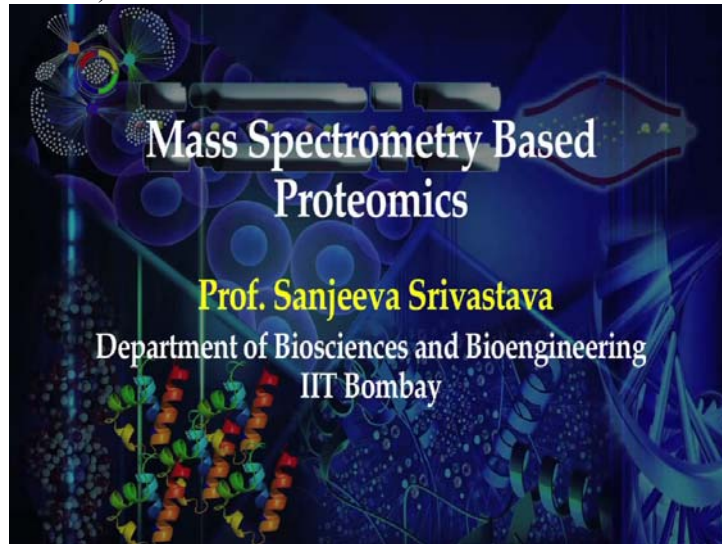


Mass Spectrometry Based Proteomics
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Mod 01 Introductory Lecture

(Refer Slide Time 00:11)



(Refer Slide Time 00:15)



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

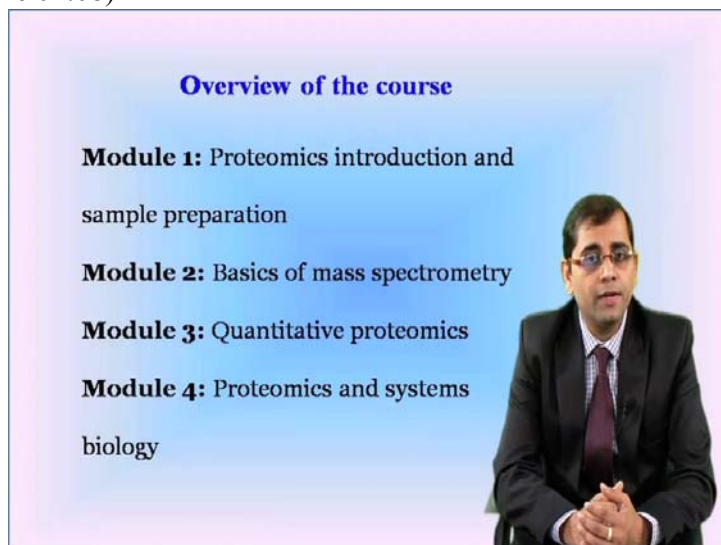
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The proteome describes the protein complement expressed by a genome. The extent of diversity and complexity due to alternatively splicing and post translational modification is tremendous. Therefore, the studying proteins and proteome becomes very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

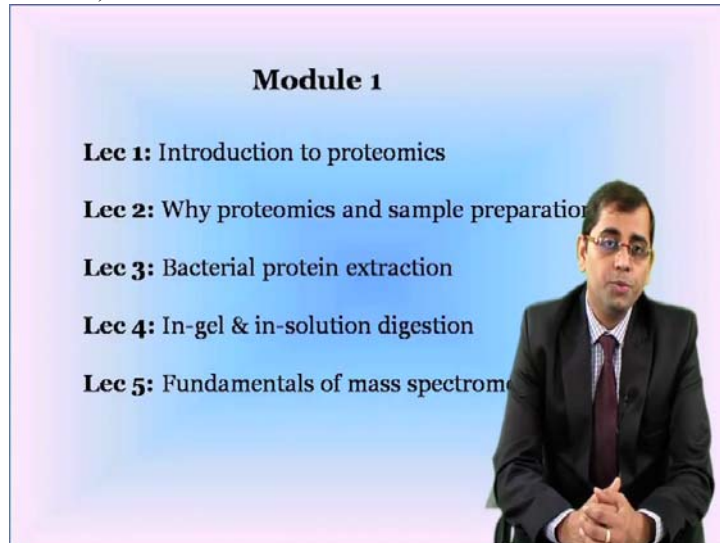
In recent years, mass spectrometry has played a major role in Proteomics level investigation. And in 2014, 2 human proteome reference maps were published using high resolution mass spectrometry.

(Refer Slide Time 01:08)



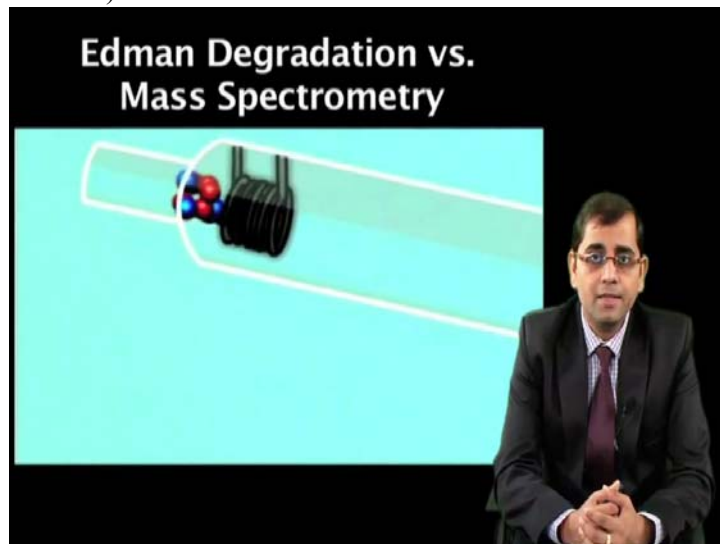
This course is divided into 4 modules. Each module, we will be finishing in 1 week. There will be 20 lectures of around 30 minutes duration which will cover the key concepts, experiments and laboratory demonstration to explain the concept effectively to the students.

(Refer Slide Time 01:35)



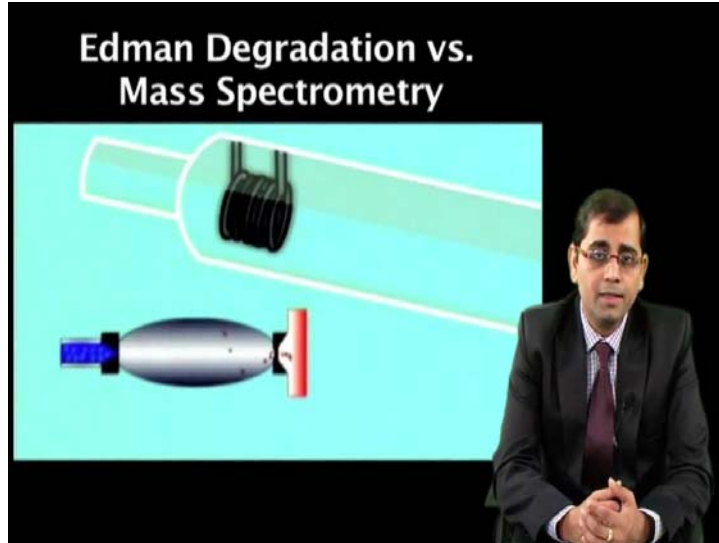
In first module, we will discuss about basic concepts of proteomics and the importance of sample preparation. Further we will discuss the fundamentals of mass spectrometry and the advancements in technology.

(Refer Slide Time 01:55)



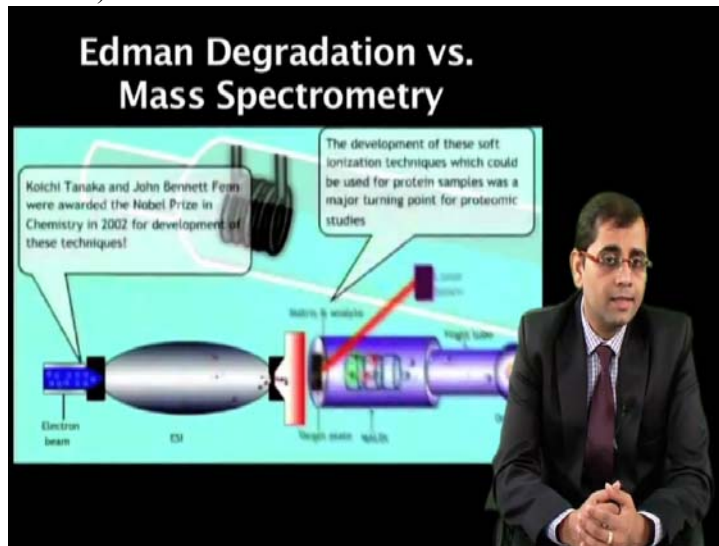
Lecture 1 will focus on basic understanding of proteomics, the technological advancements....

(Refer Slide Time 02:01)



...in analytical techniques with increased sensitivity...

(Refer Slide Time 02:06)



... resolution ...

(Refer Slide Time 02:07)

Edman Degradation vs. Mass Spectrometry

The diagram illustrates the workflow of a mass spectrometer. It starts with an 'Electron beam' that ionizes a sample. The ions pass through an 'ESI' (Electrospray Ionization) stage. They then hit a 'Target plate' where 'Matrix & analyte' are present. The ions are then accelerated through a 'Flight tube' and finally detected by a 'Mass sensor'. A callout box notes: 'The development of these soft ionization techniques which could be used for protein samples was a major turning point for proteomic studies'. Another callout box states: 'Koichi Tanaka and John Bennett Fenn were awarded the Nobel Prize in Chemistry in 2002 for development of these techniques!'.

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

(Refer Slide Time 02:13)

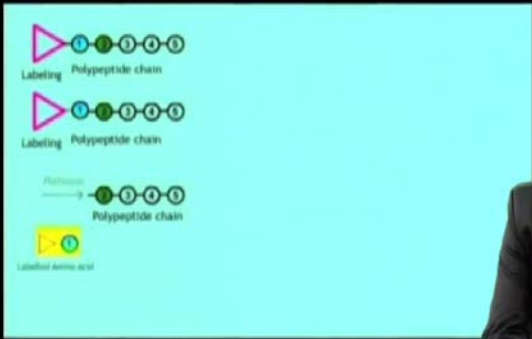
Edman Degradation vs. Mass Spectrometry

The slide displays a protein sequence represented by a horizontal chain of five colored circles: a blue circle followed by four green circles.

.... have led to the transition from...

(Refer Slide Time 02:17)

Edman Degradation vs. Mass Spectrometry



The diagram illustrates the first step of Edman degradation. It shows a polypeptide chain with four amino acids: a pink one (labeled 'Labeling'), followed by three green ones. A pink triangle (phenyl isothiocyanate) is attached to the N-terminus. An arrow labeled 'Phenyl isothiocyanate' points to the N-terminus. Below, a yellow triangle (labeled 'Labelled amino acid') is shown with a pink circle (the N-terminal amino acid) inside it, indicating its release from the chain.

Labeling Polypeptide chain

Labeling Polypeptide chain

Phenyl isothiocyanate

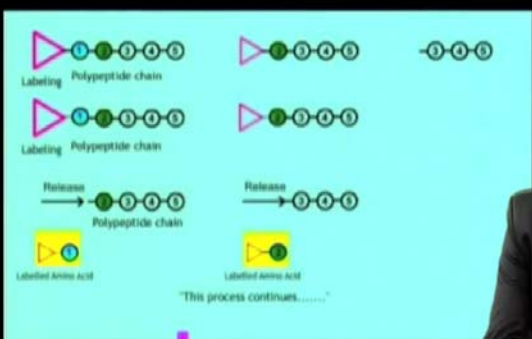
Polypeptide chain

Labelled amino acid

... protein chemistry...

(Refer Slide Time 02:25)

Edman Degradation vs. Mass Spectrometry



The diagram shows the sequential steps of Edman degradation. It starts with a polypeptide chain of four amino acids (pink, green, green, green). The first step shows the N-terminal amino acid (pink) being released as a 'Labelled Amino acid' (yellow triangle with pink circle). The second step shows the second amino acid (green) being released as a 'Labelled Amino acid' (yellow triangle with green circle). The process continues, with the third and fourth amino acids being released in subsequent steps. The text 'This process continues.....' is written at the bottom.

Labeling Polypeptide chain

Labeling Polypeptide chain

Release Polypeptide chain

Labelled Amino acid

Labelled Amino acid

"This process continues....."

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

...

(Refer Slide Time 02:28)

Edman Degradation vs. Mass Spectrometry

The diagram illustrates the components of a mass spectrometer. It starts with a 'Sample inlet' (a red circle) leading into an 'Ionization source' (a cylinder with internal structures). From there, 'Charged peptide fragments' (represented by green dots) move through a 'Mass analyzer' (a series of parallel plates) and are detected by a 'Detector' (a yellow circle). The final step is 'MS data analysis', shown as a laptop displaying a mass spectrum graph.

.... was overcome by development of soft ionization techniques...

(Refer Slide Time 02:31)

Edman Degradation vs. Mass Spectrometry

This diagram is identical to the one in the previous slide, showing the workflow of a mass spectrometer: Sample inlet, Ionization source, Mass analyzer, Detector, and MS data analysis.

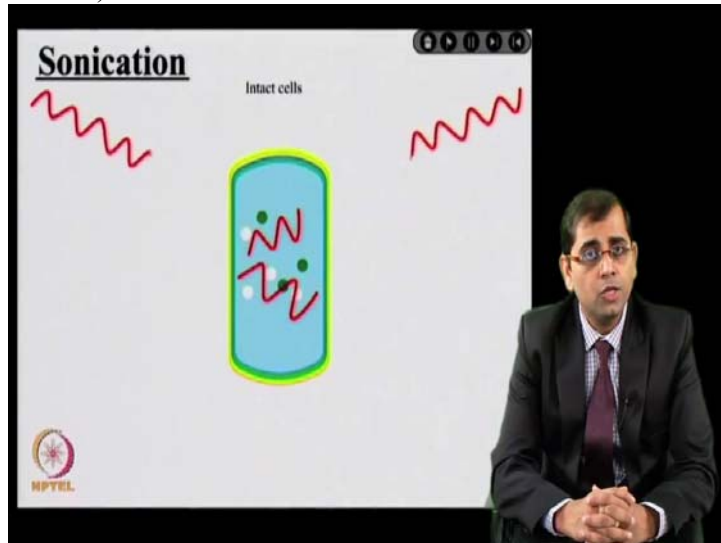
...such as MALDI and ESI will be discussed in this lecture

(Refer Slide Time 02:38)



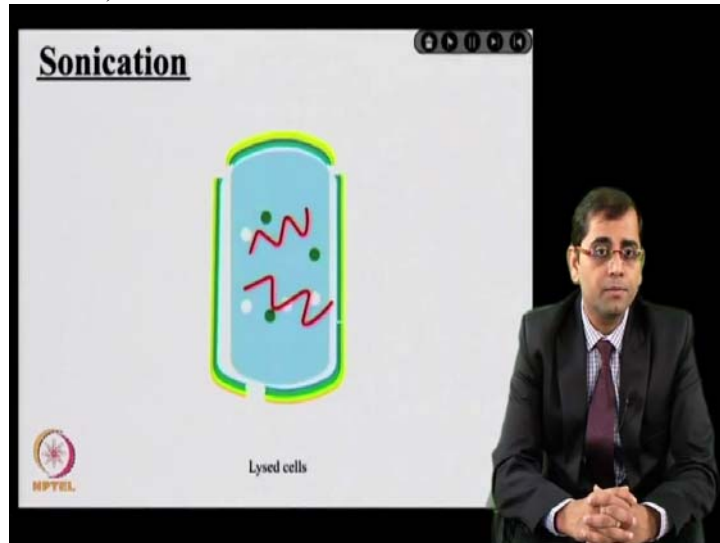
In lecture 2, we will talk about sample preparations for proteomics application. Protein extraction is crucial step for any proteomic investigation. The protein extraction method involves cell lysis, prevention of proteolysis during lysis, different types of protein precipitation methods...

(Refer Slide Time 03:06)



...protein solubilization...

(Refer Slide Time 03:08)



...and removal of ...

(Refer Slide Time 03:12)



...various interfering components

The protocol standardization becomes a major challenge in proteomics ...

(Refer Slide Time 03:19)



...and there is no generic protocol...

(Refer Slide Time 03:23)



...which exists in literature ...

(Refer Slide Time 03:24)



....which can be used ...

(Refer Slide Time 03:26)



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

(Refer Slide Time 03:36)



...and how the good sample...

(Refer Slide Time 03:38)



...can be prepared ...

(Refer Slide Time 03:40)



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

(Refer Slide Time 03:44)



... using bacteria and discuss about cell lysis...

(Refer Slide Time 03:49)



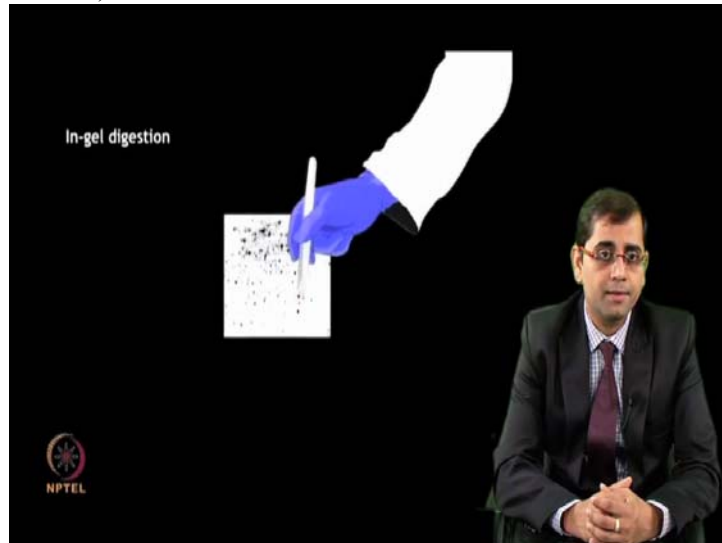
.... protein precipitation and quantification and discuss about trizol extraction protocol.

(Refer Slide Time 03:58)

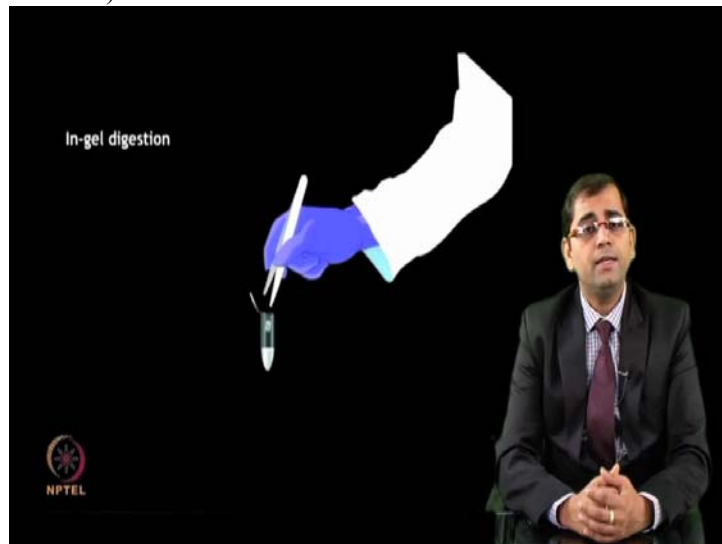


In lecture 4, we will talk about in-gel digestion or in-solution digestion of the protein which is essential prior to the mass spectrometry analysis.

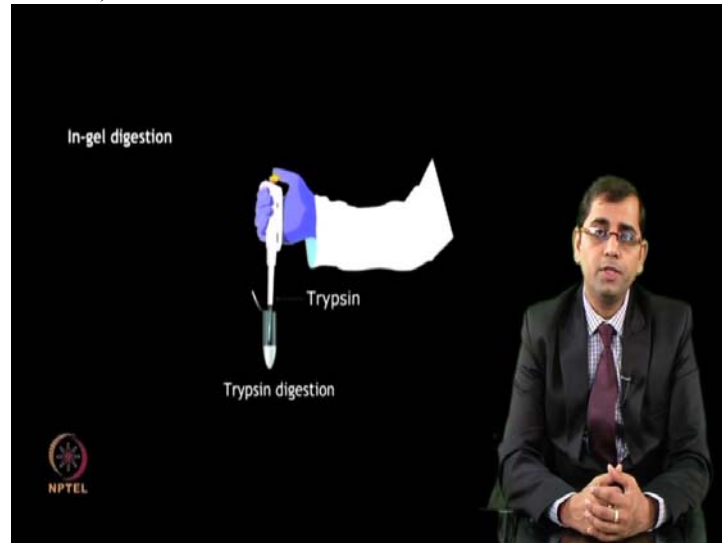
(Refer Slide Time 04:09)



(Refer Slide Time 04:11)

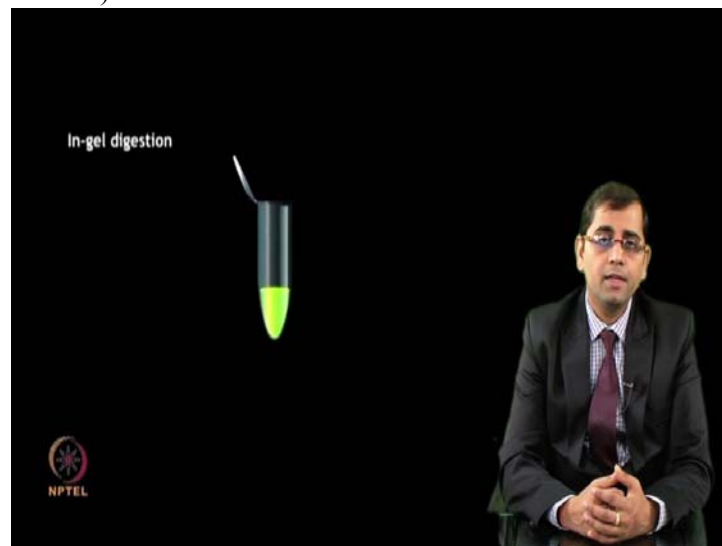


(Refer Slide Time 04:13)



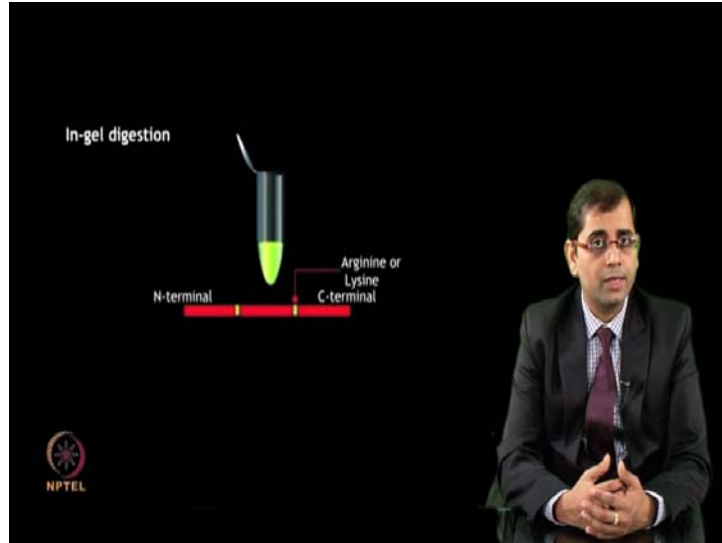
Often the in-gel digestion is used to extract proteins...

(Refer Slide Time 04:15)



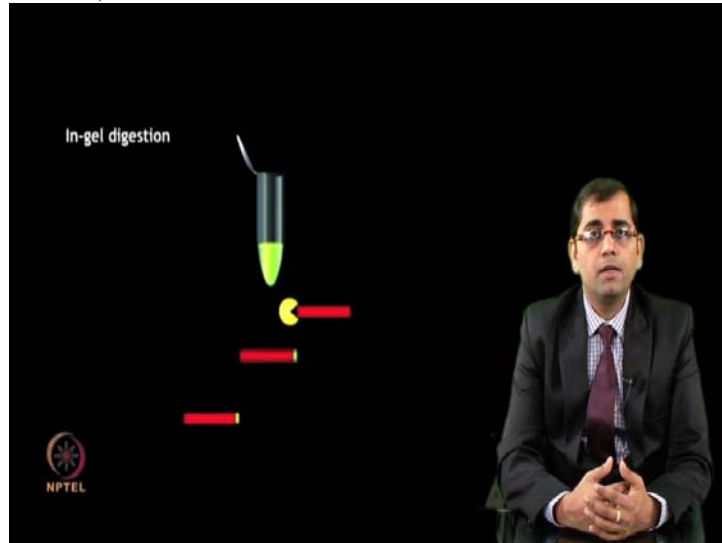
or peptides...

(Refer Slide Time 04:17)



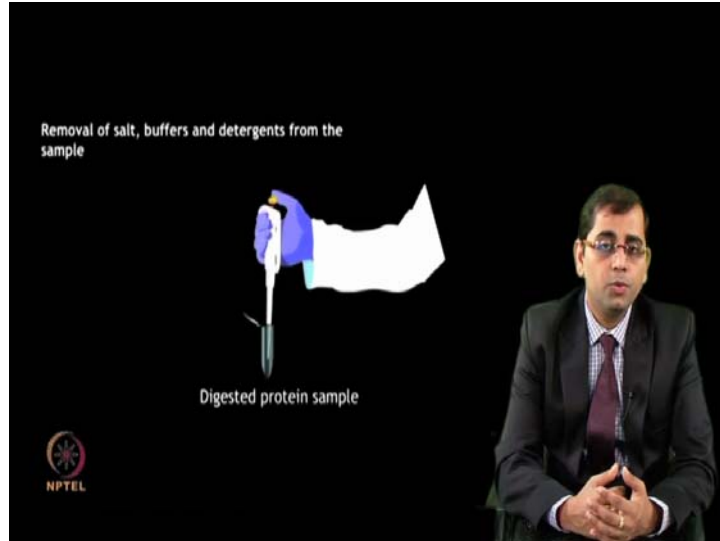
... separated on the gel electrophoresis. In gel proteolytic digestion

(Refer Slide Time 04:22)



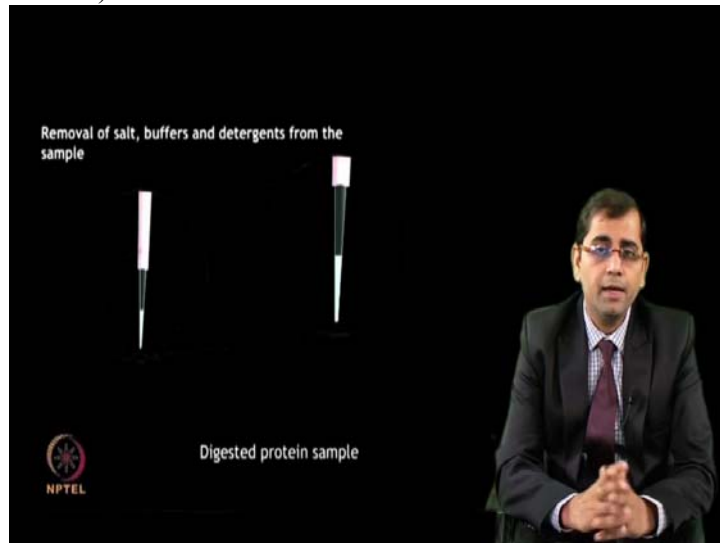
the in-gel proteolytic digestion is performed ...

(Refer Slide Time 04:26)



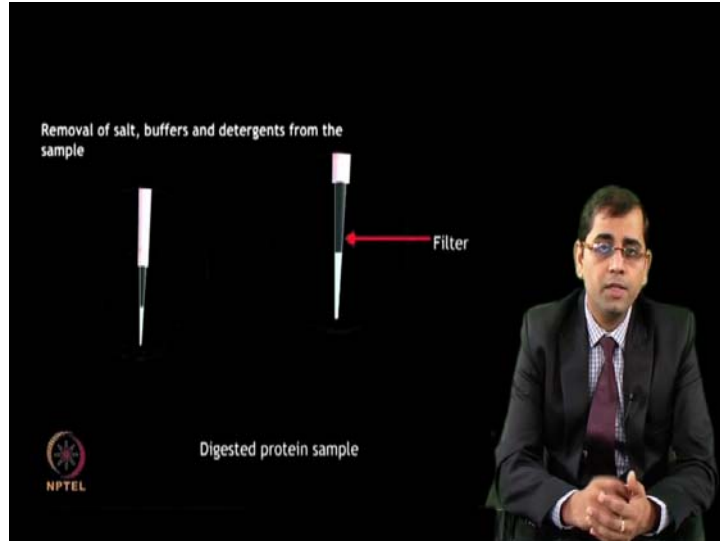
.... to cleave the protein of interest present within the polyacrylamide matrix

(Refer Slide Time 04:37)



Mass spectrometric identification of the target protein greatly depends on...

(Refer Slide Time 04:39)



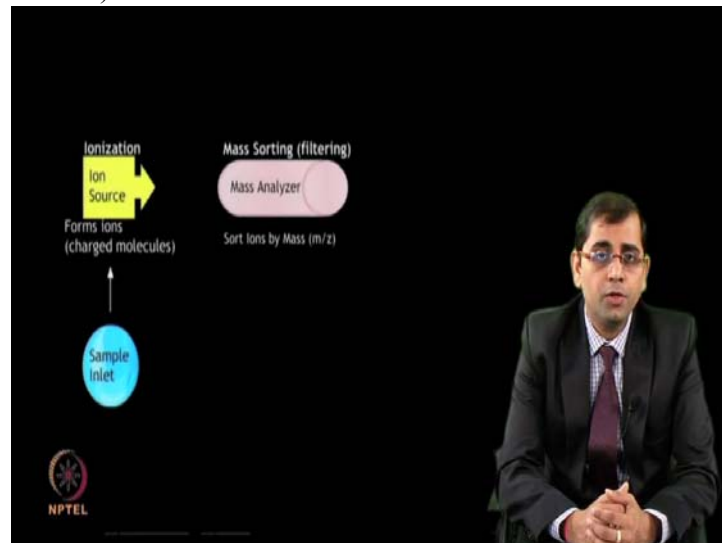
... the efficacy of the in-gel digestion protocol which generates mixture of peptides from the target protein through proteolytic digestion.

(Refer Slide Time 04:51)



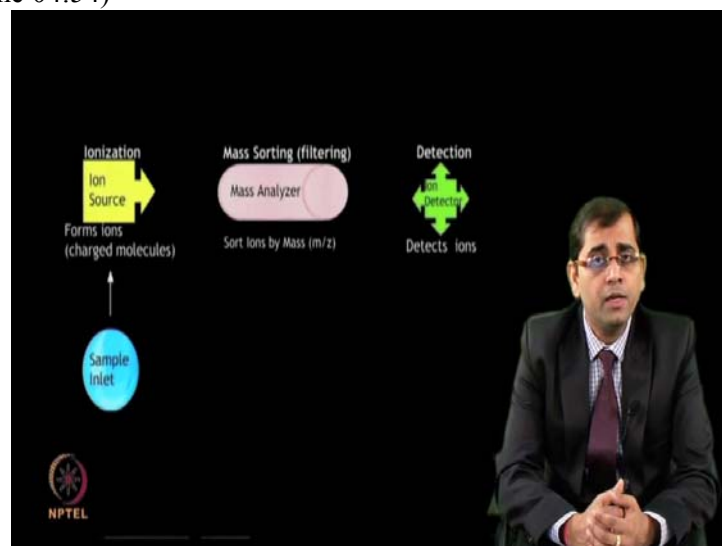
In lecture 5...

(Refer Slide Time 04:53)



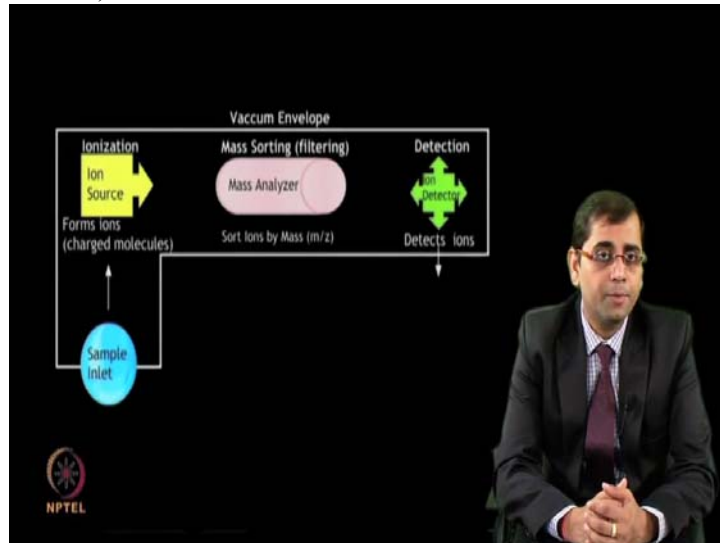
...we will talk ...

(Refer Slide Time 04:54)



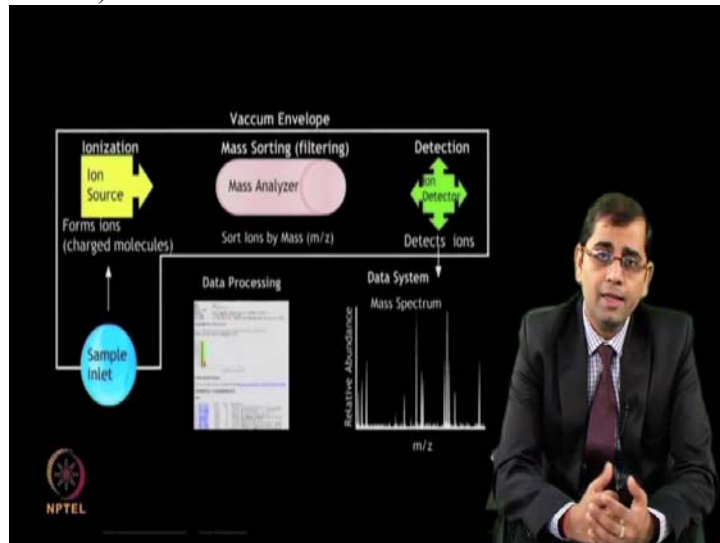
... about ...

(Refer Slide Time 04:55)



...the fundamentals of mass spectrometry

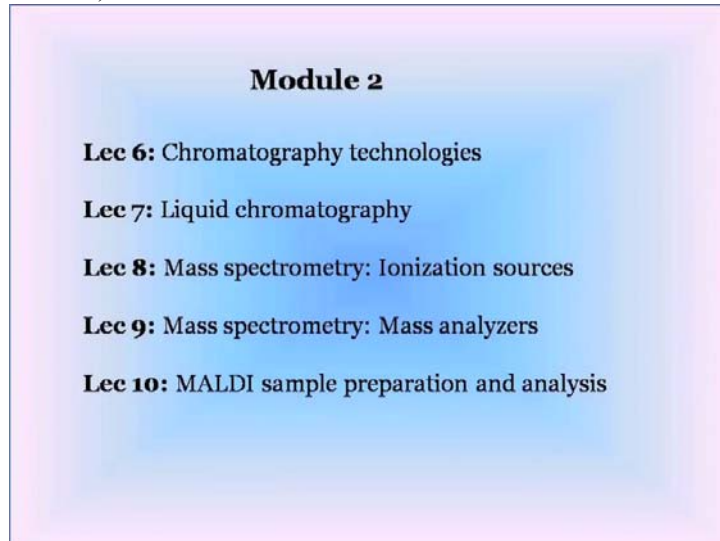
(Refer Slide Time 04:58)



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.

(Refer Slide Time 05:45)



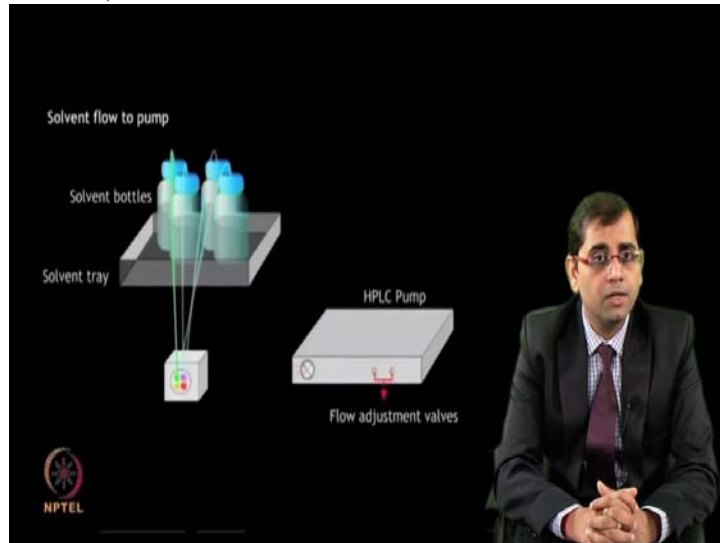
In second week, we will discuss in detail about different types of chromatographic techniques...

(Refer Slide Time 05:54)



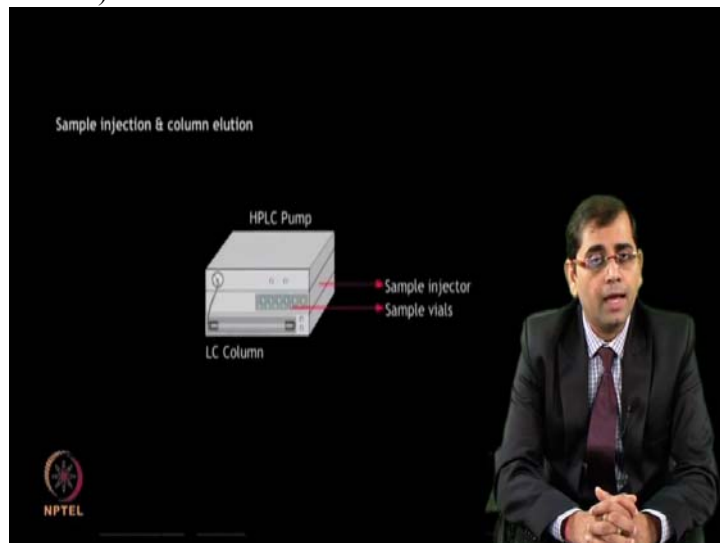
... like gel filtration, ion exchange chromatography...

(Refer Slide Time 05:57)



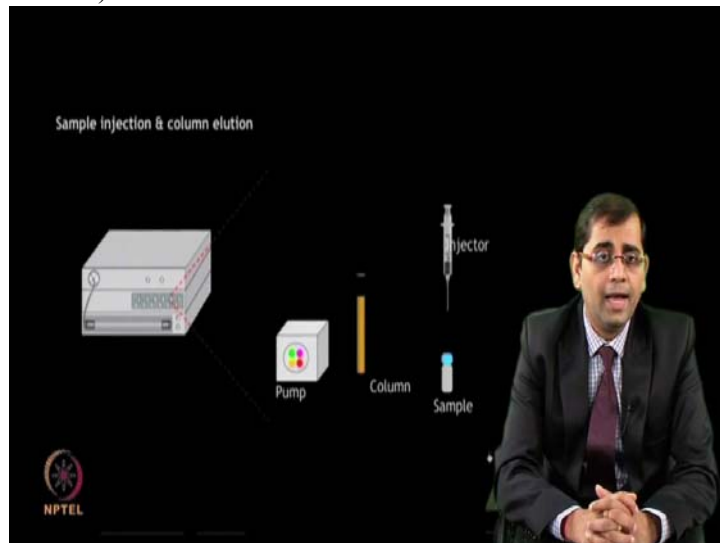
...affinity chromatography, SCX or Strong cation exchange...

(Refer Slide Time 06:03)



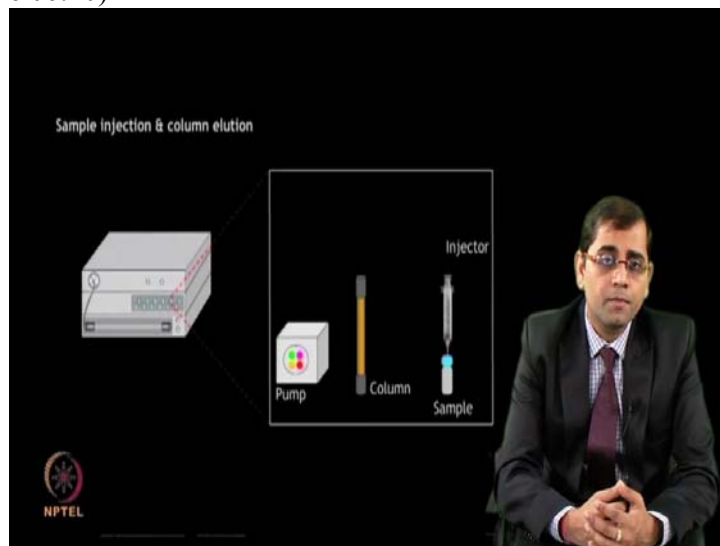
...and reverse phase chromatography.

(Refer Slide Time 06:06)



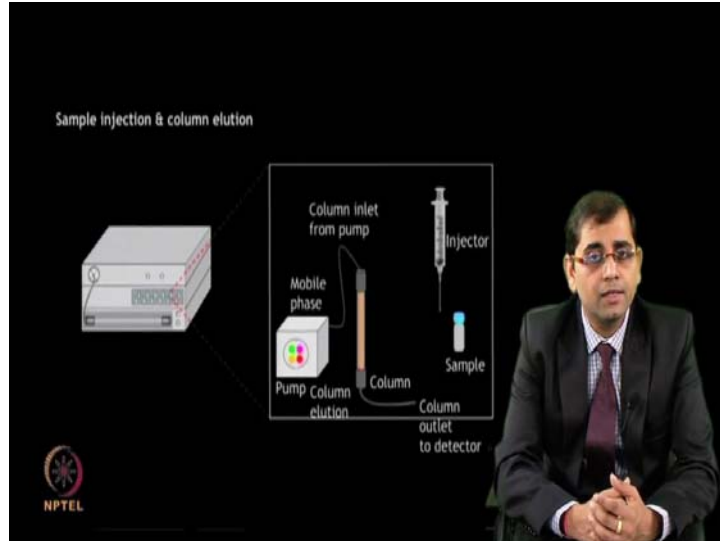
As we will progress...

(Refer Slide Time 06:10)



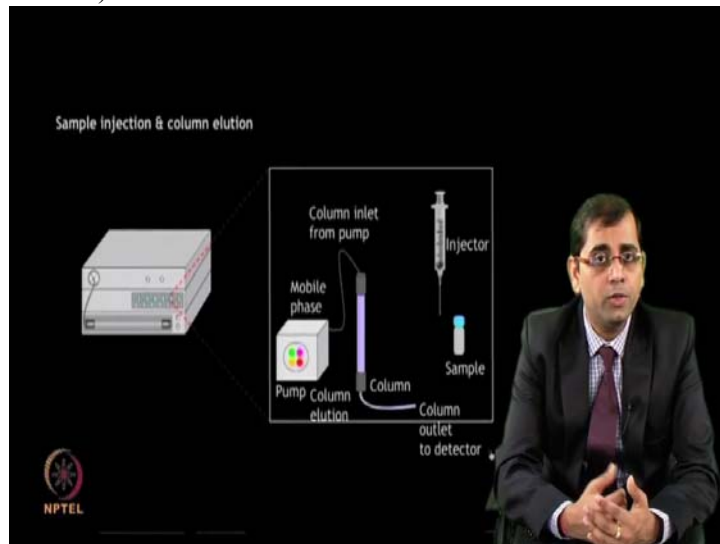
... through lectures 6 and 7, we will discuss...

(Refer Slide Time 06:12)



... how different chromatographic techniques work based on different principles...

(Refer Slide Time 06:18)



...and how one could employ those ...

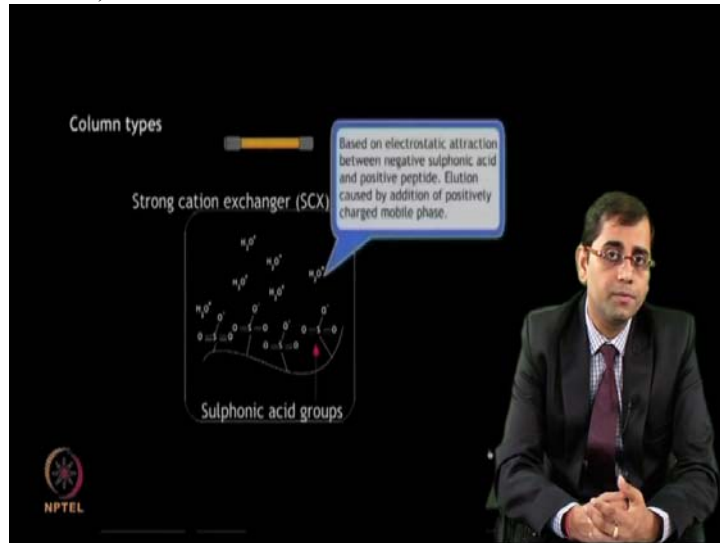
(Refer Slide Time 06:20)

Column types

Strong cation exchanger (SCX)

Based on electrostatic attraction between negative sulphonic acid and positive peptide. Elution caused by addition of positively charged mobile phase.

Sulphonic acid groups



...in proteomic workflow

(Refer Slide Time 06:22)

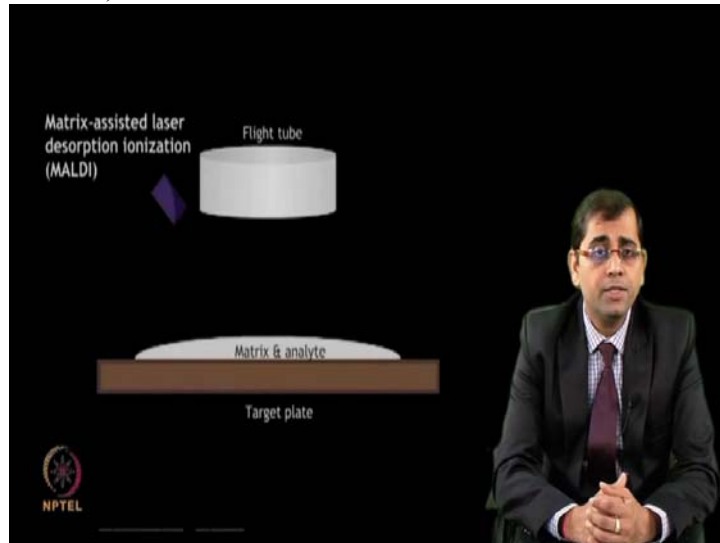
Matrix-assisted laser desorption ionization (MALDI)

Matrix & analyte

Target plate

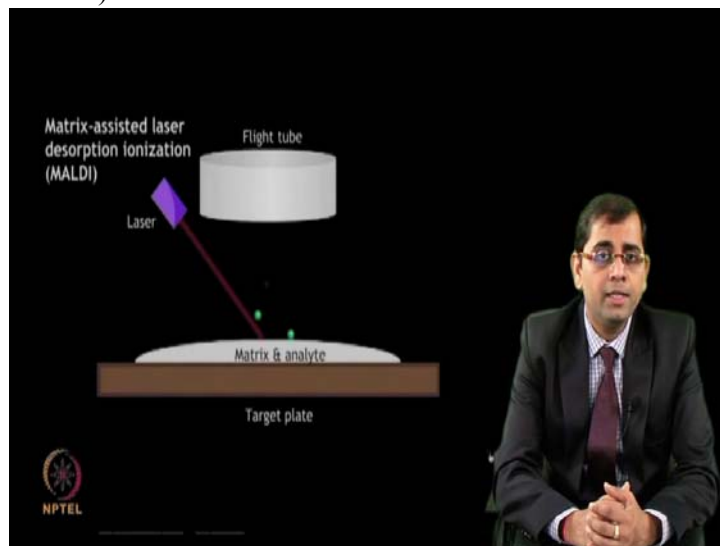


(Refer Slide Time 06:23)



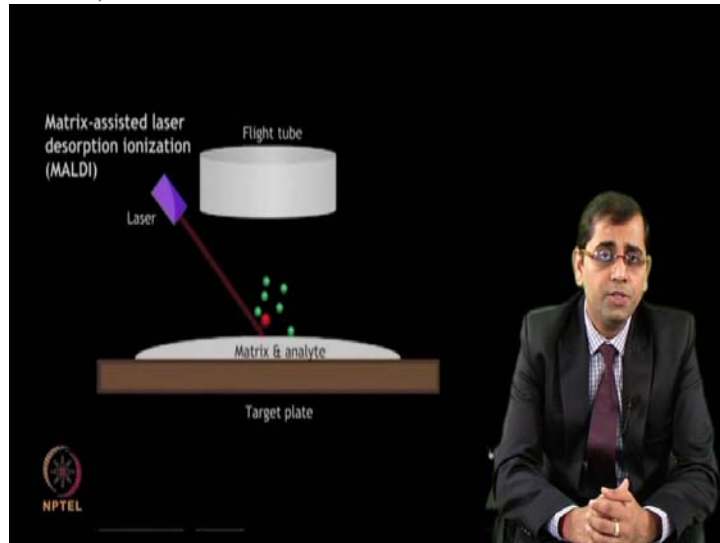
Lecture 8 will focus on....

(Refer Slide Time 06:25)



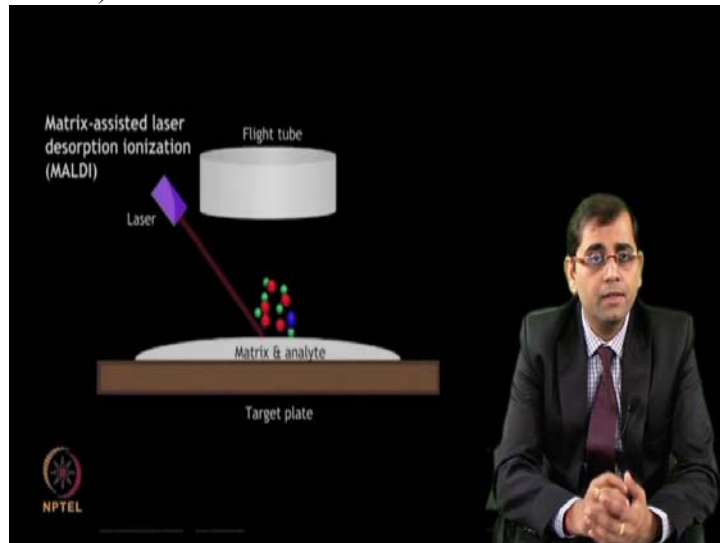
...ionization sources

(Refer Slide Time 06:27)



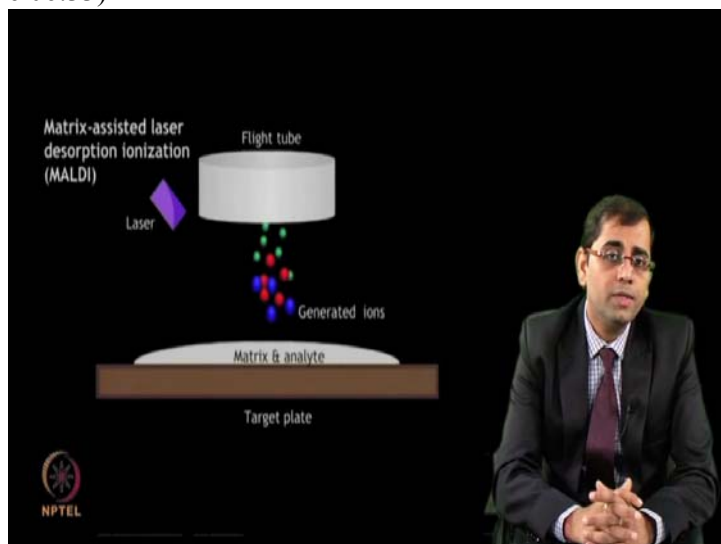
The ionization sources are responsible for

(Refer Slide Time 06:31)



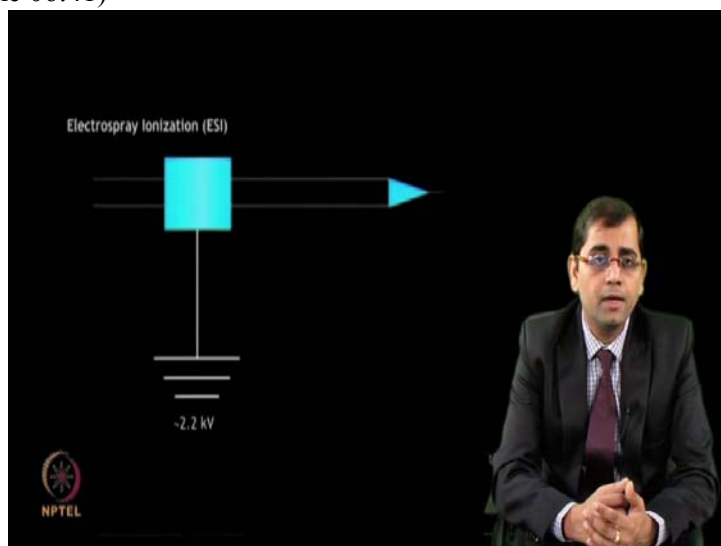
...converting the analyzed molecule into ...

(Refer Slide Time 06:33)



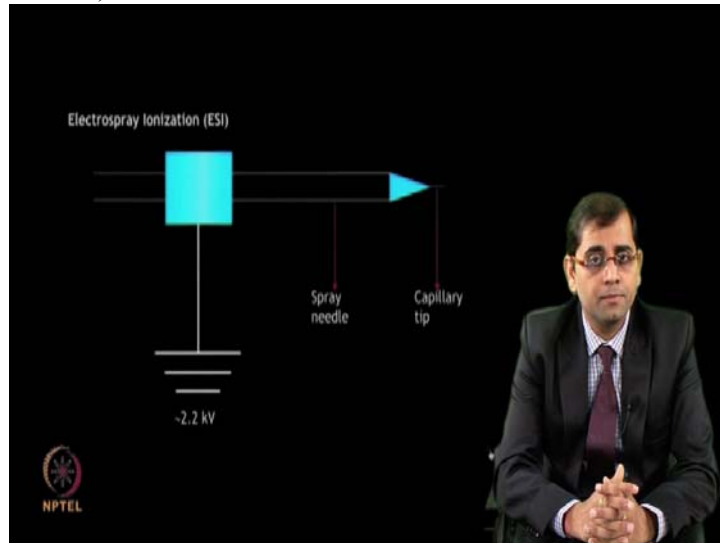
...gas cations into vacuum. The ions generated by the ionization source ...

(Refer Slide Time 06:41)



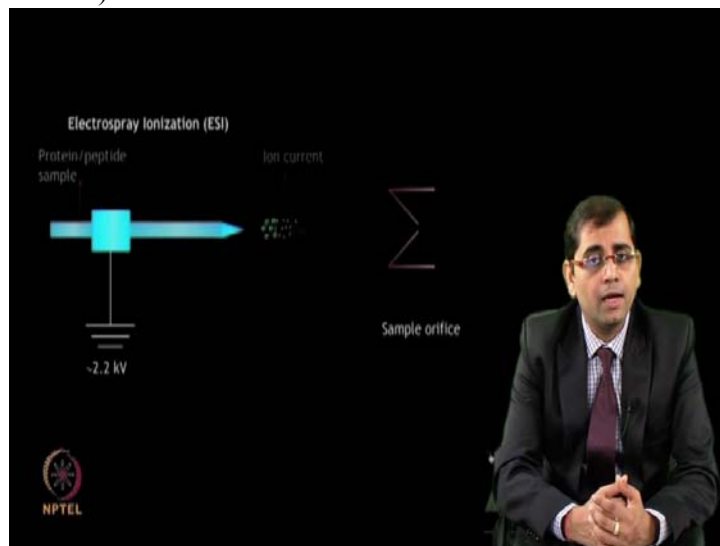
...are then integrated with the mass analyzer ...

(Refer Slide Time 06:45)



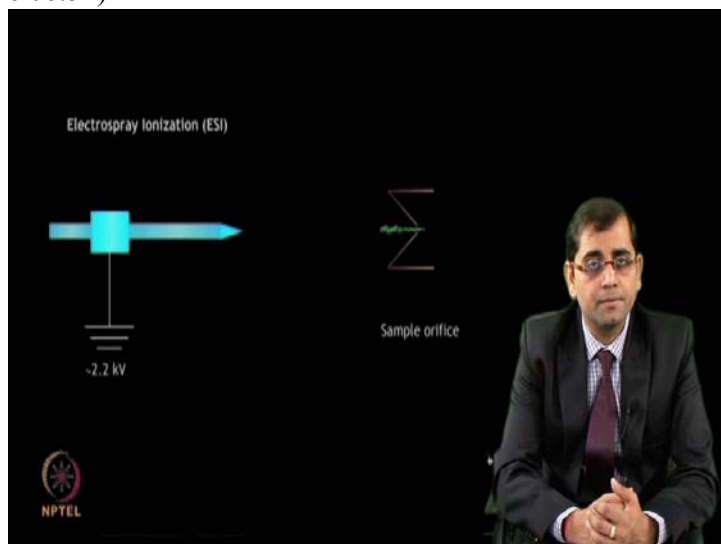
. The commonly used ...

(Refer Slide Time 06:47)



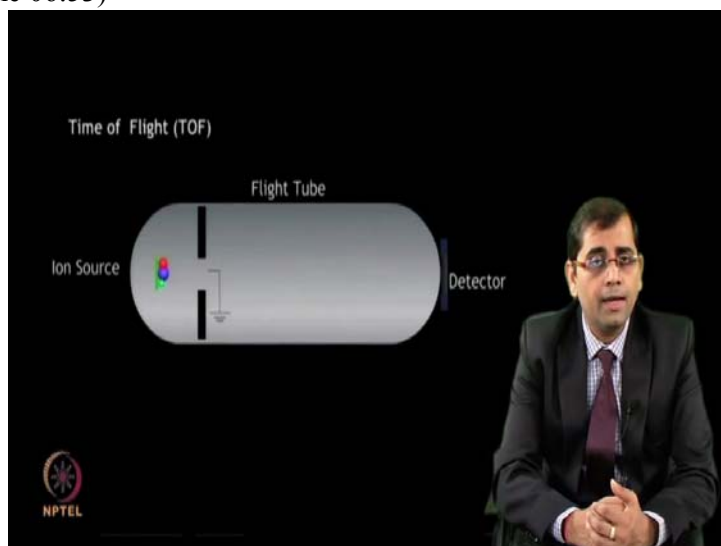
.... ionization sources are MALDI ...

(Refer Slide Time 06:51)

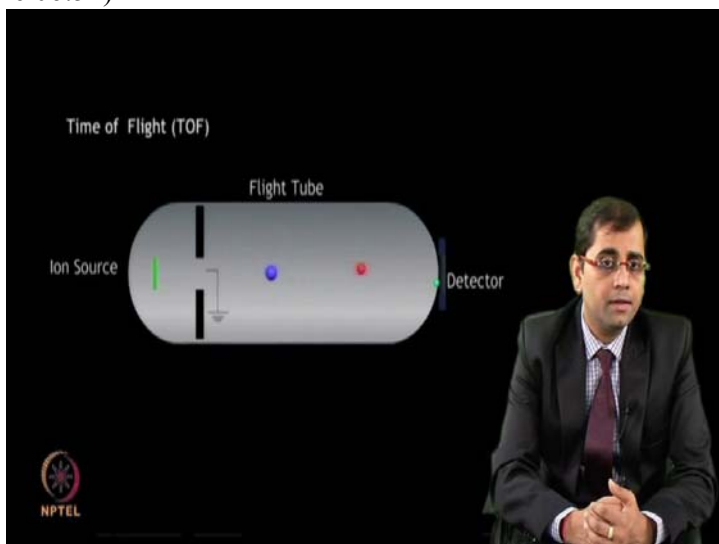


...and ESI

(Refer Slide Time 06:53)

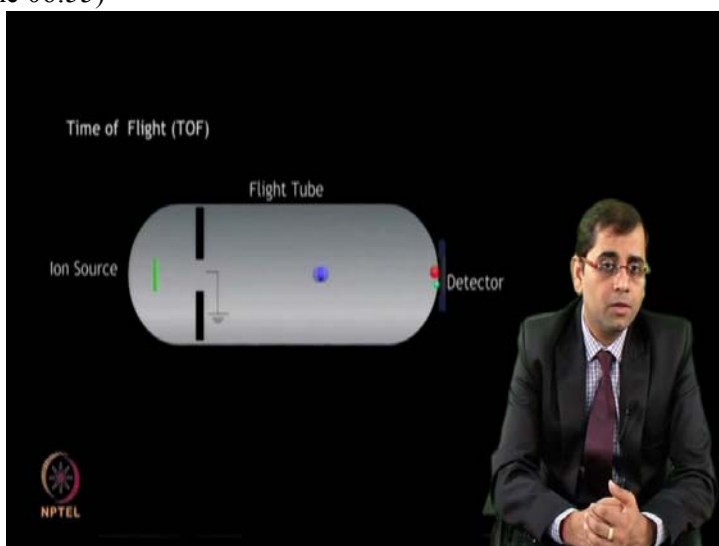


(Refer Slide Time 06:54)



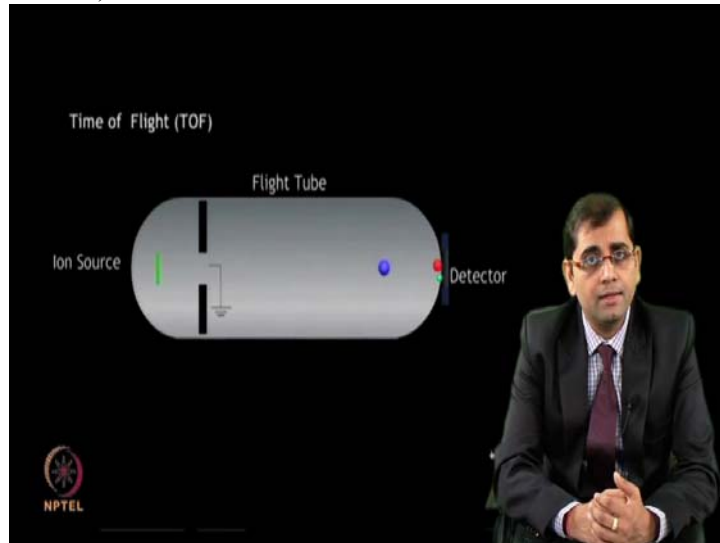
In lecture 9,

(Refer Slide Time 06:55)



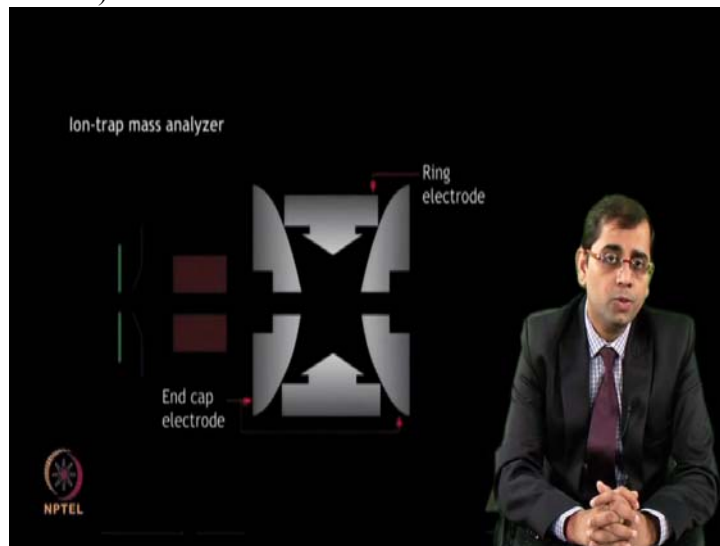
...we will talk about mass analyzers. The mass analyzers....

(Refer Slide Time 07:00)



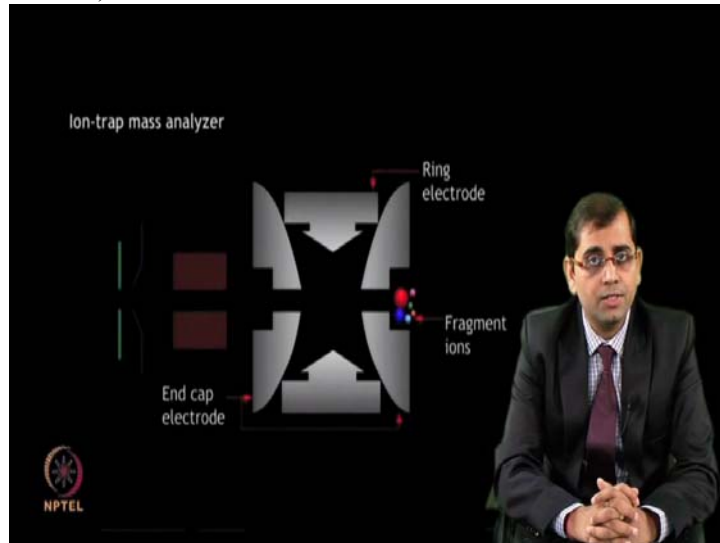
...resolve the ions produced by ionization source ...

(Refer Slide Time 07:04)



...on the basis of ...

(Refer Slide Time 07:11)



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

(Refer Slide Time 07:15)



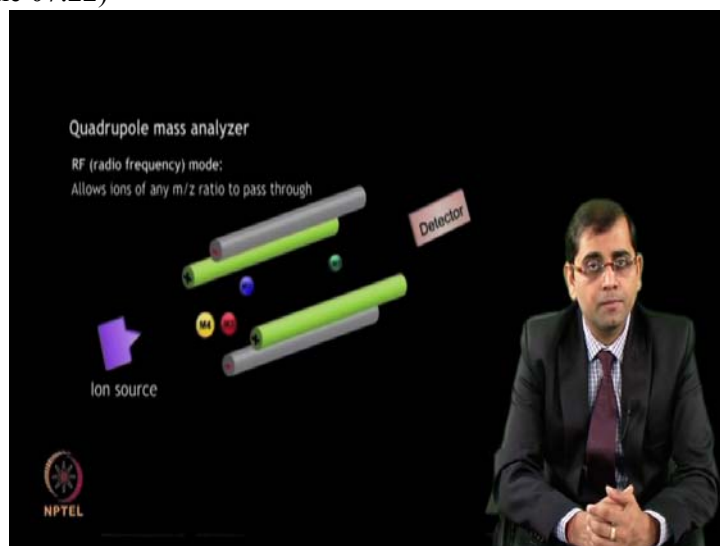
...accuracy, mass range and speed determine the...

(Refer Slide Time 07:20)



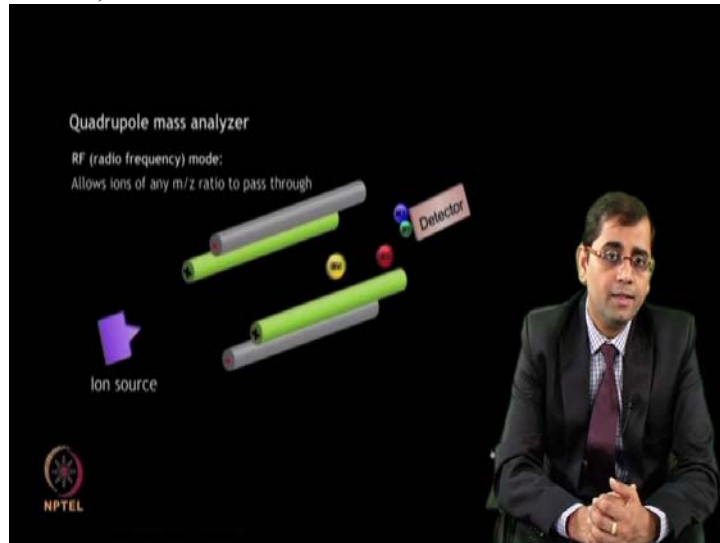
...efficiency of the analyzers

(Refer Slide Time 07:22)



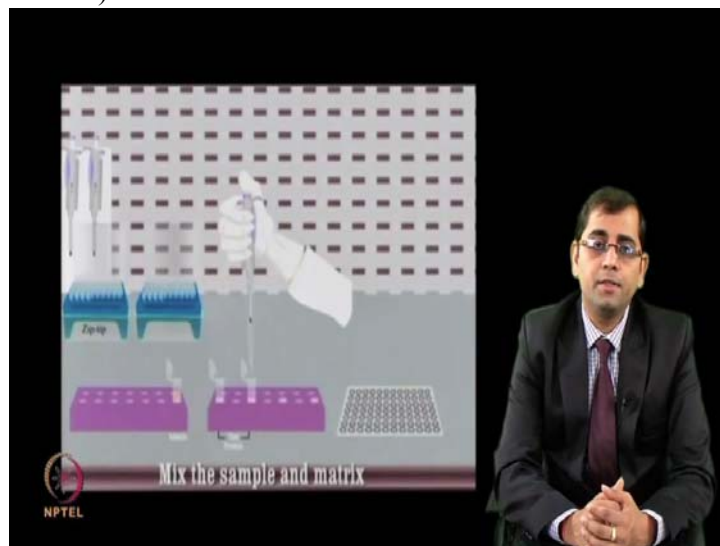
The commonly used mass analyzers are ...

(Refer Slide Time 07:25)



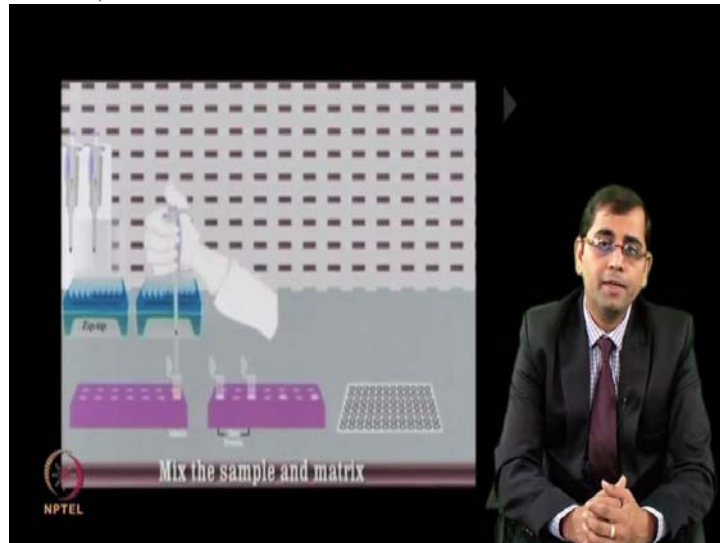
... Time of Flight TOF, Quadrupoles and Ion Traps

(Refer Slide Time 07:34)



The sample preparation strategy prior to mass spec analysis ...

(Refer Slide Time 07:36)



...will be discussed...

(Refer Slide Time 07:39)



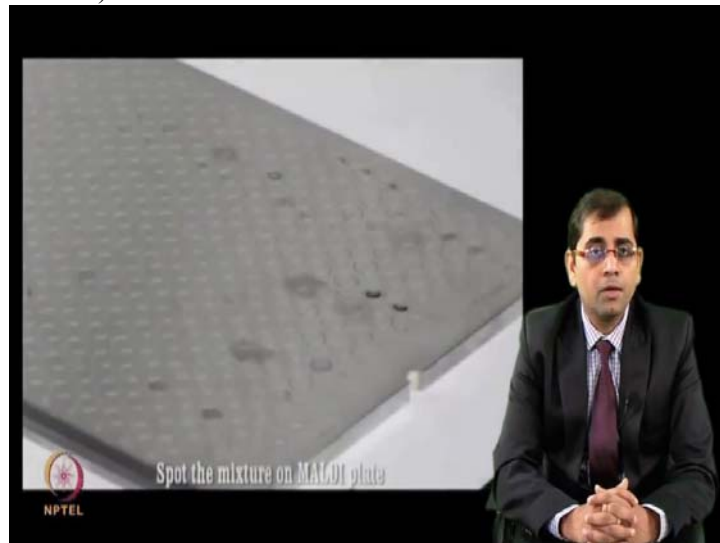
...in lecture 10

(Refer Slide Time 07:40)



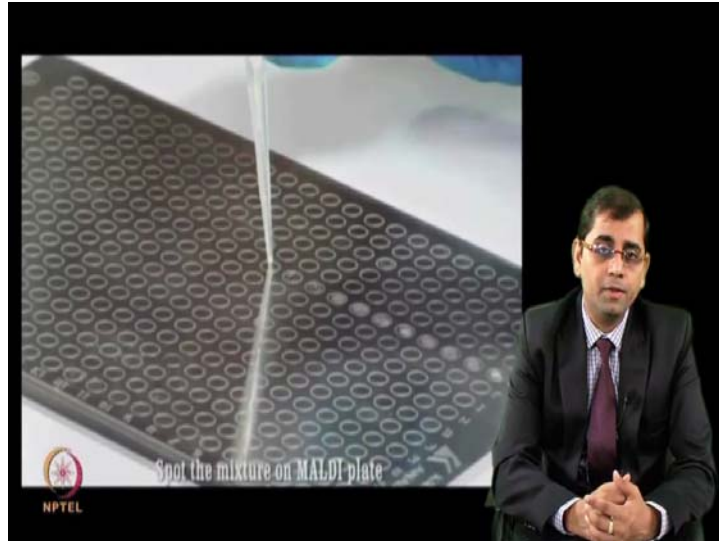
Once...

(Refer Slide Time 07:41)



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

(Refer Slide Time 07:49)



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

(Refer Slide Time 07:53)



...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)



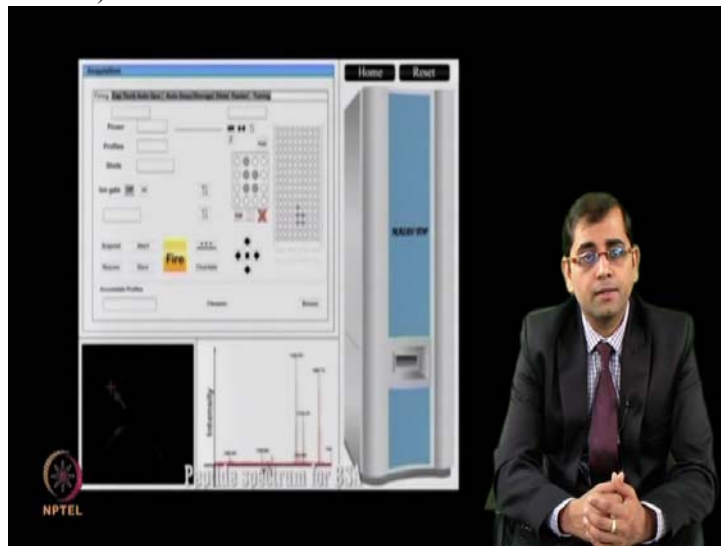
However it can purify only a limited volume of the sample and ...

(Refer Slide Time 08:17)



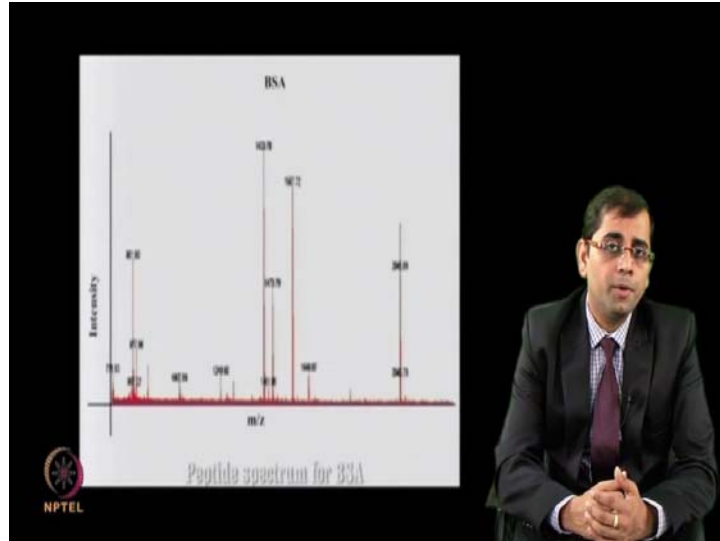
...also adsorbs some amount of protein sample ...

(Refer Slide Time 08:22)



....thereby leading to losses

(Refer Slide Time 08:25)



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

(Refer Slide Time 08:32)

| |
|--|
| Module 3 |
| Lec 11: Introduction to quantitative proteomics |
| Lec 12: Hybrid mass spectrometry configurations |
| Lec 13: SILAC: <i>In vivo</i> labeling |
| Lec 14: iTRAQ: <i>In vitro</i> labeling |
| Lec 15: TMT: <i>In vitro</i> labeling |

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)

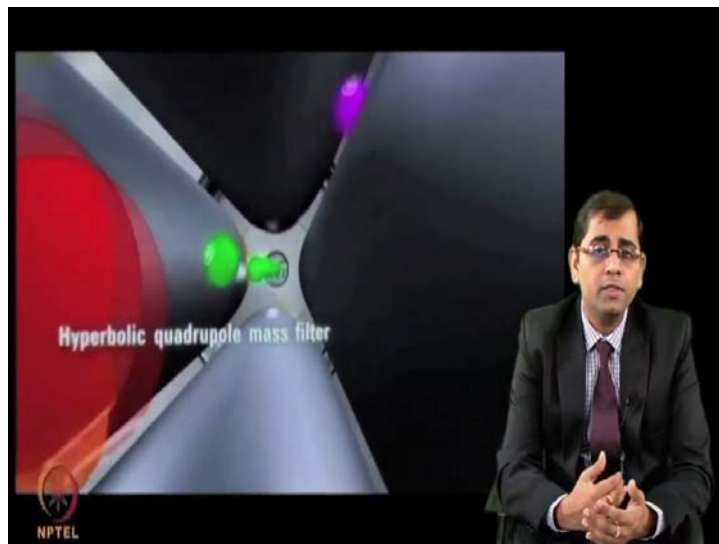


(Refer Slide Time 09:25)



The basics of quantitative proteomic analysis ...

(Refer Slide Time 09:27)



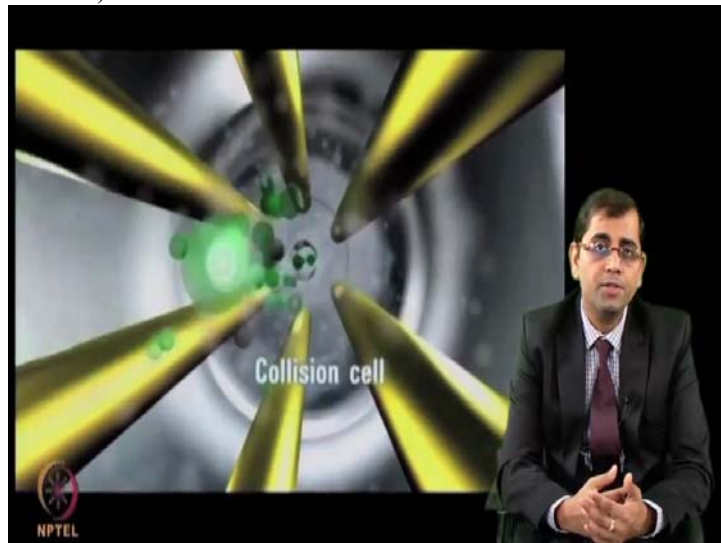
and what are the different types of

(Refer Slide Time 09:29)



...quantitative methods exist....

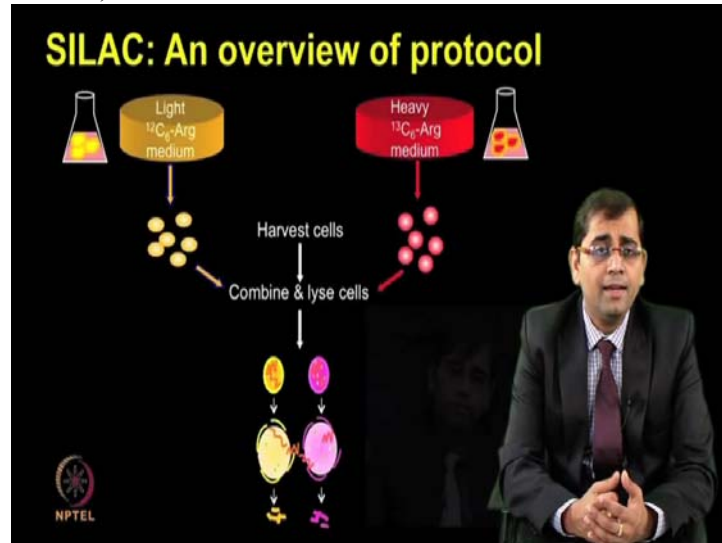
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... in literature using mass spectrometry will be discussed.

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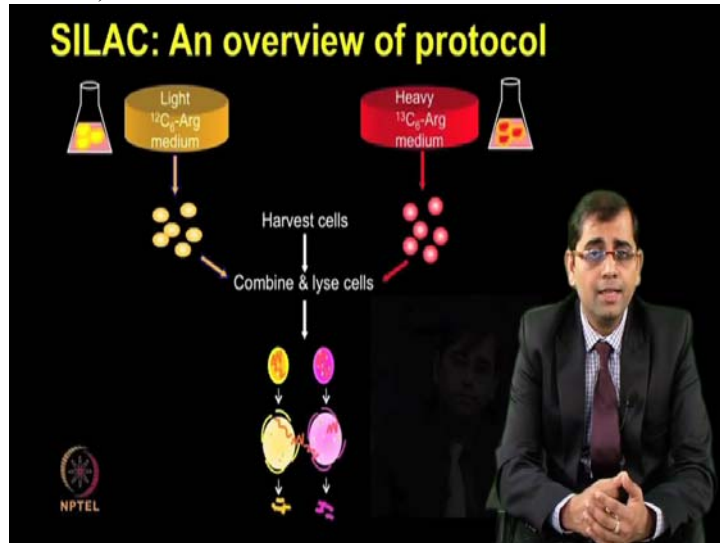
SILAC is an in vivo labeling method; the labels can be introduced in vivo by growing an organism in the media enriched with specific isotopes.

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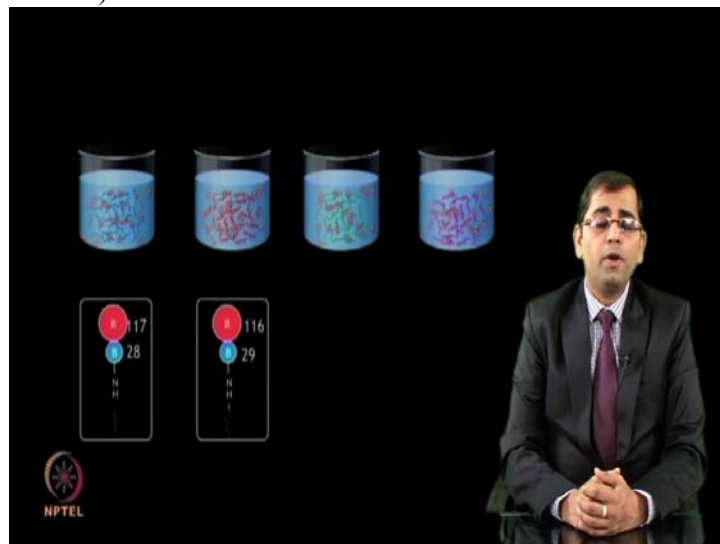
There are different ways of in vivo labeling such as enrichment of ^{15}N Nitrogen media, Culture Derived Isotope Tags known as CDIT or SILAC.

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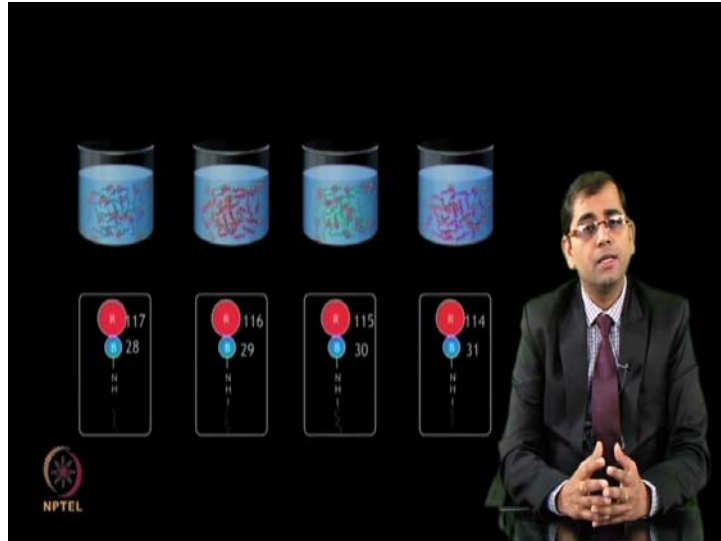
In this lecture, the major emphasis will be on the SILAC method for quantitative proteomic analysis.

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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 ...

(Refer Slide Time 11:12)



.... using iCAT where only 2 samples

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can be labeled and analyzed.

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In ITRAQ, 4 plates reagent sets...

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...the anodizing value ranging

(Refer Slide Time 11:26)



...from 114 to 117,...

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...there is a balance group...

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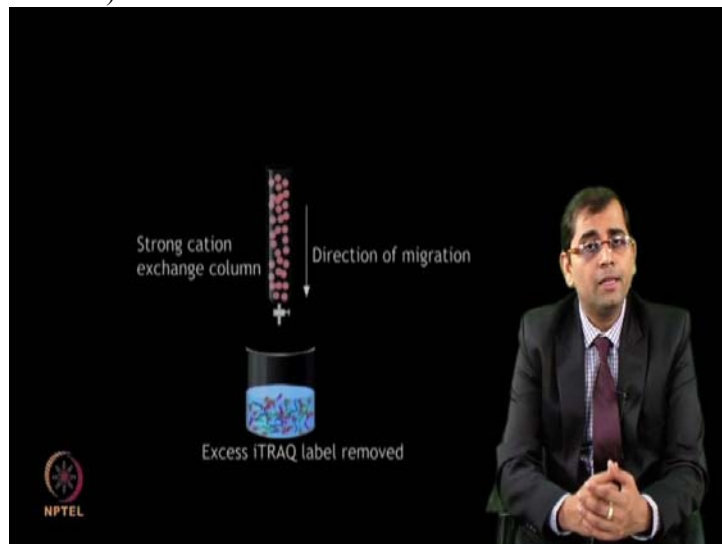
...of mass 28 to 31 Dalton, therefore the overall mass of the reporter and balancer component
...

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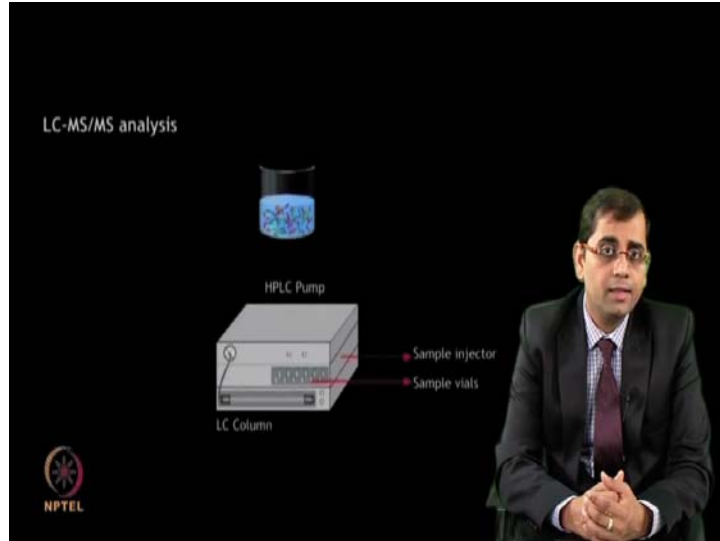
... remains constant.

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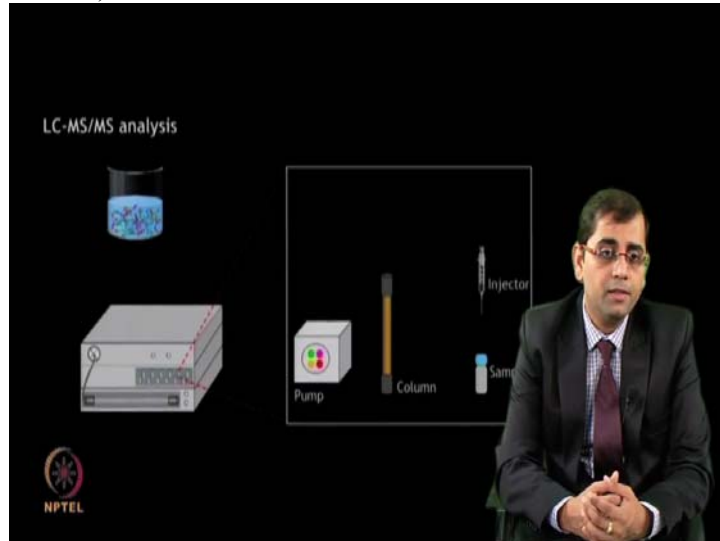
During the MS/MS fragmentation, the reporter ion gives the peak at 114, 15, 16, and 17...

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...which provides the information about ...

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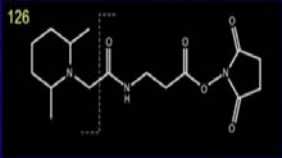


... the peptide or protein abundance.



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

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TMT⁰



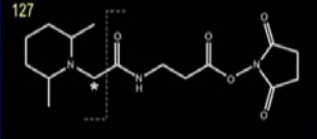
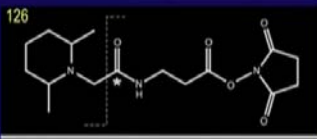
| | |
|----------------|--------|
| Modification | 224 Da |
| MS/MS Reporter | 126 Da |





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

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TMT²

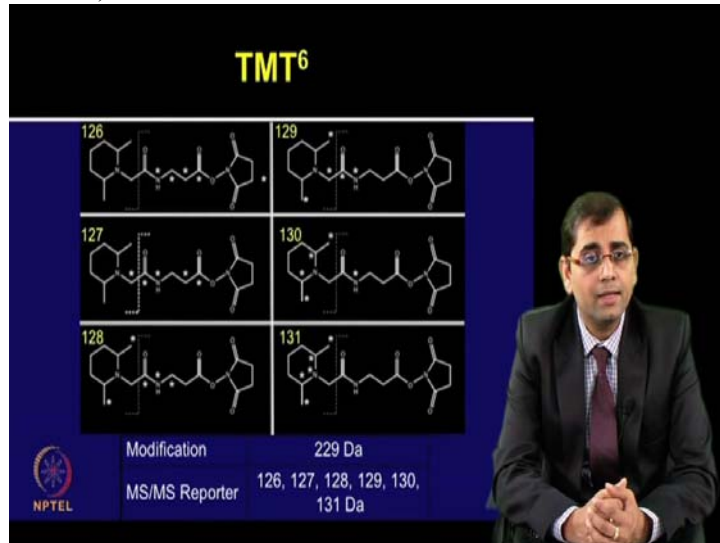


| | |
|----------------|-------------|
| Modification | 225 Da |
| MS/MS Reporter | 126, 127 Da |



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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Module 4

- Lec 16:** Quantitative proteomics data analysis
- Lec 17:** Proteomics and systems biology-I
- Lec 18:** Proteomics and systems biology-II
- Lec 19:** Proteomics applications
- Lec 20:** Challenges in proteomics

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

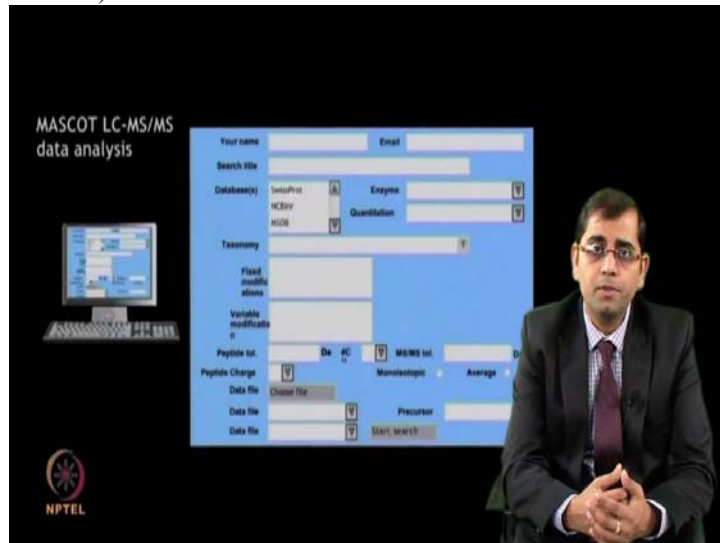
The quantitative proteomic technology aims to identify the differentially expressed protein in a biological sample. The differential expression of proteins can be caused by a disease state or various external factors like stress, drugs or different experimental conditions.

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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

(Refer Slide Time 13:50)

MASCOT LC-MS/MS data analysis

Your name: Proteomics Email: proteomics@gmail.com

Search title: Sample proteins

Database(s): SwissProt A Enzyme: Trypsin V
NCBI V Quantitation: SILAC V
K026 V

Taxonomy: Bacterial T

Fixed modifications: Carbonylmethyl (C) V
oxidation (M) V
Phospho (S) V

Variable modifications: Oxidation (M) V

Peptide list: 1.1 De AC V MS/MS list: 0.2 B V

Peptide Charge: V Monoisotopic Average

Data file: Choose file V Precursor: V

Data file: V V

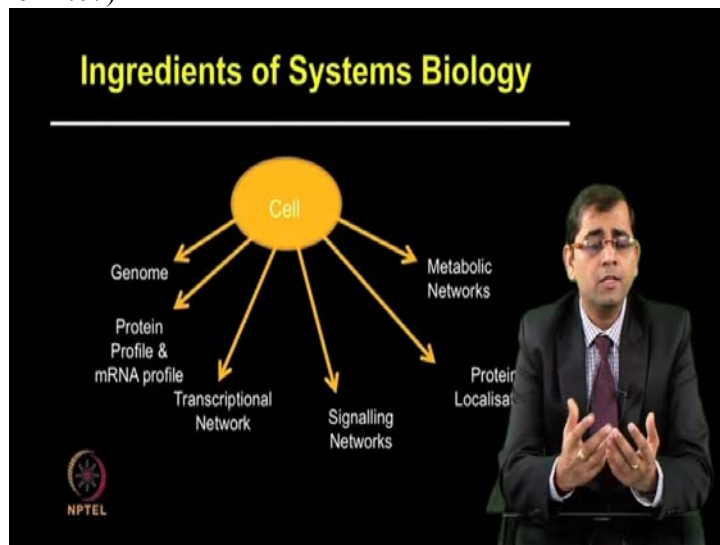
Data file: FSI-Q.TOF V Start search

NPTEL

.... Mascot and protein quantification using iTRAQ based workflow.

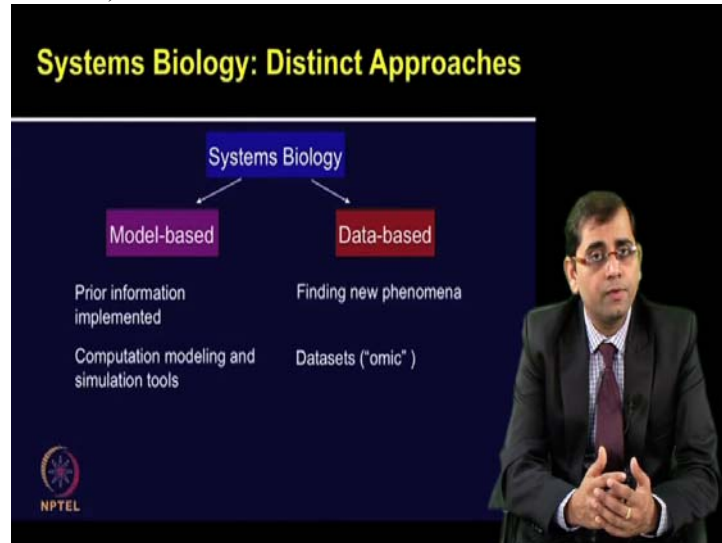
The next 2 lectures 17 and 18 will cover proteomics and System Biology.

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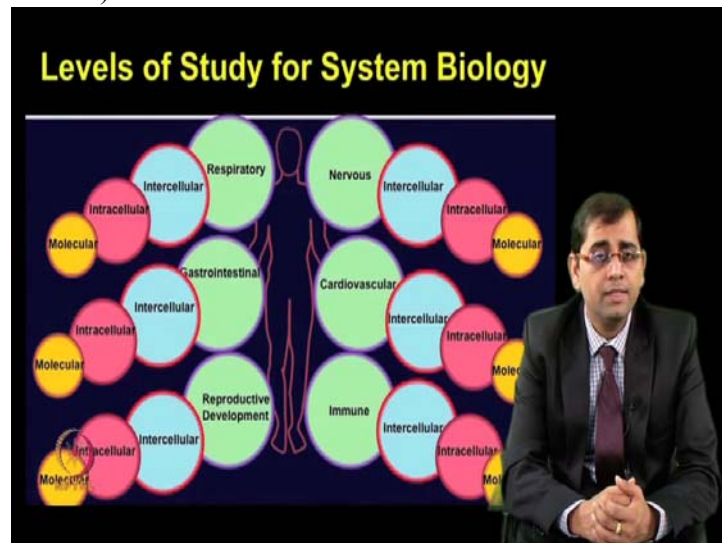
So what is System Biology? The System Biology is the examination of the biological entity as an integrated system rather than studying its individual characteristics, reactions and components. And that is what is termed as System Biology.

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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.

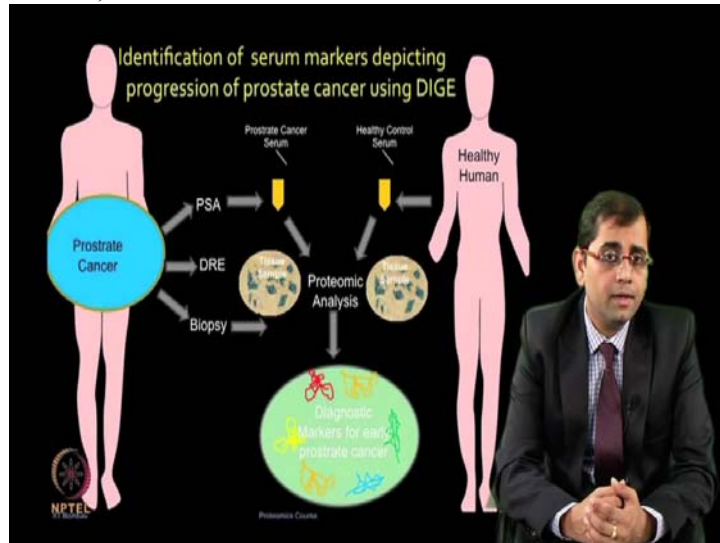
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Some of these details will be covered in System Biology.

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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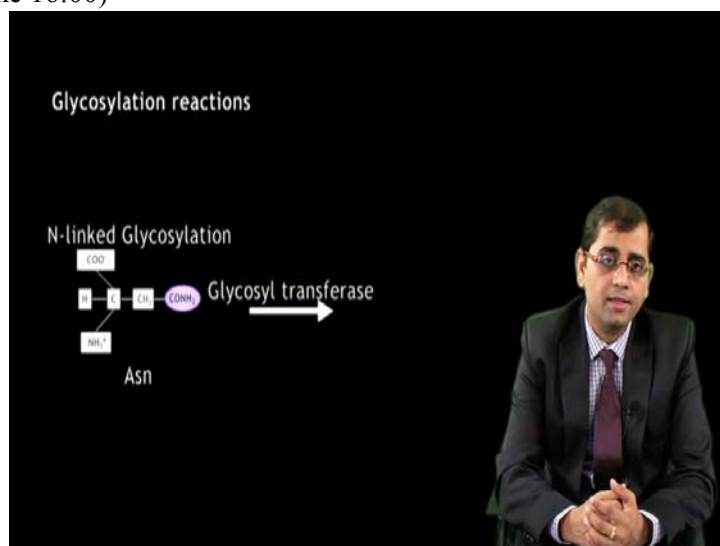


Towards the end I would like to cover proteomics applications and challenges.

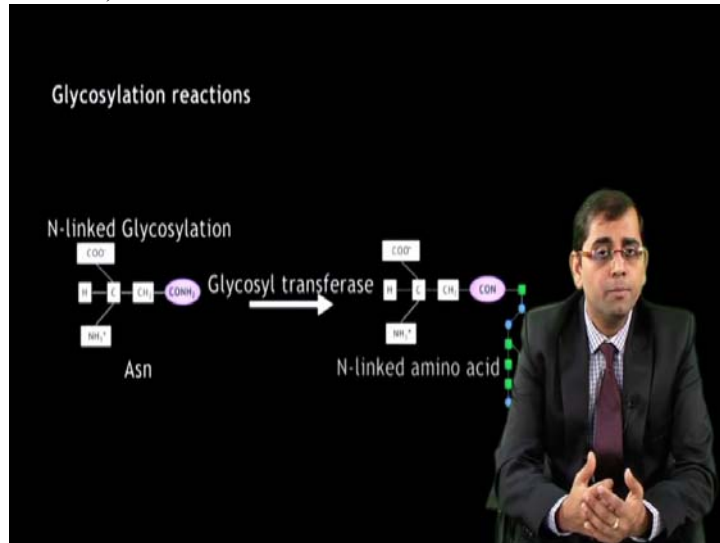
The last lecture will focus on proteomics challenges. The mass spectrometry is one of the best inventions in proteomics field in recent years. It is able to achieve many milestones including the draft human proteome maps.

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.

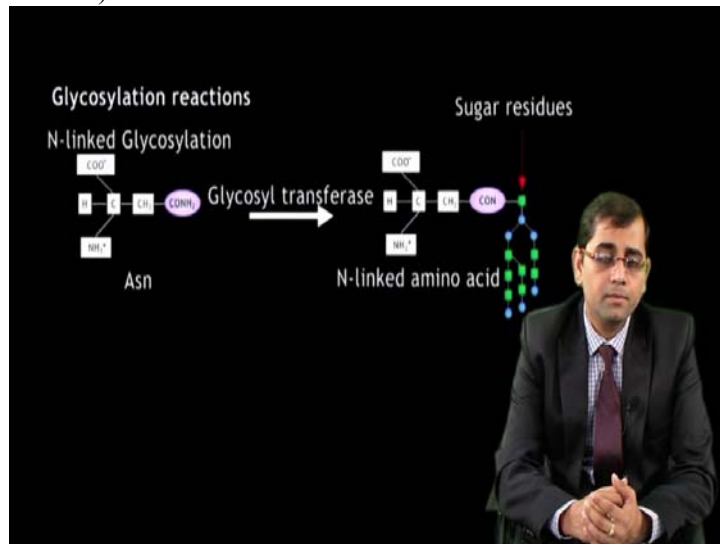
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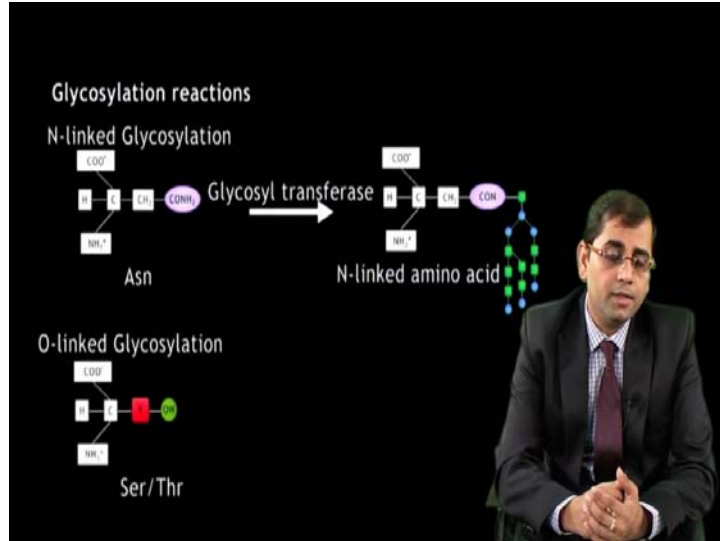
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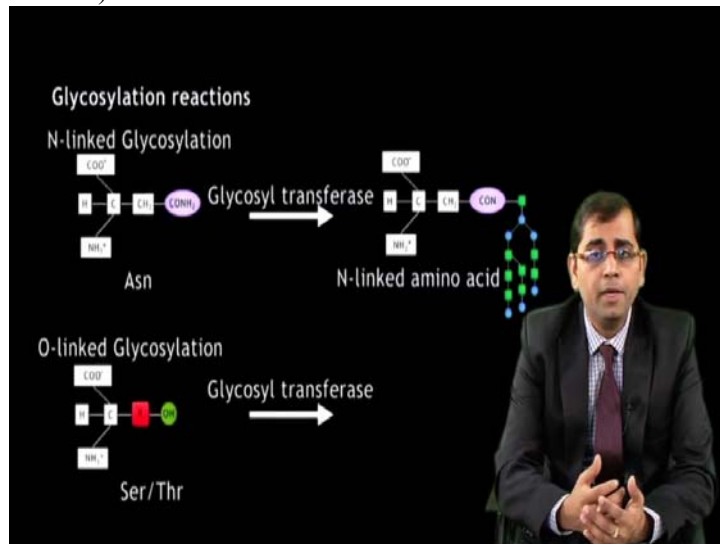
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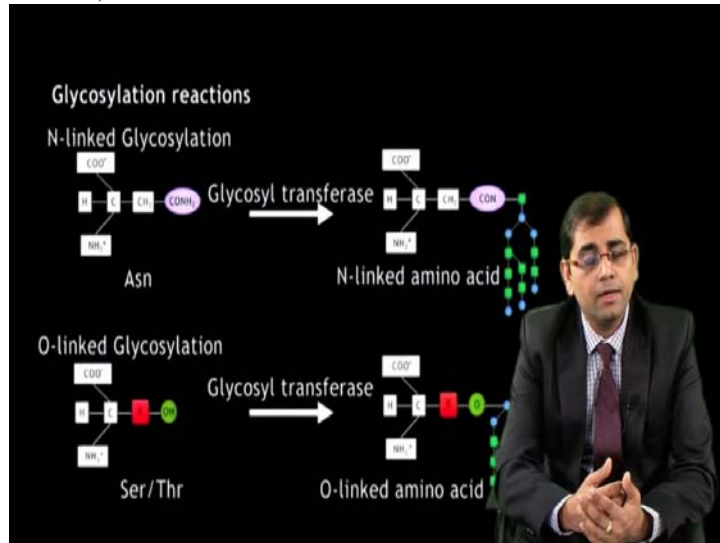


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The post translational modification analysis using Mass Spec is one of the challenges.

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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