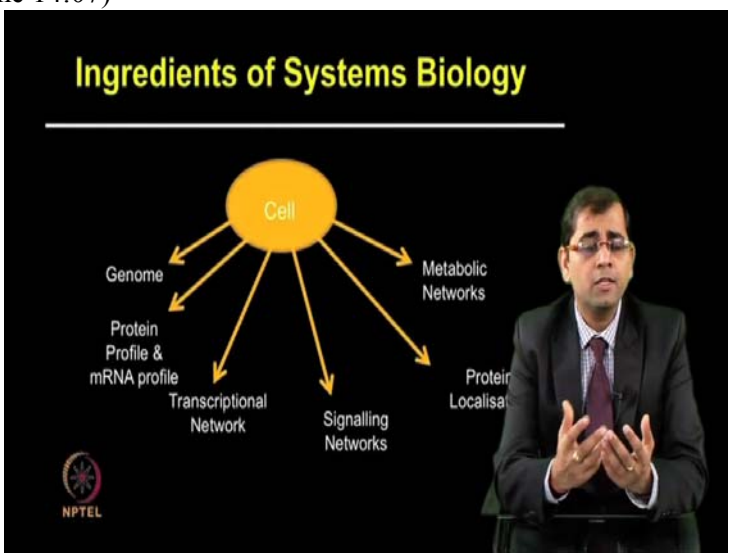
…. Mascot and protein quantification using iTRAQ based workflow.

The next 2 lectures 17 and 18 will cover proteomics and System Biology.

(Refer Slide Time 14:07)



So what is System Biology? The System Biology is the examination of the biological entity as an integrated system rather than studying its individual characteristics, reactions and components. And that is what is termed as System Biology.

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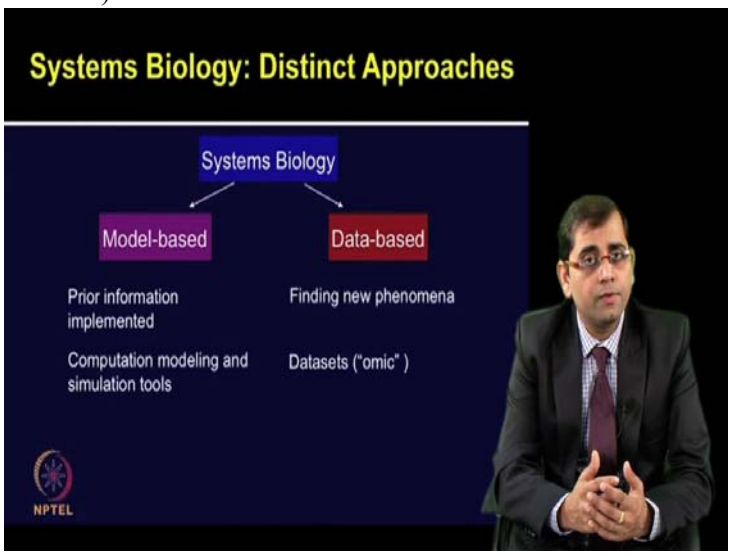

The distinct approaches of System Biology include the model based and data-based methods. The model-based approach involves some prior information which can be implemented in these models where as the data-based methodology; the objective is to find the new phenomena.

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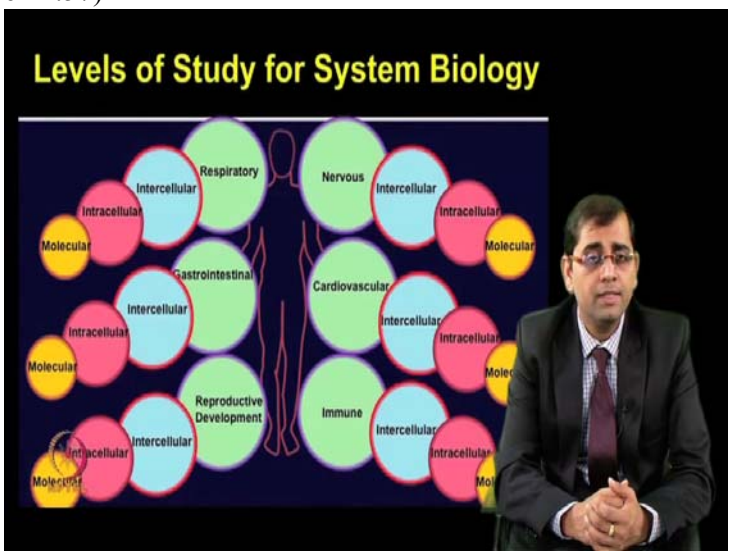

Some of these details will be covered in System Biology.

Proteomics has wide range of applications in understanding the physiology of micro-organism to biomarker discovery for cancer and other diseases.

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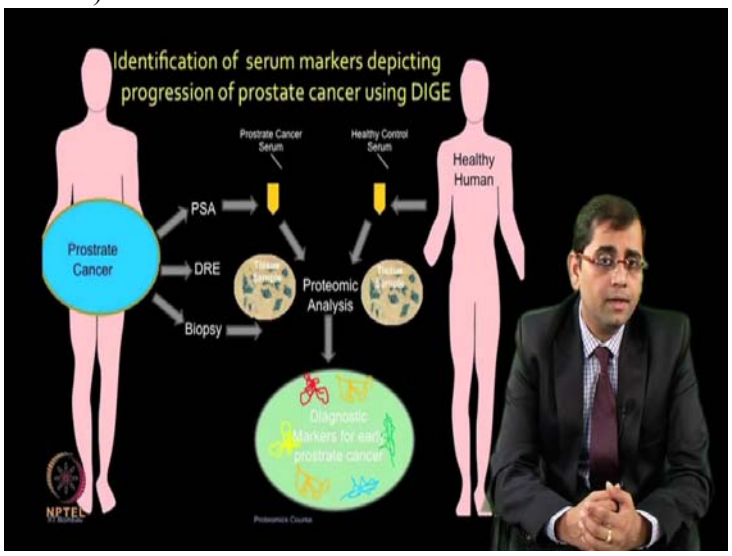

Towards the end I would like to cover proteomics applications and challenges.

The last lecture will focus on proteomics challenges. The mass spectrometry is one of the best inventions in proteomics field in recent years. It is able to achieve many milestones including the draft human proteome maps.

The Mass Spec is sophisticated instrument and provides very high throughput robust capability of analyzing proteome; still it has many challenges to overcome in the future.

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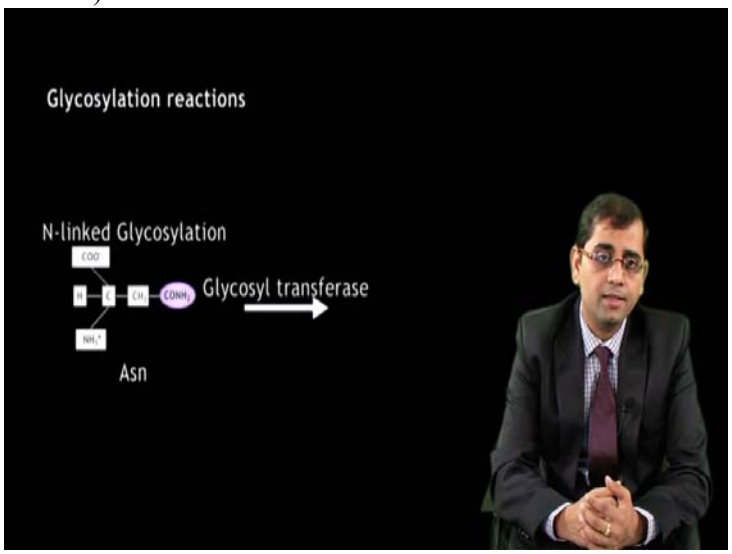

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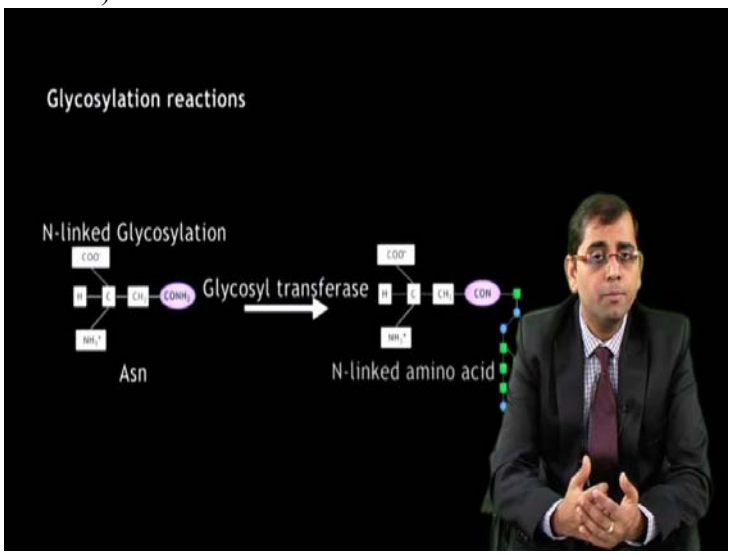

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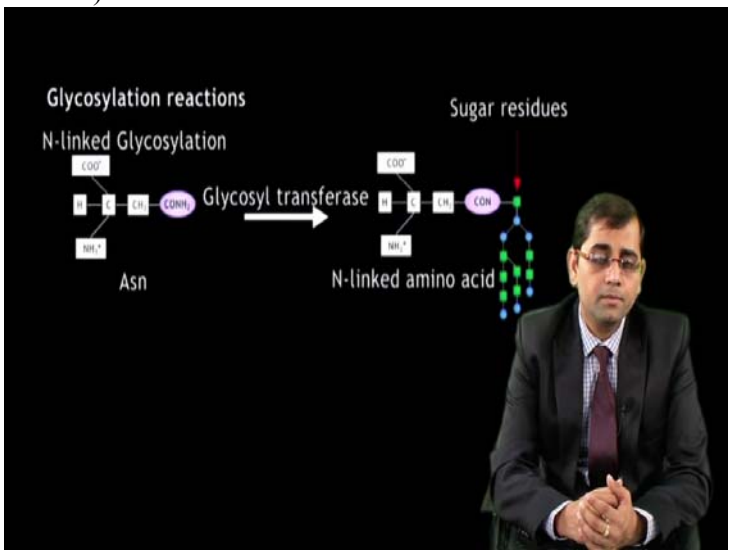

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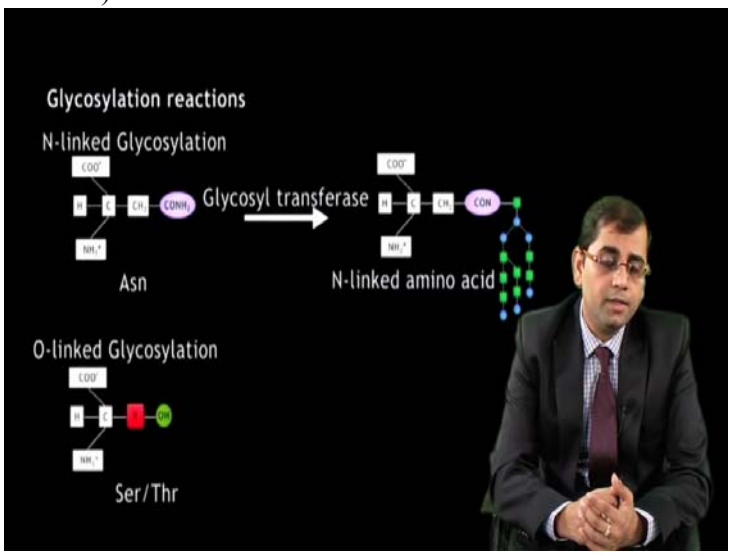

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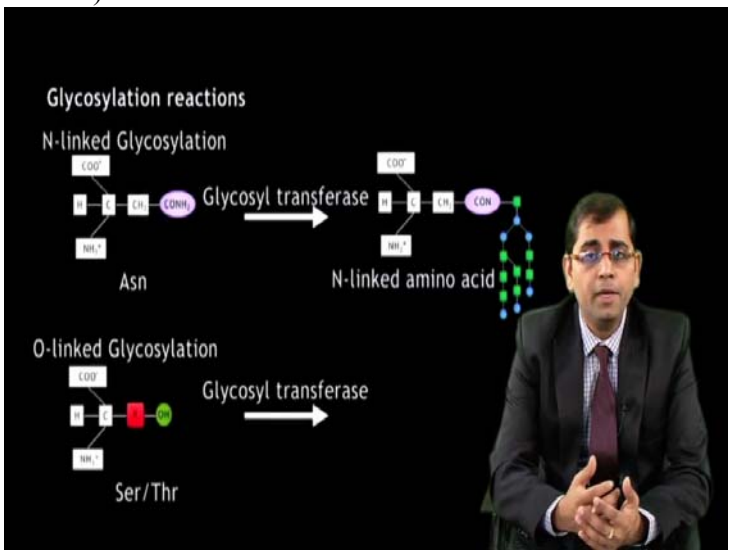

The post translational modification analysis using Mass Spec is one of the challenges.

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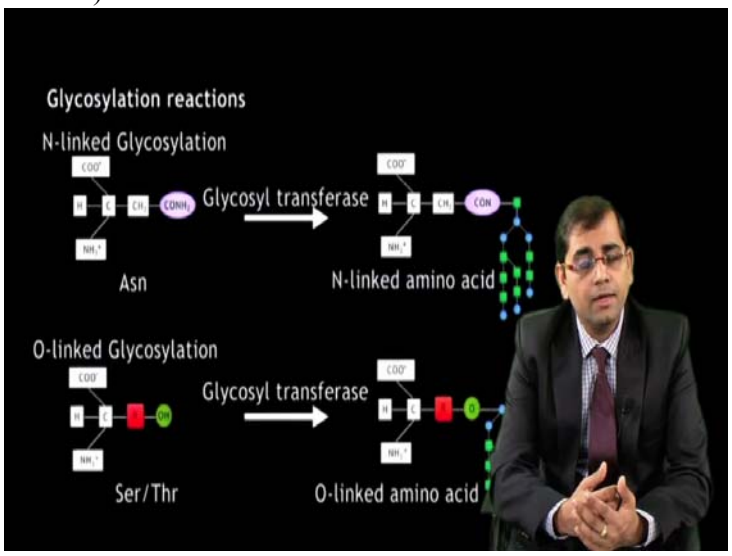

Additionally the dynamic range of proteins, inadequate coverage of whole proteome and accuracy of quantification are challenging in this field.

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Overall this course will provide the basic knowledge of mass spectrometry with focus on quantitative proteomics. Hope these concepts and understanding will be useful for your research. Thank you