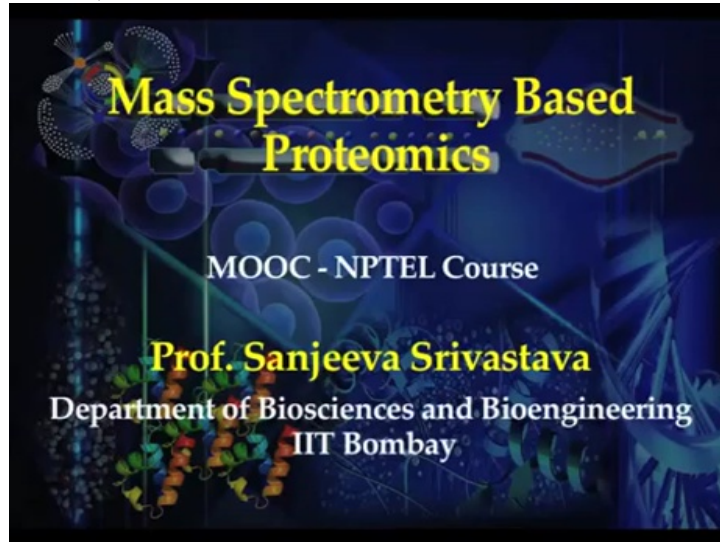
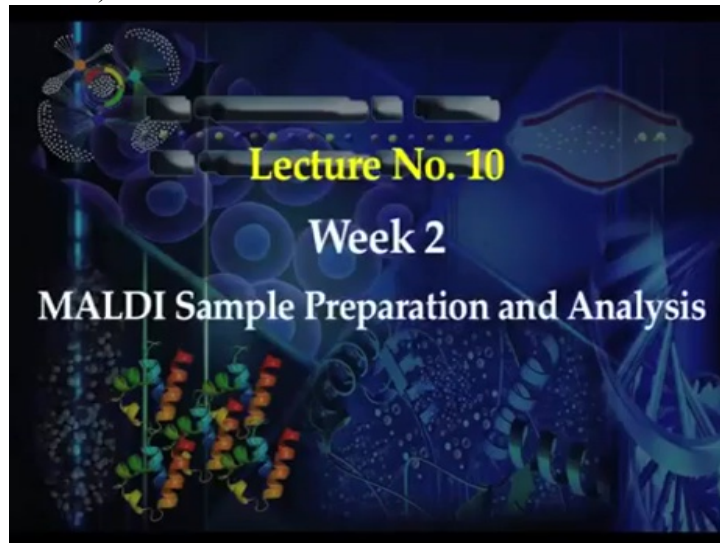


Mass Spectrometry Based Proteomics
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Mod 02 Lecture Number 10

(Refer Slide Time 00:10)



(Refer Slide Time 00:14)



(Refer Slide Time 00:18)

Topics to be Discussed Today:

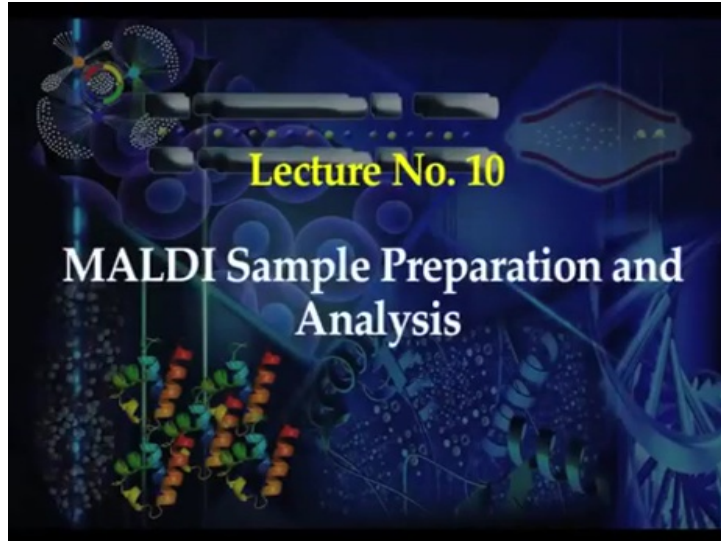
- # Basics of MALDI-TOF MS
- # An overview of typical Proteomics experiments
- # MALDI Experiment Sample Preparation
- # MALDI-TOF Instrumentation
- # Data Analysis for Protein Identification

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Welcome to the proteomics course, today we will talk about ...

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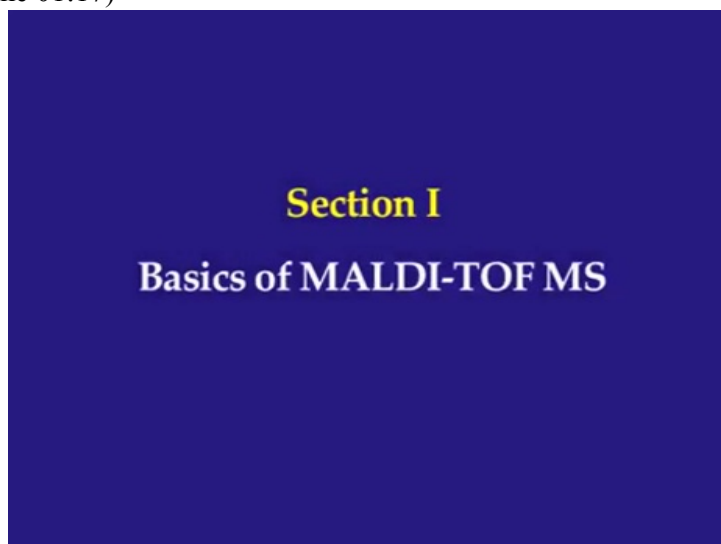


Matrix Assisted Laser Desorption Ionization Time Of Flight MALDI TOF.

In previous lecture, we talked about basics of mass spectrometry, the various combinations of mass analyzers and ionization sources. Now it is time for us to combine those and start discussing these in more detail.

So, today let us focus on the MALDI TOF which is one of the very widely used techniques in proteomics. This provides a high throughput platform for several applications, including molecular weight determination, protein identification as well as post translational modification studies.

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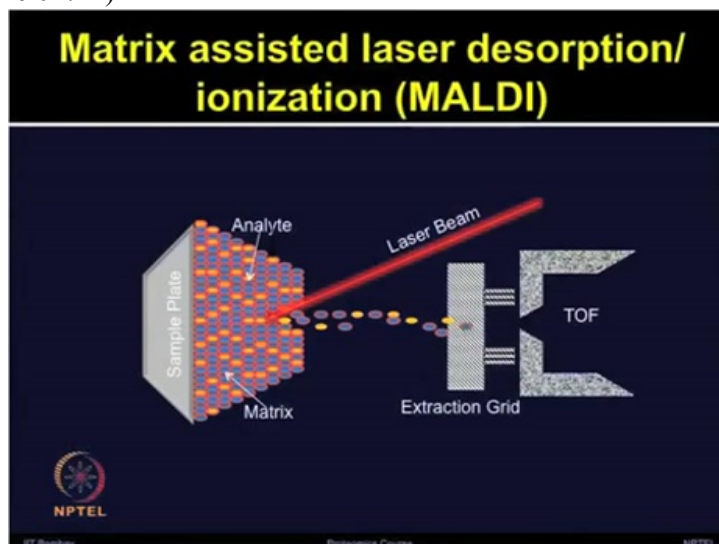


Let us first start about basics of MALDI-TOF. So, MALDI is an efficient process for generating gas phase ions of peptides and proteins for mass spectrometric detection. MALDI is one of the most widely used ionization technique, currently applicable in the proteomics area.

This ionization method was independently developed by two scientist Koichi Tanaka and Hillenkamp. Tanaka also received the Nobel Prize for his novel contribution into soft ionization technique such as MALDI.

So, let us go through the some of the basic concepts involved in the MALDI TOF. We can split that in two parts one is MALDI which is ionization source another is TOF which is a mass analyzer

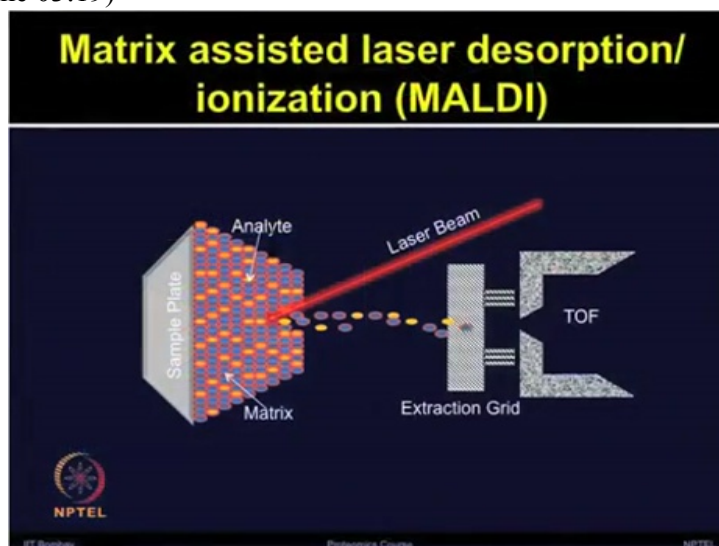
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Let's first talk about Matrix Assisted Laser Desorption Ionization or MALDI. So analyte or the proteins of interest are mixed with matrix which is usually an aromatic compound.

There are various types of matrices available, which we talk about in more detail when we come to the sample preparation and matrix selection. But just for your reference we can use 2-5-dihydroxy benzoic acid, we can use sinipinic acid and there are several other choice.

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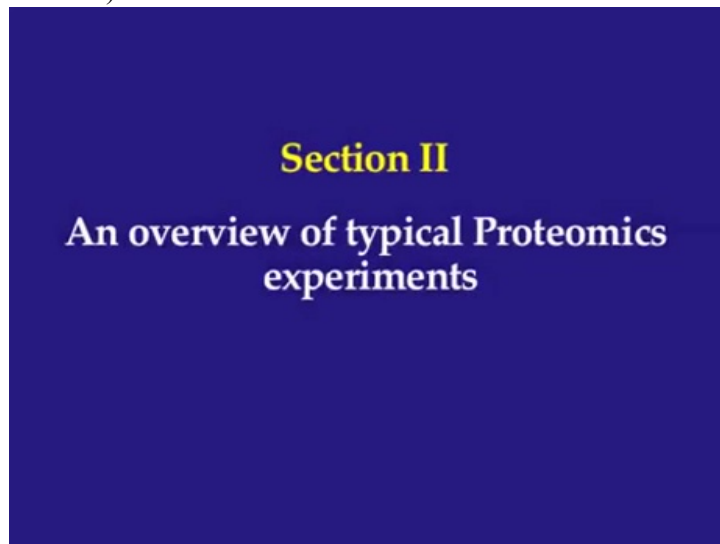
Once you have selected a matrix for the experiment then analyte and matrix can be dissolved in an organic solvent after which then it can be placed on a metallic target.

As you can see in the slide, the first left section shows you how to place the analyte and matrix together on the sample plate. Now, once you have placed the matrix and the analyte on the target plate you can put that in the vacuum chamber and apply high voltage.

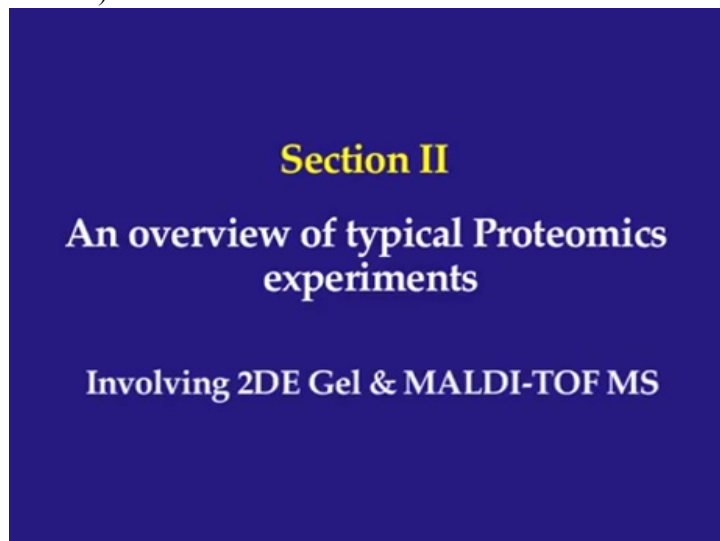
Now these crystals are targeted with short laser beams, as you can see in the slide, then rapid sublimation can convert analyte into gas phase ions.

Now these ions once generated, they can accelerate away from the target plate through the mass analyzer which is Time of Flight TOF tube and they can reach towards the detector.

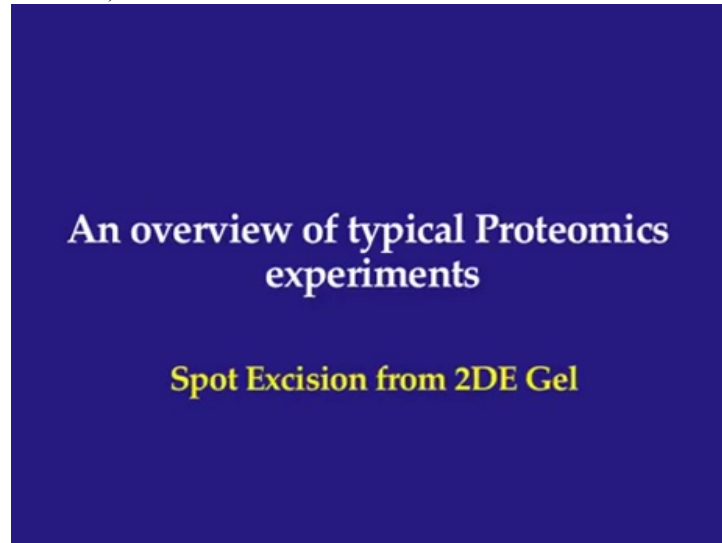
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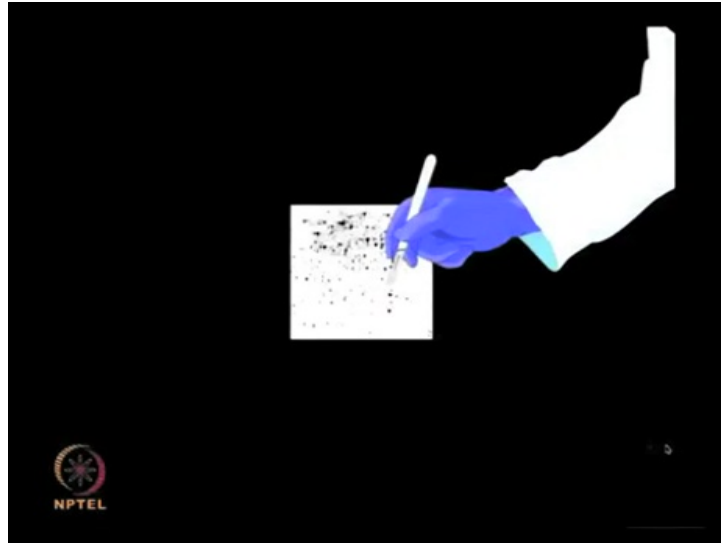


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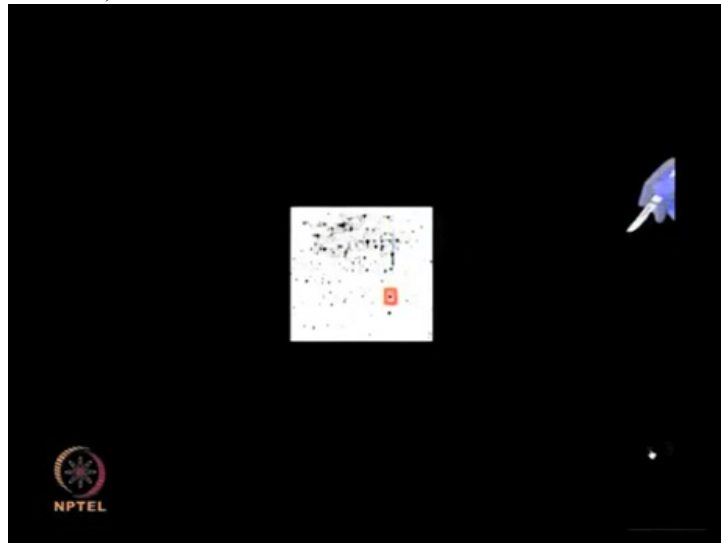
Sample preparation

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... and spotting

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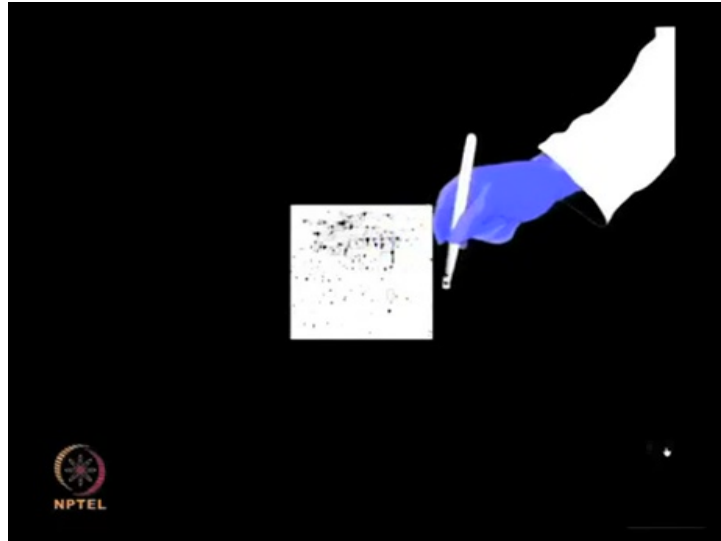


The protein sample must be prepared suitably before it can be analyzed by mass the spectrometer.

If you have run the 2D Gel, first of all, the purified protein of interest need to be excised from the gel on which it has been electrophoresed and dissolved in a suitable buffer.

Depending upon the application, if you have purified a protein, you can separate that on the gel and cut that band. Or if you have a mixture of the proteins in 2D gel, you can just excise that particular spot.

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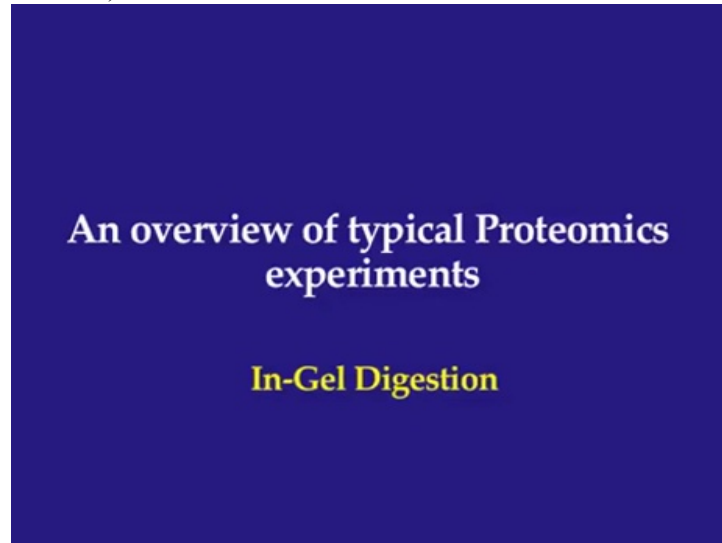
Spot can be dissolved ...

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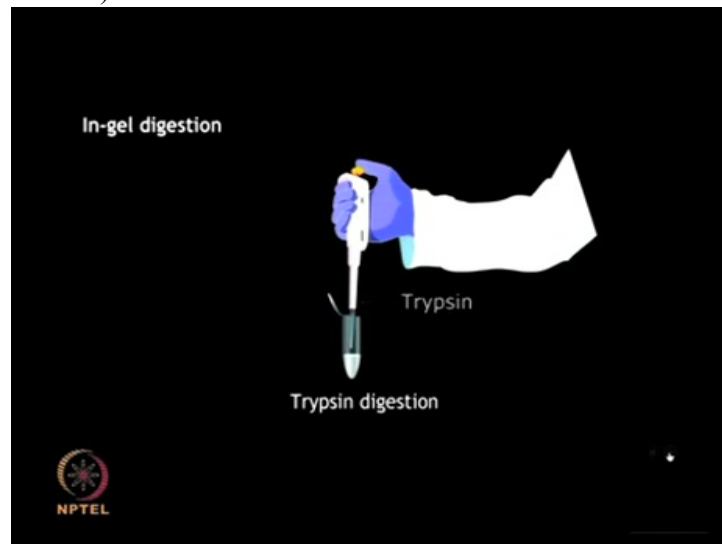


... in the suitable buffer

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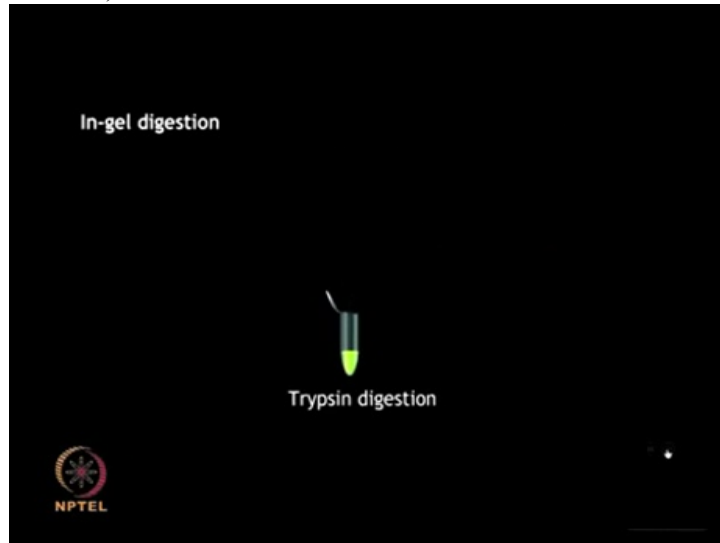


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Trypsin is then added to this mixture in order to carry out...

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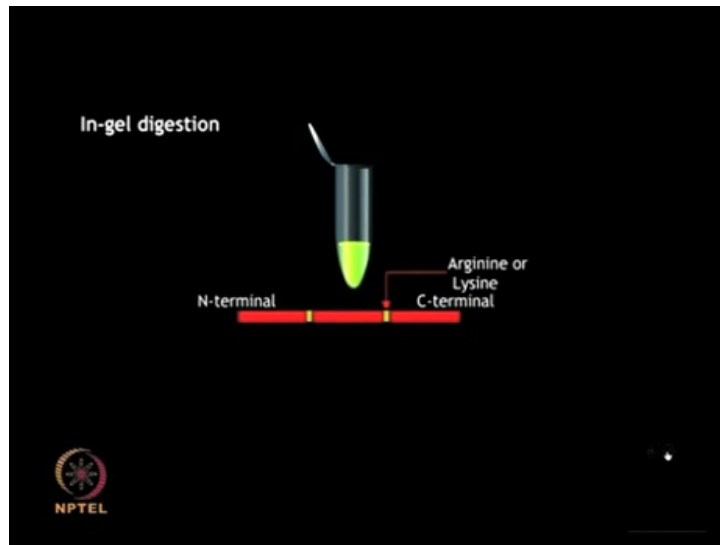


...digestion of the protein

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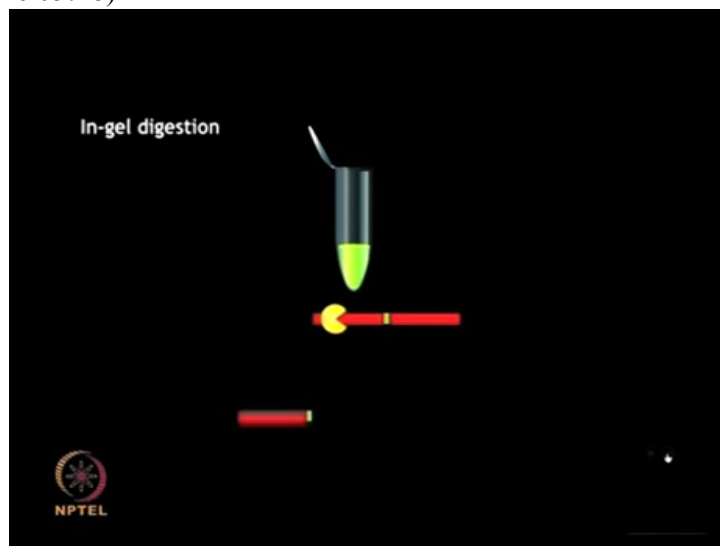


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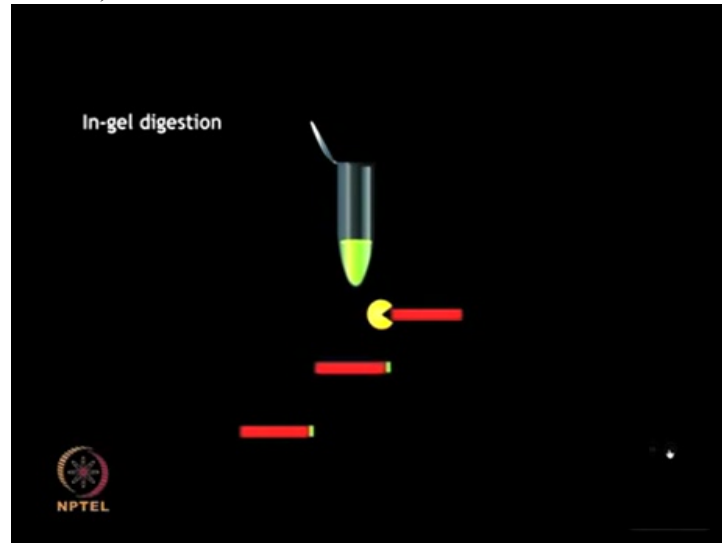
Trypsin cleaves the protein at the C-terminal of its arginine and lysine residues. But that is not always universal. If you have a proline present immediately after ...

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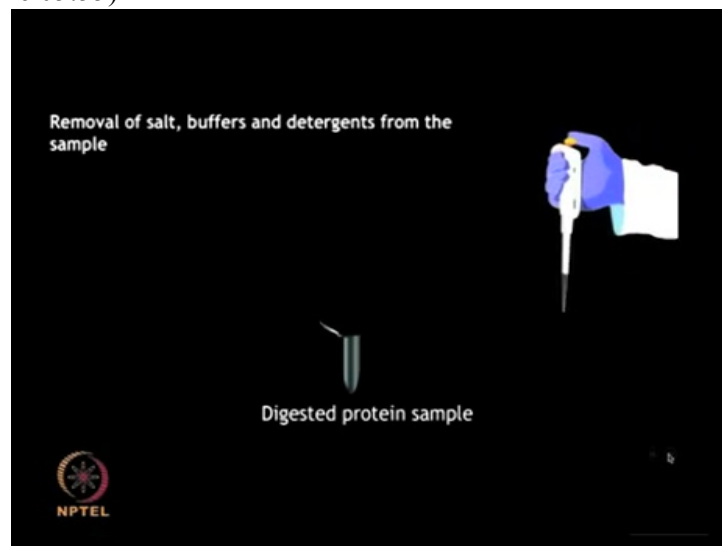
... then it will hinder that.

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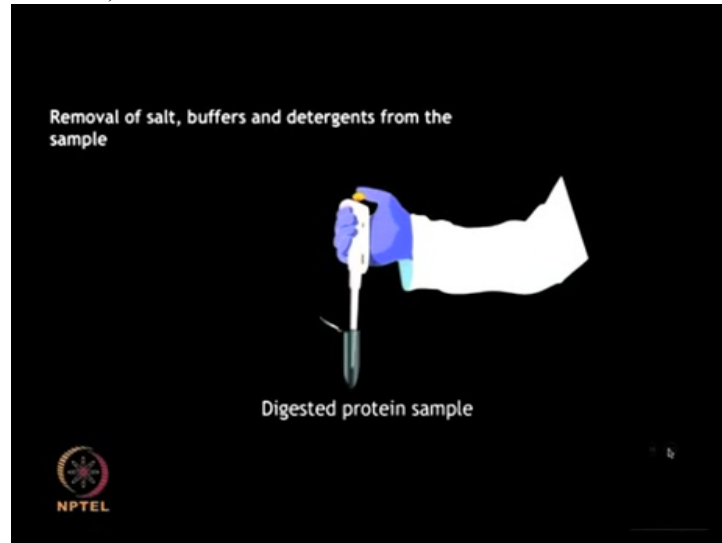
But overall, the protein is

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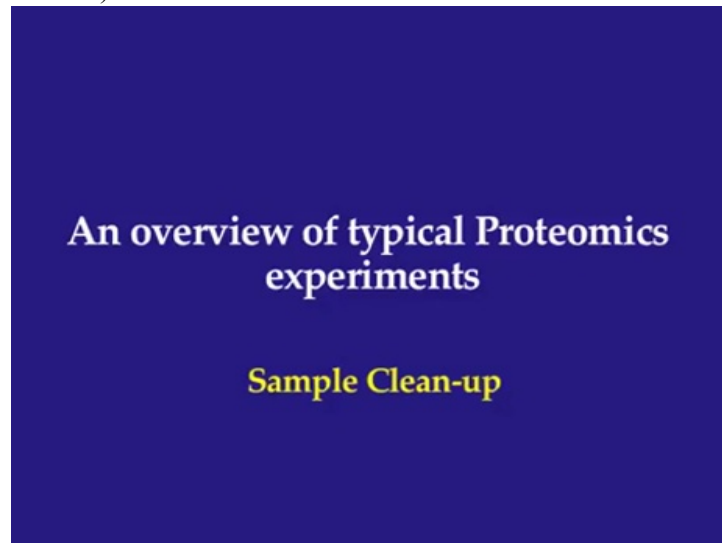
... digested into smaller fragments ...

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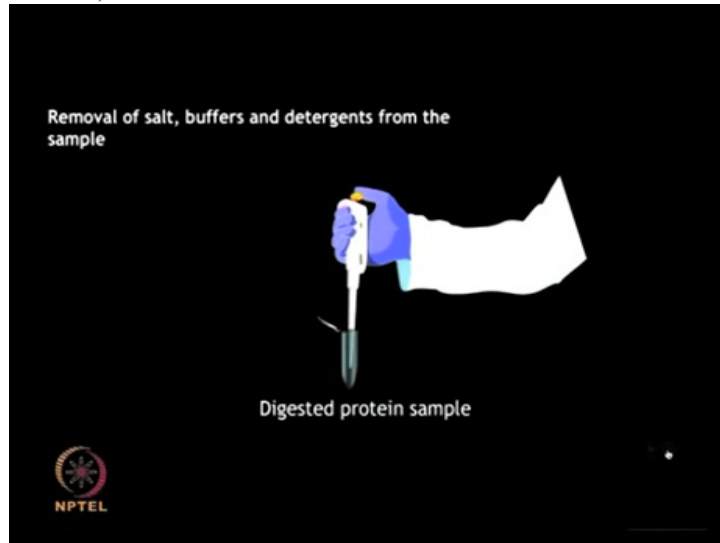


... of manageable size.

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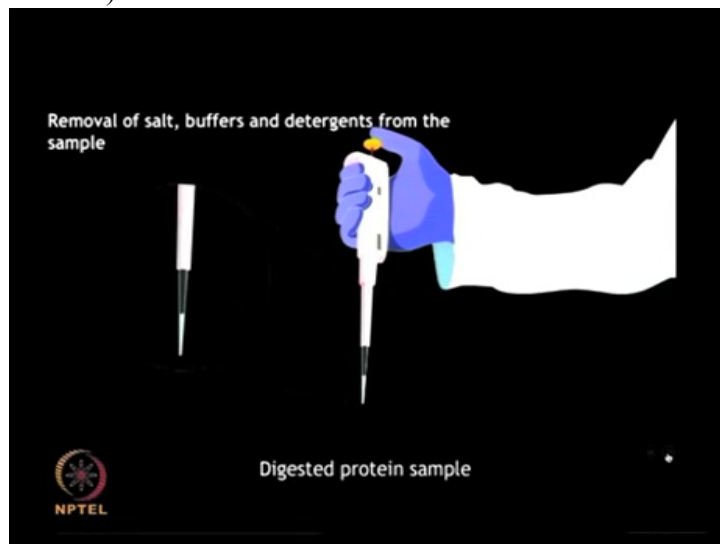


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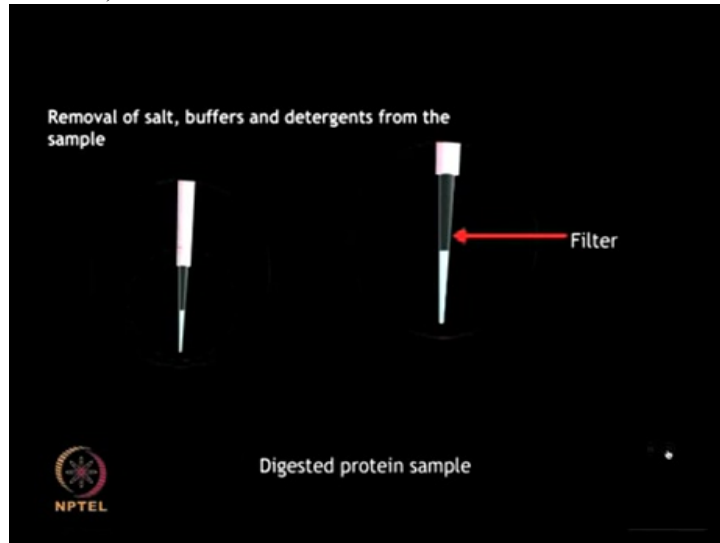
Once the protein sample has been digested, all the salt, buffers and any detergents must be removed from the sample. After doing in-gel digestion...

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...and before proceeding for the mass spectrometry analysis, in between an efficient step is to use some filters...

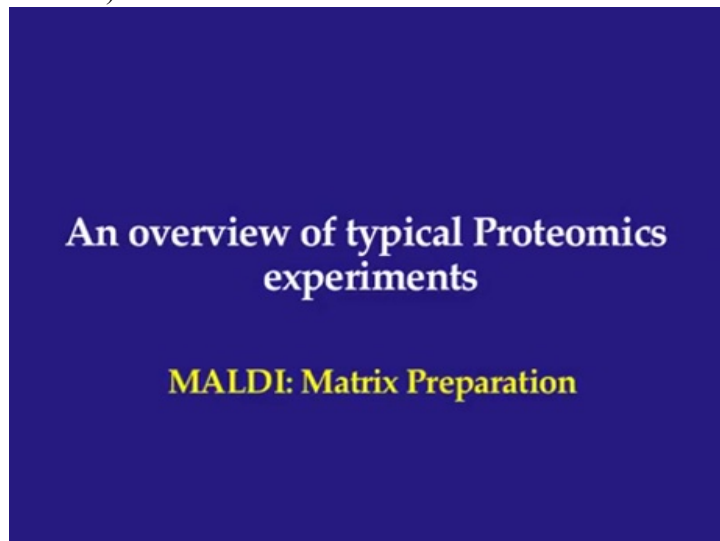
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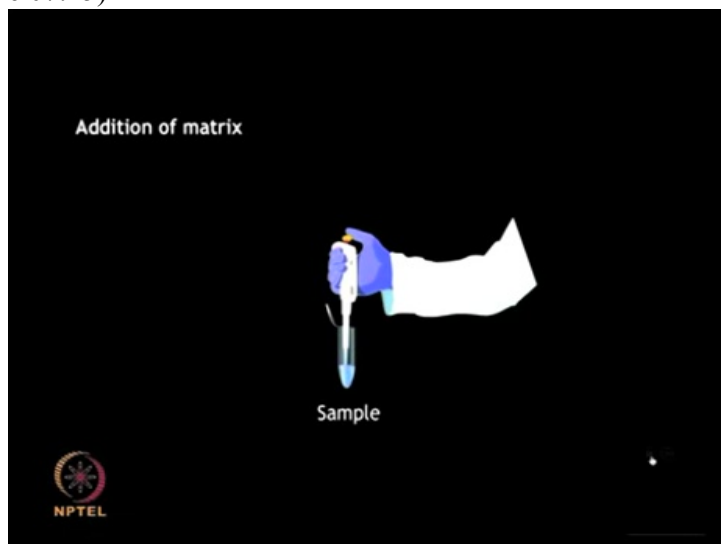
... or ZipTip which can eliminate some of these contaminants and salts. It offers several advantages such as quick verification, sample enrichment and ensures that there is no contamination.

So there are multiple advantages of using ZipTips. However it can purify only limited volume of the sample and also it adsorbs some amount of protein sample thereby leading to losses.

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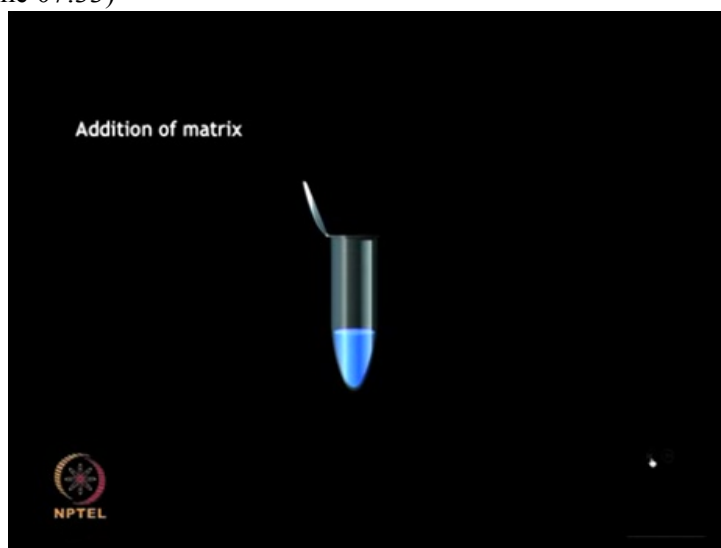


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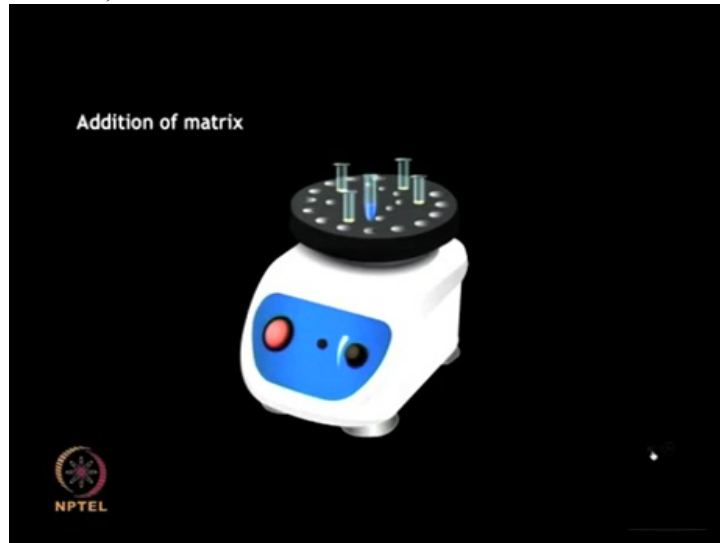
The purified protein sample can be mixed with an aromatic matrix compound such as ...

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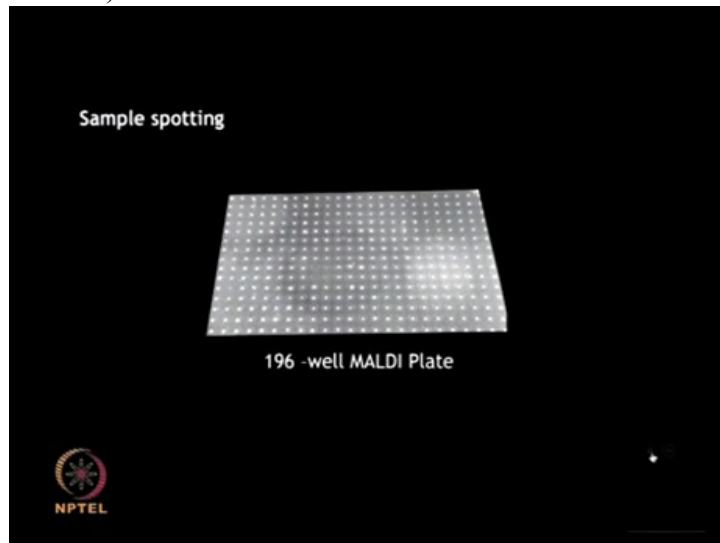
... alpha-cyano-4-Hydroxycinnamic acid or Sinapinic acid in the presence of an organic solvent.

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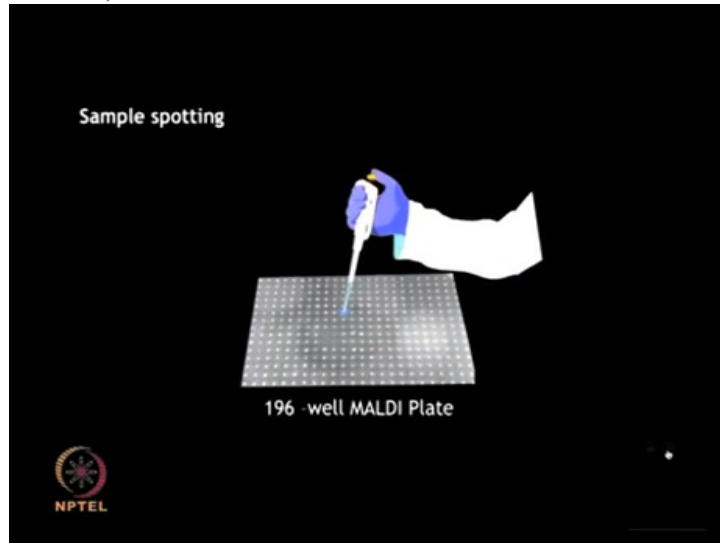
The components are mixed thoroughly.

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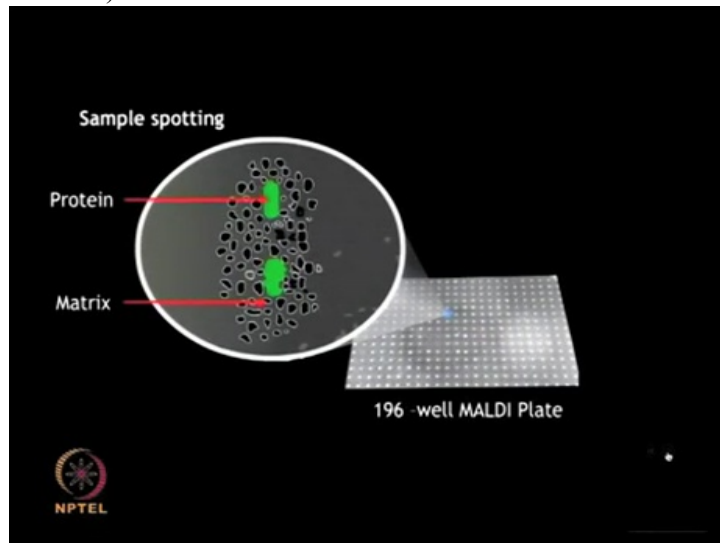
And then the solution containing the organic matrix with the embedded analyte of interest can be spotted on to a metallic MALDI sample plate.

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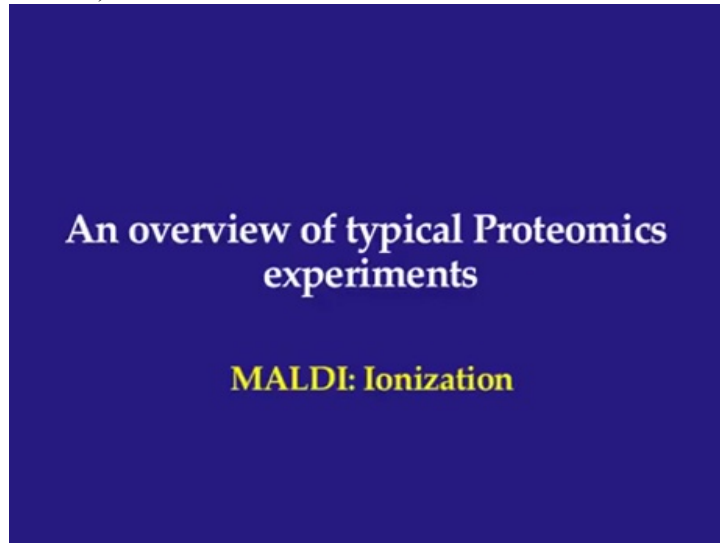
MALDI gives you an opportunity to analyze large number of samples ...

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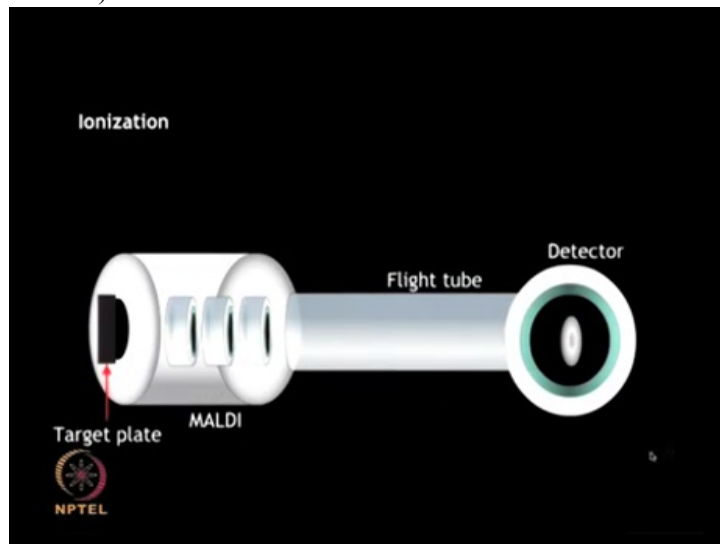


...in a high-throughput fashion.

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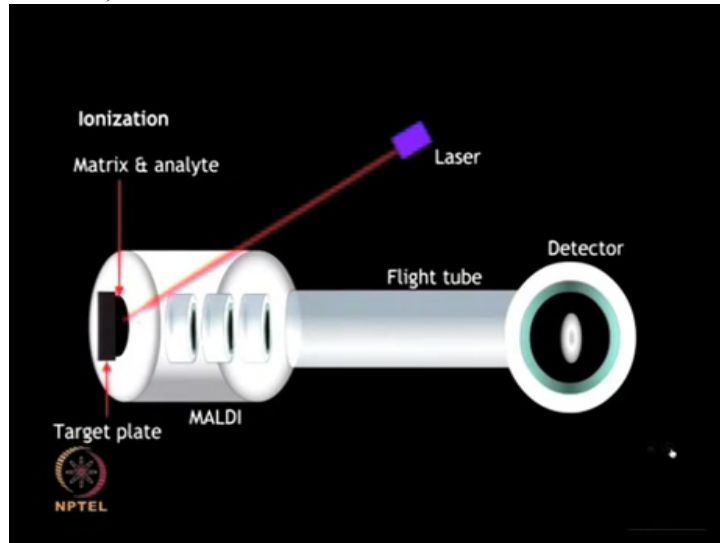


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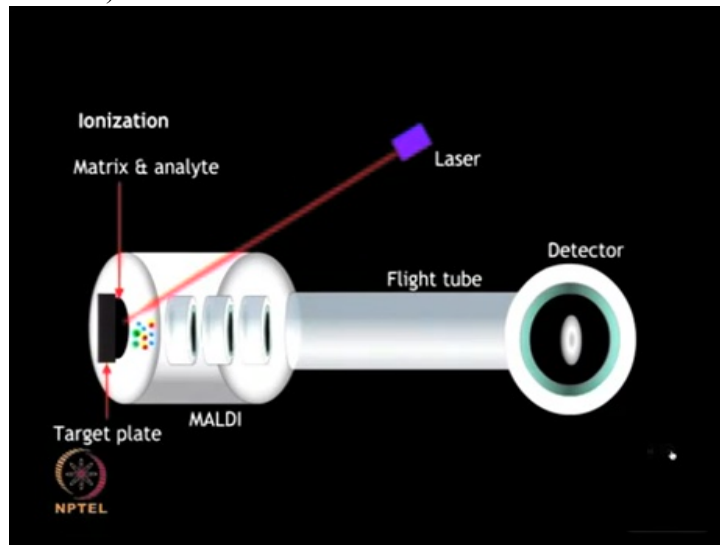
The target plate containing the spotted matrix and analyte can be further placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy ...

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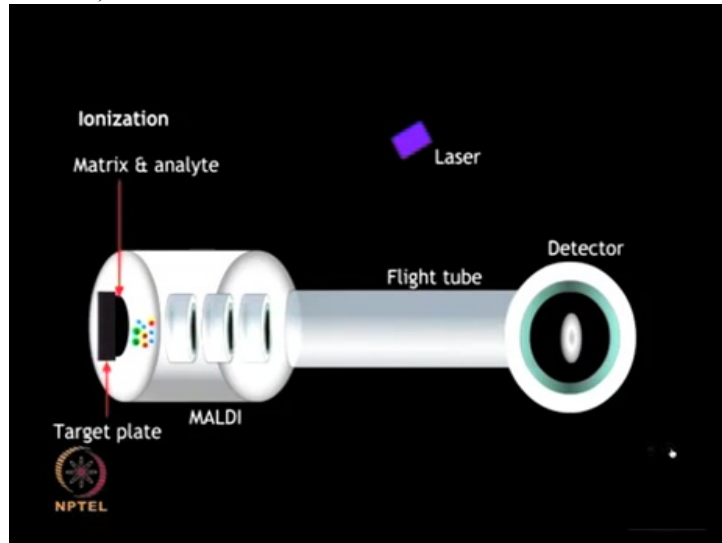
...gets absorbed by the matrix and is transferred to the analyte molecules ...

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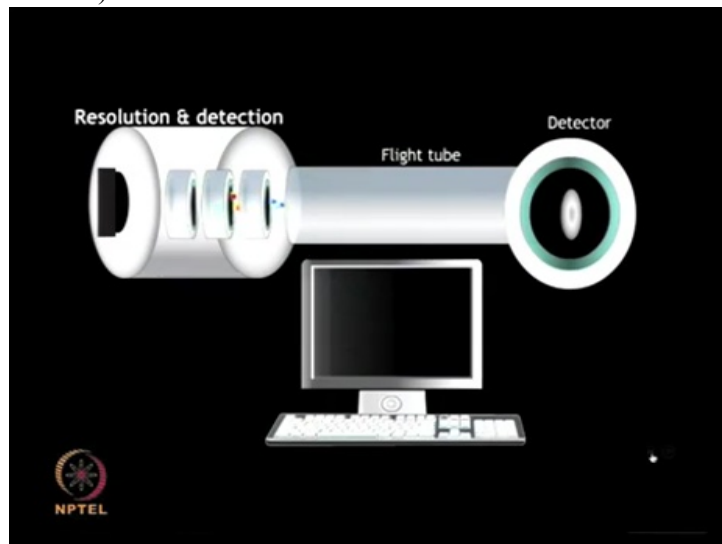


... which undergo rapid sublimation resulting in gas phase ions

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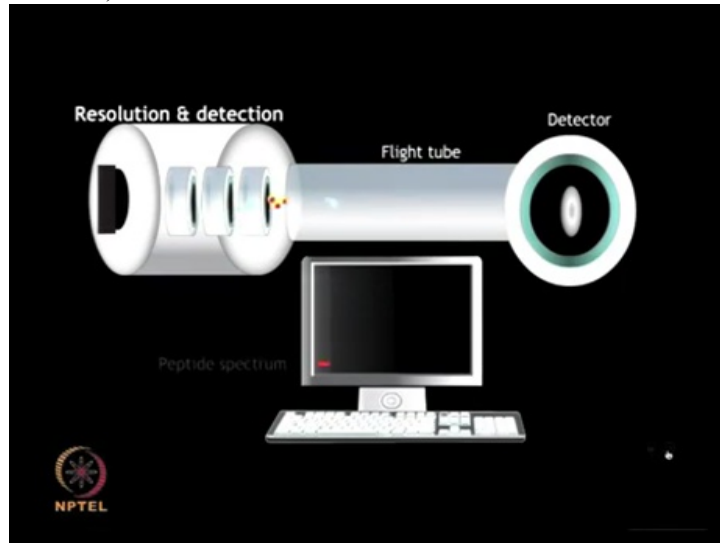


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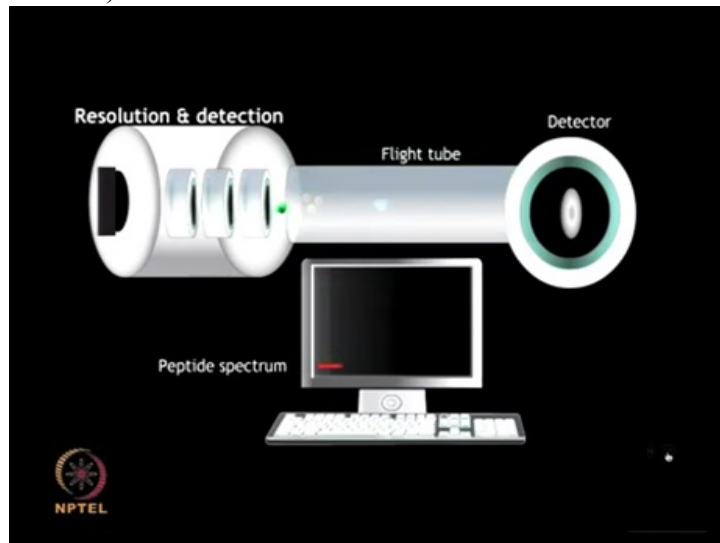
The gas phase ions generated are accelerated and travel through the flight tube at different rates.

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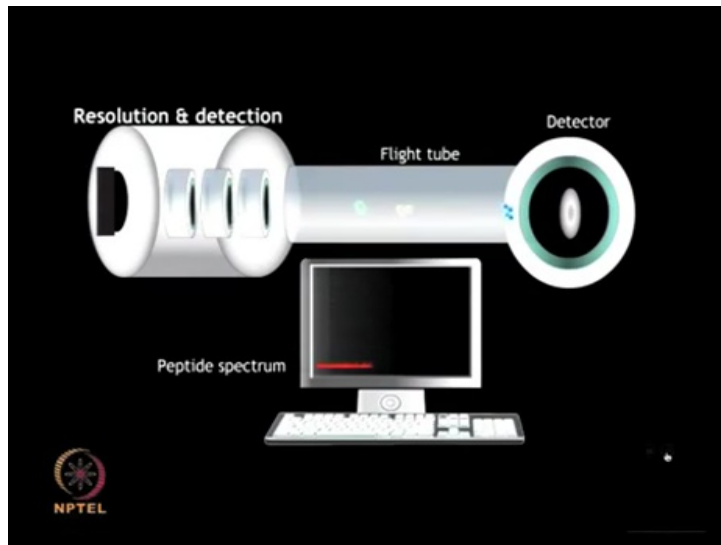
The lighter ion moves rapidly ...

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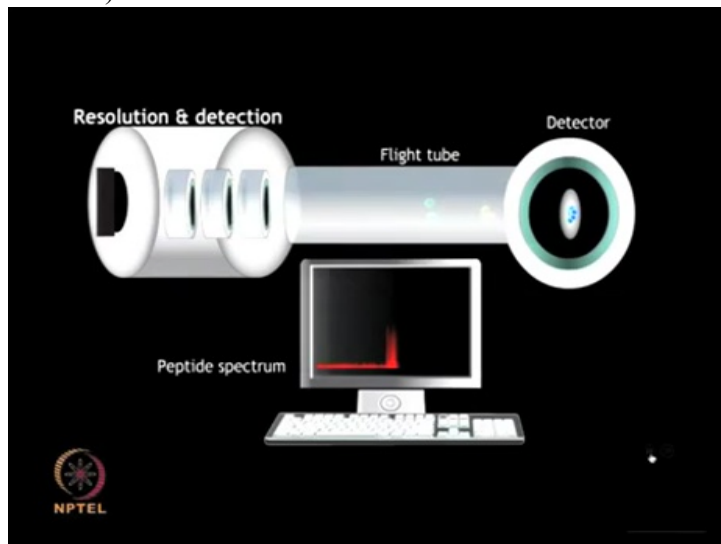
...and reaches the detector first while the heavier ions ...

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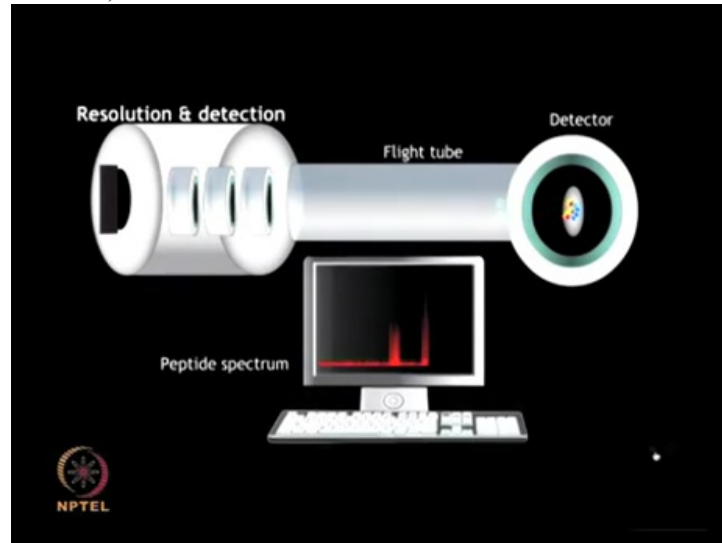
...migrate slowly. These ions are resolved and detected ...

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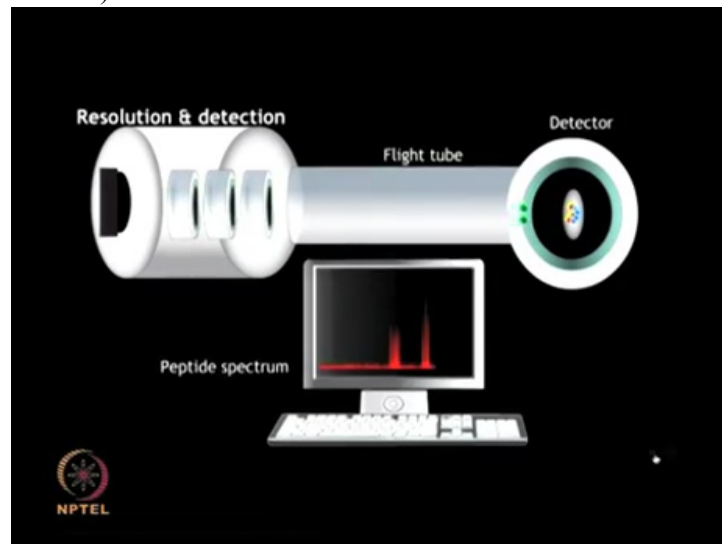


...on the basis of their mass to charge ratio and a mass spectrum is generated.

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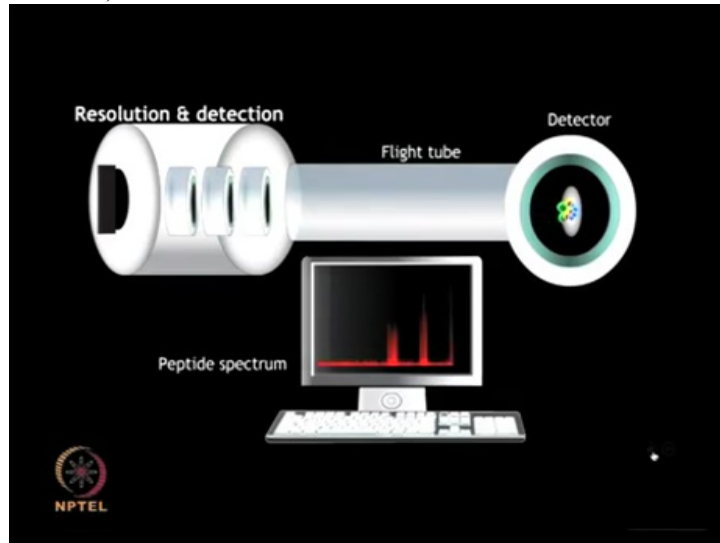


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Parameters such as geometric design....

(Refer Slide Time 09:39)



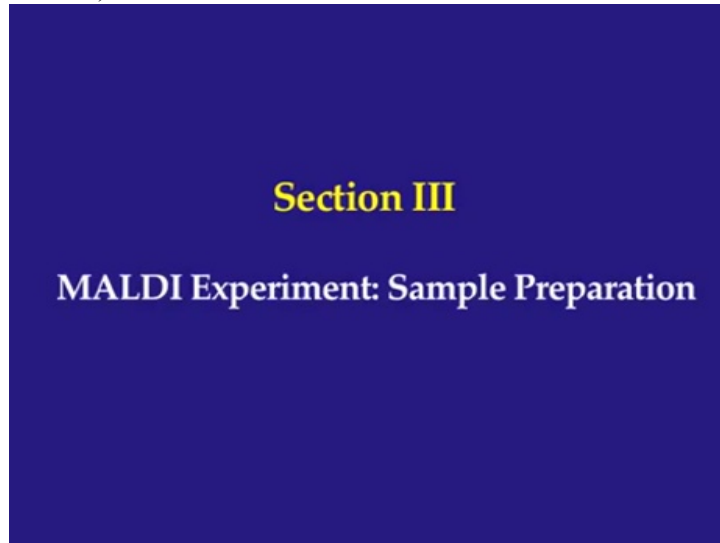
... power supply quality, calibration method, sample morphology, ion beam velocity etc. all of these factors affect the accuracy of mass detection.

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Points to Ponder

- # Spot of interest excised from the gel
- # Protein was digested with trypsin
- # Samples were cleaned with C18 tips
- # Samples were deposited on plate with suitable matrix
- # MALDI-TOF was used for data acquisition

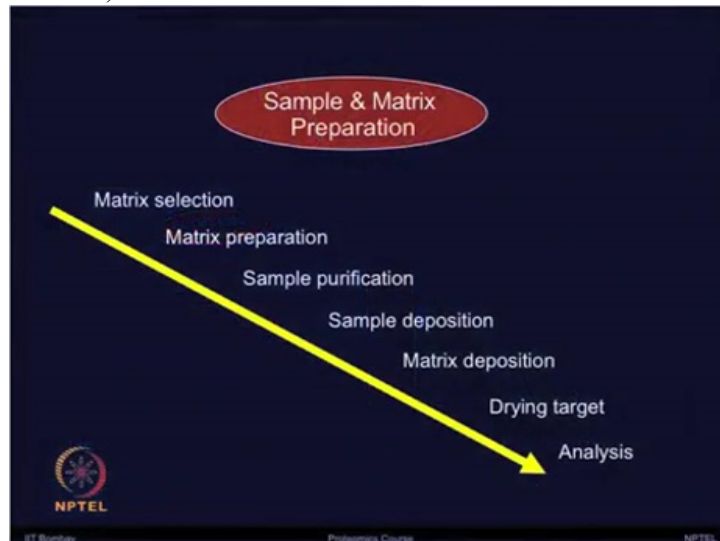
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Section III
MALDI Experiment: Sample Preparation

So now you know how to perform the cleaning step by using ZipTips. Now you have the sample ready and you have selected the matrix. So now let me show you these various steps involved before you can actually start the MALDI experiment

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So, you need to select the matrix, you need to prepare the matrix. You have already done the sample purification.

Now sample need to be deposited on the MALDI plate. Either you can mix it with matrix or you can do this separately. There are various combinations one can try and then once both sample and matrix are deposited on the MALDI target plate then you are ready to do the drying and then plate can be used for MALDI-TOF uh instrument for further analysis.

Let's first talk about matrix selection. So the important step in MALDI-TOF analysis is the selection of appropriate matrix for the sample. The matrix selection mostly depends on the molecular weight of the target to be analyzed and often the type of application which you intend to do by using these instruments.

So these matrices are low molecular weight organic compounds with low vapor pressure and volatile nature. Most of the matrices are acidic in nature so it can easily excite the photon and ionize analyte for the analysis.

However there are few basic matrices are also available.

In the slides I am giving you an overview of few matrices and some of their properties. But there are many more properties which is not mentioned here. But just to give you certain major features of these matrices commonly used for various applications.

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Matrix selection

Peptides less than 5000 daltons, lipids and nucleic acids

N#CC(=O)C=Cc1ccc(O)cc1

α -cyano-4-hydroxycinnamic acid (α -cyano)

Peptides and proteins having higher than 5000 daltons and sometimes also use for lipids

OC(=O)/C=C/c1cc(OC)c(O)c(OC)c1

Sinapinic acid

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So, the one is alpha-cyano-4- hydroxycinnamic acid. When you have peptides less than 5000 Daltons or lipids and nucleic acids one can use this matrix. One can also use Sinapinic acid if peptides and proteins are having more than 5000 Daltons and it can also be used sometimes for the lipids.

Then you have options such as 2-5-dihydroxybenzoic acid also known as DHB ...

(Refer Slide Time 12:56)

Matrix selection

Small molecules and peptides which are not ionized by other matrices	 2,5-Dihydroxybenzoic acid (DHB)
Used for small nucleotides and phosphorylation studies on proteins	 Trihydroxyacetophenone (THAP)
Generally used for nucleotides	 Picolinic acid

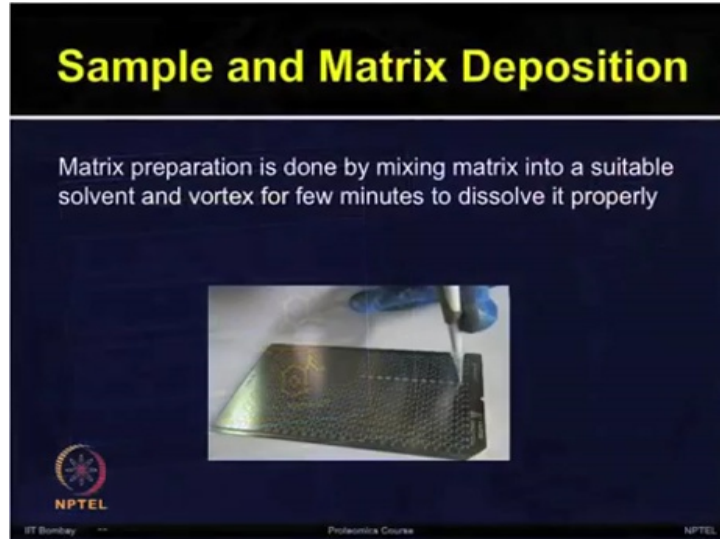
NPTEL Professor's Course NPTEL

...small molecules and peptides which are not ionized by the other molecules can be analyzed by using this matrix. TriHydroxyAcetoPhenone, THAP, this is used for small nucleotides and also used for phosphorylation and specialized applications.

Then we have picolinic acid which is generally used for the nucleotides. So these are only few representative matrices. As you can see there are many options available for selecting the matrix depending on the molecular weight and the type of applications.

But regardless of this, these matrices absorb energy from the laser source and converts both matrix and analyte into the gaseous phase. Matrix can also analyze analyte molecule by providing energy which comes from the laser bombardment.

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Now once you have selected the matrix, matrix can be prepared by mixing it into a suitable solvent and vortex it for few minutes so that it can dissolve properly.

Now you are ready with both, your analyte, the protein which you want to analyze as well as the matrix which we have selected for your application.

Now, one needs to think how to deposit sample on the MALDI target plate. So there are many ways of deposition of sample and matrix onto the MALDI plate.

Mostly sample and matrix are mixed in an eppendorf tube and then mixture is directly deposited by using a micropipette onto the MALDI plate.

But one can also try various combinations. In one approach the sample is first deposited to the MALDI plate followed by the matrices deposited above it and then it is properly mixed before drying process can happen.

Other way of doing it is to apply that with the sandwich-based method. In which a small amount of matrix is deposited on the plate, then you add the protein sample and again the matrix is spotted on top of it so that you have enough matrix in the below and above of the analyte.

So one can try different combinations of placing the matrix and the analyte. And then once you have placed all of this sample of interest on MALDI plate then you are ready to dry the target plate.

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After spotting is done and the MALDI plate is dried almost 30 minutes, then the instrument can be turned on and MS analysis can be performed.

Now there are various types of configurations of these instruments available as well as there are various types of commercial software which help to operate the hardware.

It's not possible to go into individual detail but I am going to show you the generic steps in the following video of MALDI-TOF instrumentation

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**How to perform analysis of
in-gel digested samples using MALDI?**

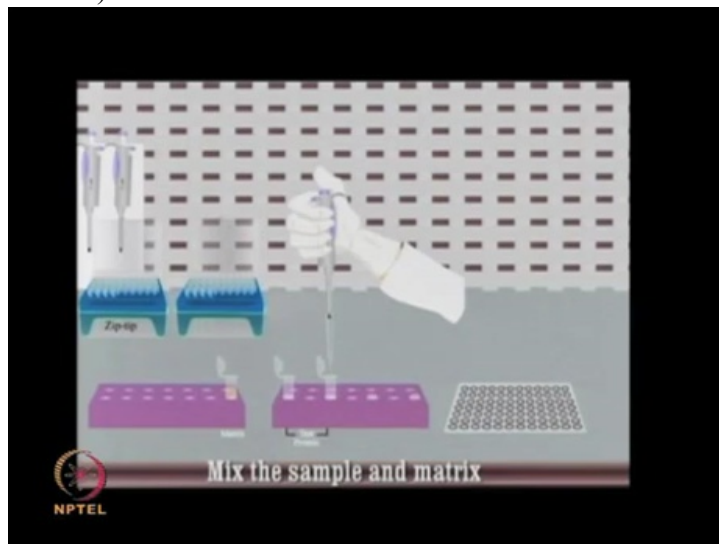
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**MALDI-TOF Instrument
Operation**

(Refer Slide Time 16:33)

After in-gel proteolytic digestion and Zip Tipping, the sample is further subjected to mass spectrometric analysis. Here we are depicting the MALDI-TOF analysis of tryptic digested proteins

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MALDI is performed in two steps. In first step, the compound for the analysis should be dissolved in a solvent containing small organic molecules, known as matrix.

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This mixture is dried before analysis and liquid solvent used in the preparation of the solution is removed.

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So in this video by depicting the matrix preparation as well as instrumentation I will try to give you the overview of MALDI-TOF instrumentation.

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Spot the mixture on the MALDI plate. How uniformly you can plate these mixtures on the MALDI plate ensures your good spectra and data quality later on

Completed on the MALDI plate

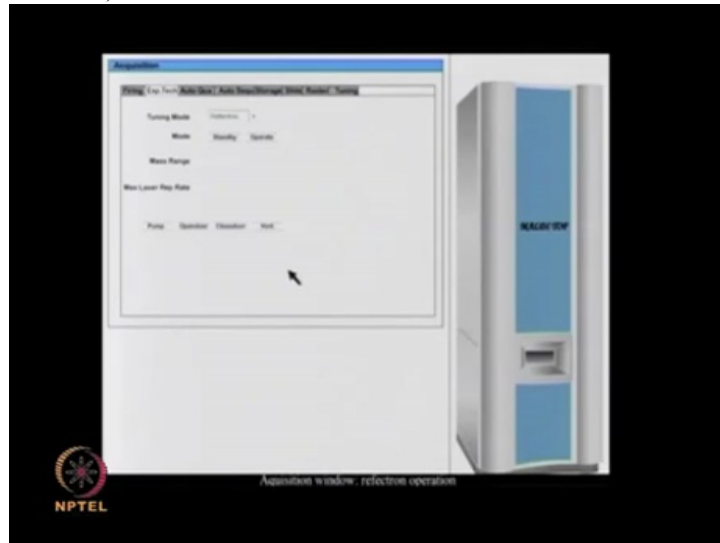
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the samples are allowed to dry for 30 minutes, after which the instrument is switched on and the MS analysis can be performed.

While these steps are happening you need to ensure

(Refer Slide Time 18:19)



... that the instrument is on.

(Refer Slide Time 18:21)



So click on the software and ...

(Refer Slide Time 18:24)



...open the acquisition window and then click on “open door”.

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Insert MALDI target plate ...

(Refer Slide Time 18:32)



...face up with the cut-off corner to the front.

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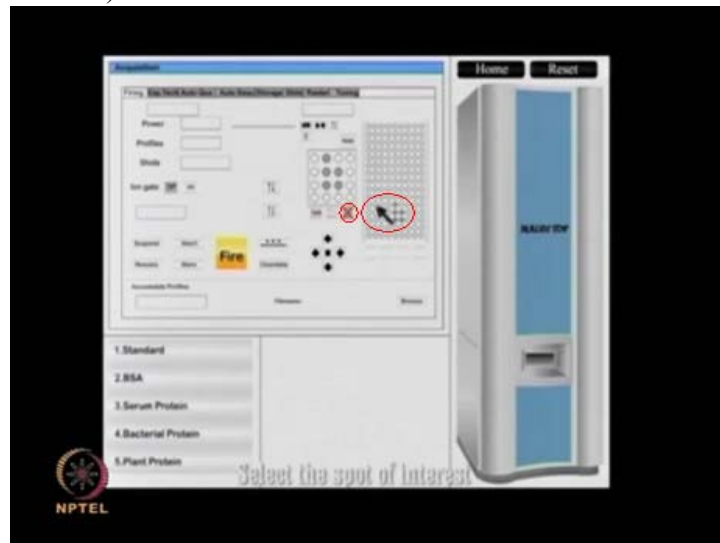
And now by using the software, close the door.

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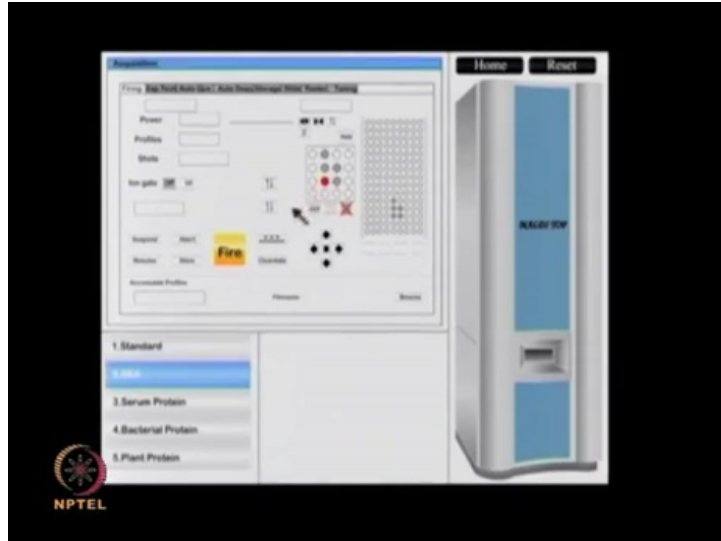
The door of the insertion chamber is now closed.

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You can select the plate; You can view the overall plate on the screen and then select a spot which you want to analyze. So click on the yellow target in the acquisition window and ...

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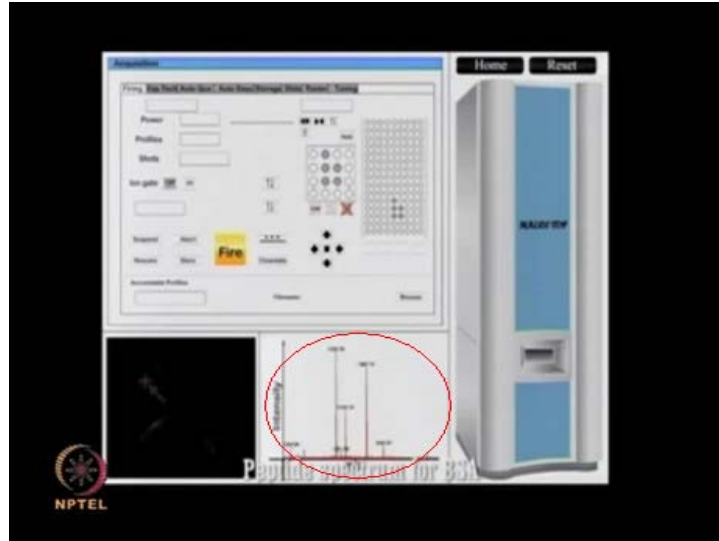
select “go to the location”.

(Refer Slide Time 19:11)



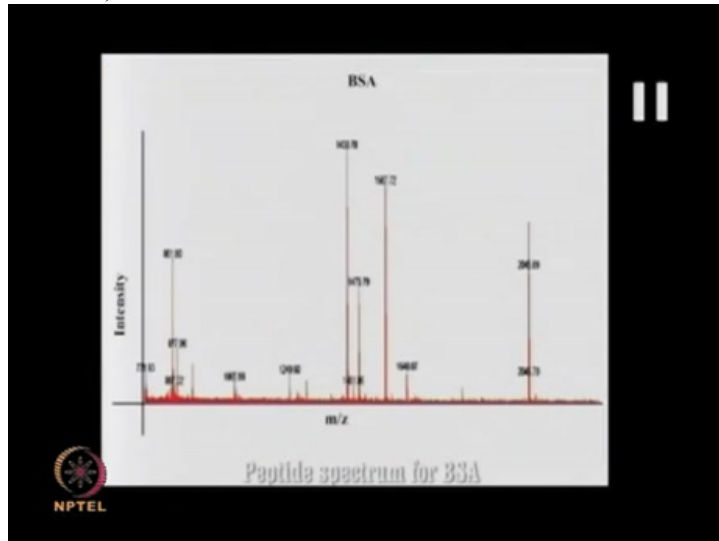
You can now do the laser bombing and

(Refer Slide Time 19:15)



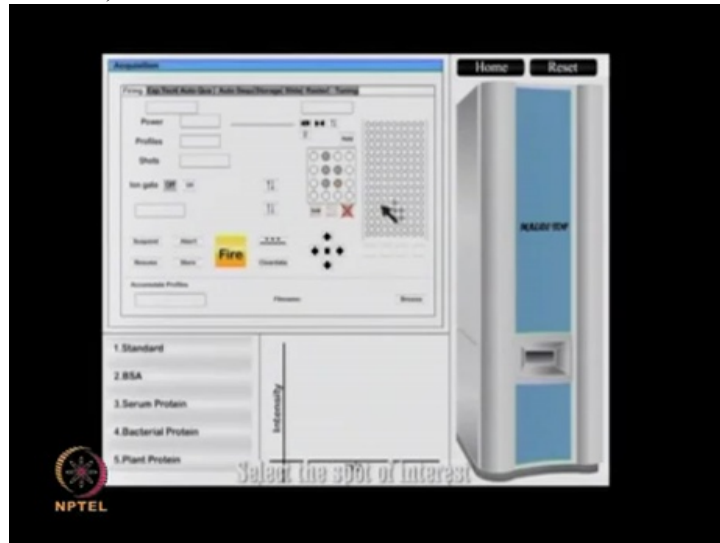
... peptide spectrum is generated.

(Refer Slide Time 19:18)

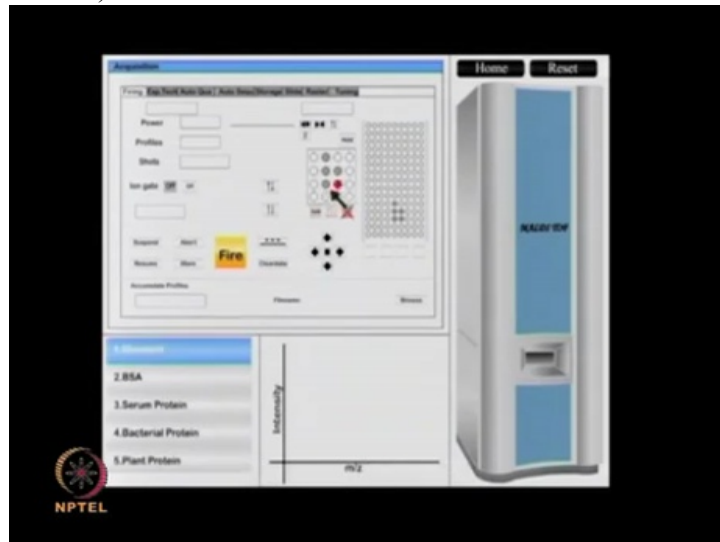


We have shown here one standard protein, bovine serum albumin. So you have to look at various locations where you can get best spectra from that spot and then you can freeze it.

(Refer Slide Time 19:38)

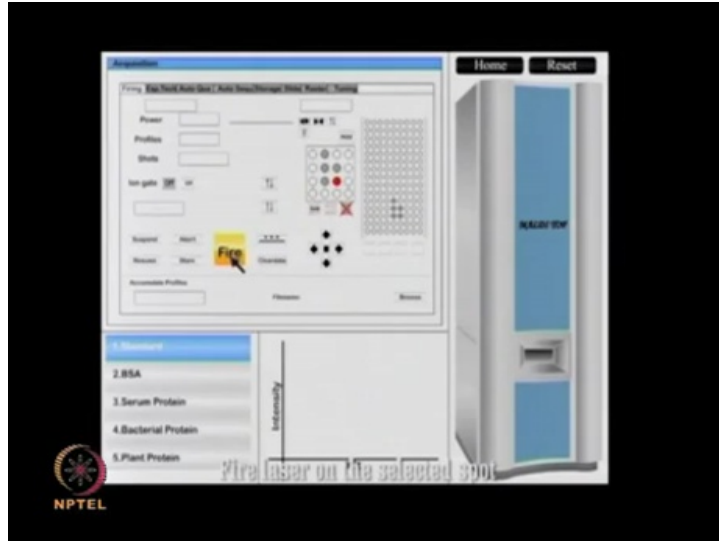


(Refer Slide Time 19:40)



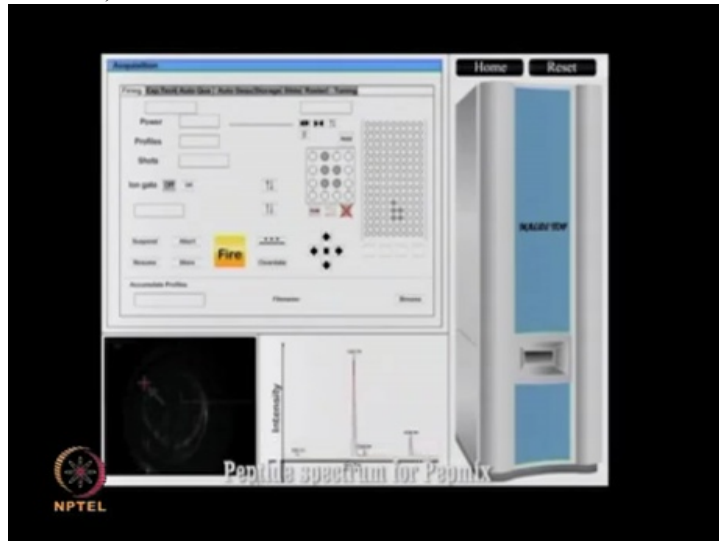
Same process can be performed for ...

(Refer Slide Time 19:42)



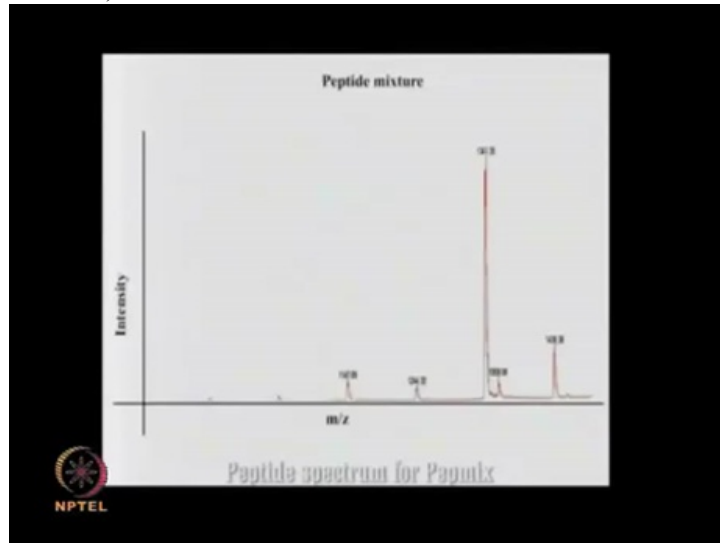
....different spots and different regions

(Refer Slide Time 19:46)



Now we have shown here ...

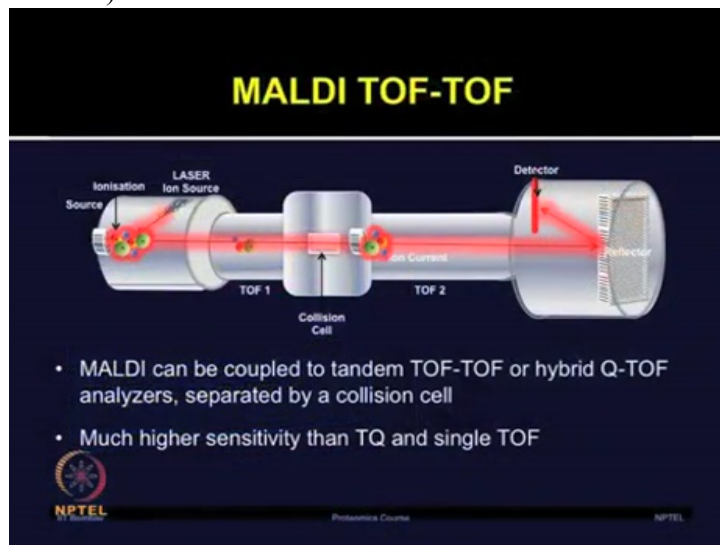
(Refer Slide Time 19:51)



...a spectra for the Pepmix

So now you are clear with how to perform the MALDI TOF experiment. Now let us add one more mass analyzer. So now we have a configuration of MALDI TOF-TOF.

(Refer Slide Time 20:05)



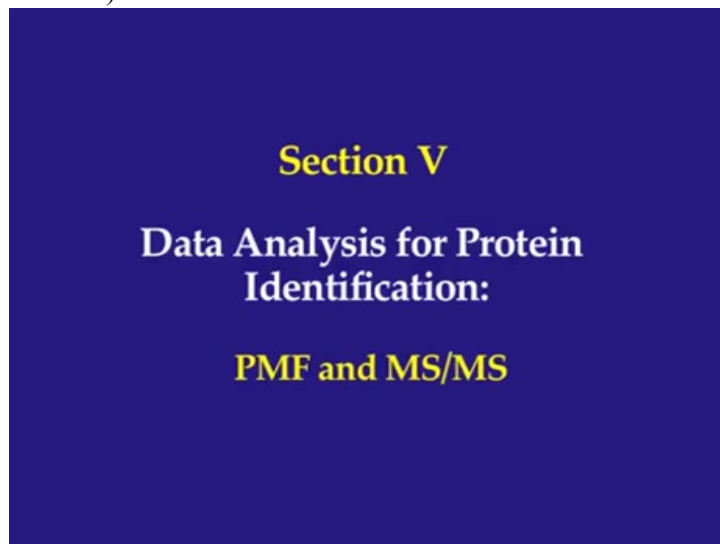
So MALDI can be coupled to the tandem Time of Flight in combination with another Time of Flight, so TOF-TOF or with hybrid Quadrupole Time of Flight analyzers which are separated by the collision cells.

Now for proteomic application, it is recommended to use the TOF-TOF or Q-TOF. The peptide ions are accelerated through the first Time of Flight tube as you can see in this slide and then they are dissociated by introducing an inert gas into the Collision cell.

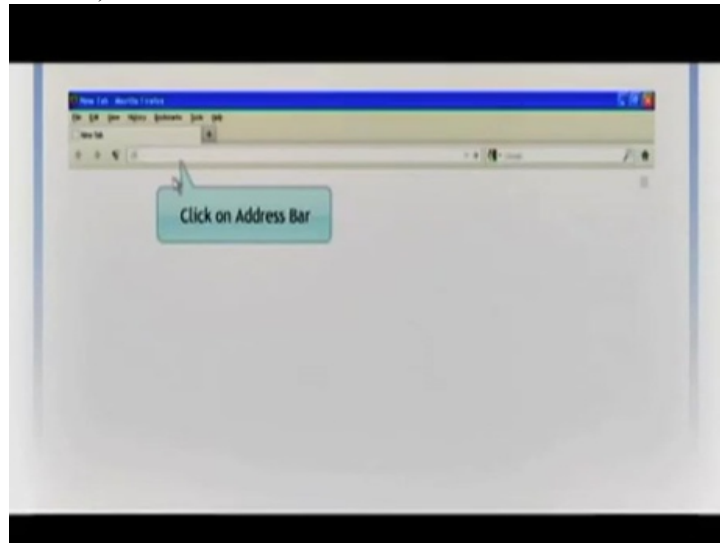
This process allows collision induced dissociation spectra from the MALDI produced from the precursor ions. Now these hybrid configurations are more sensitive than the triple quad and the single Time of Flight.

So the combination of TOF-TOF allows the protein identification through the peptide mass fingerprinting and high throughput analysis of the protein or proteome is possible with the hybrid TOF analyzers.

(Refer Slide Time 21:23)

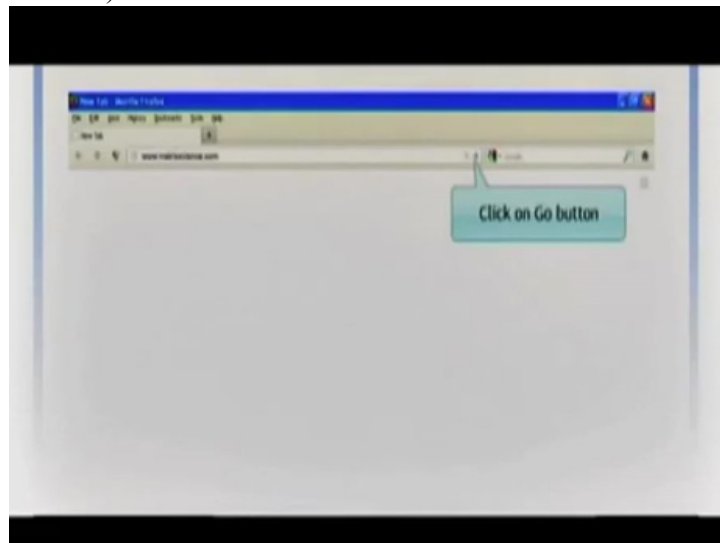


(Refer Slide Time 21:27)

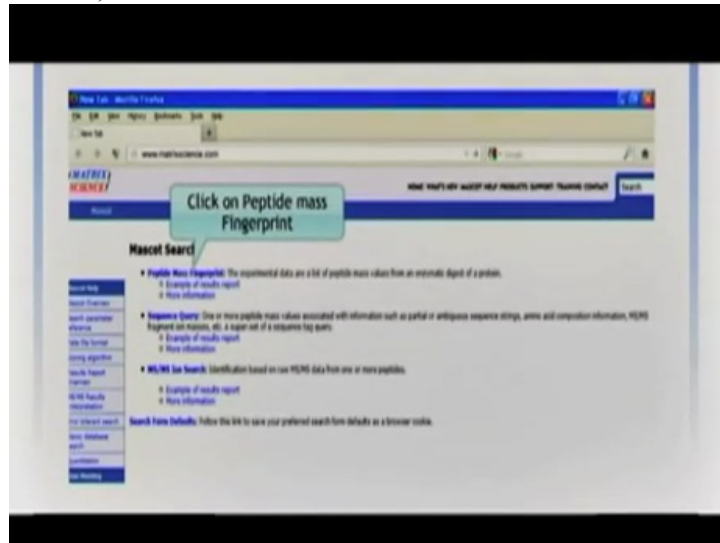


Open the Matrix Science browser window to carry out online data analysis.

(Refer Slide Time 21:37)

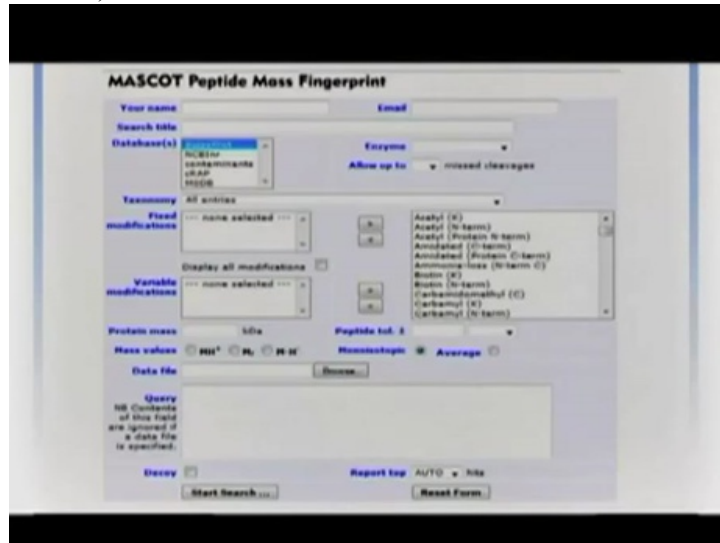


(Refer Slide Time 21:39)



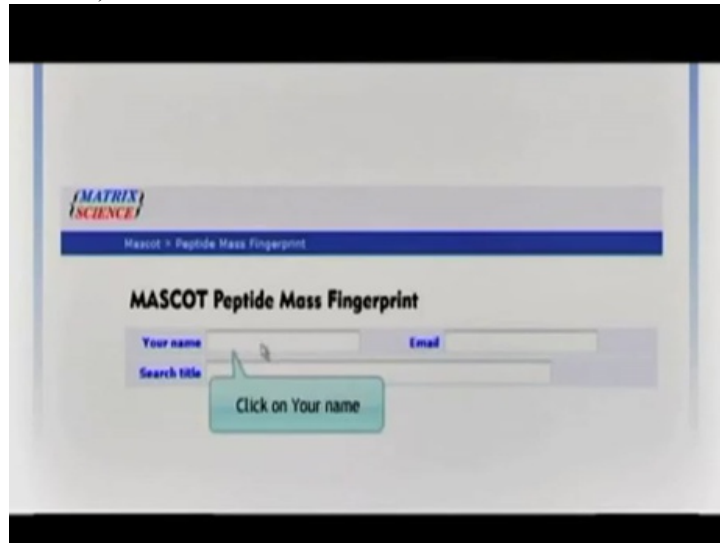
For peptide analysis, click on peptide mass fingerprinting.

(Refer Slide Time 21:45)



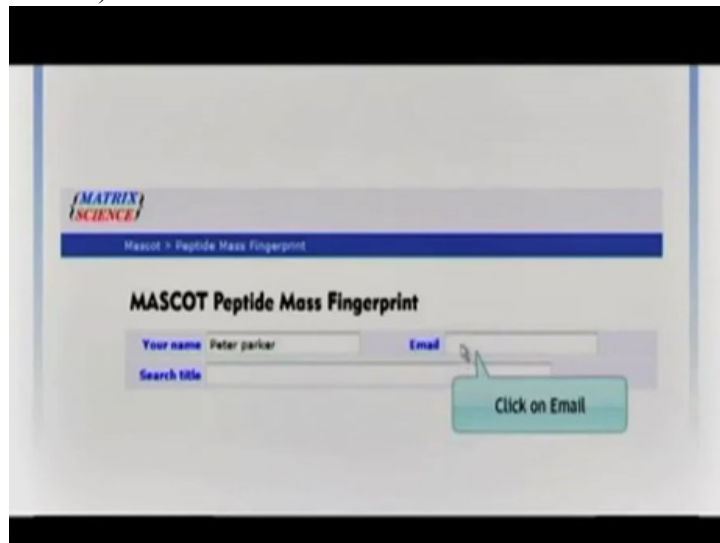
Mascot peptide mass fingerprint. Please enter the ...

(Refer Slide Time 21:50)



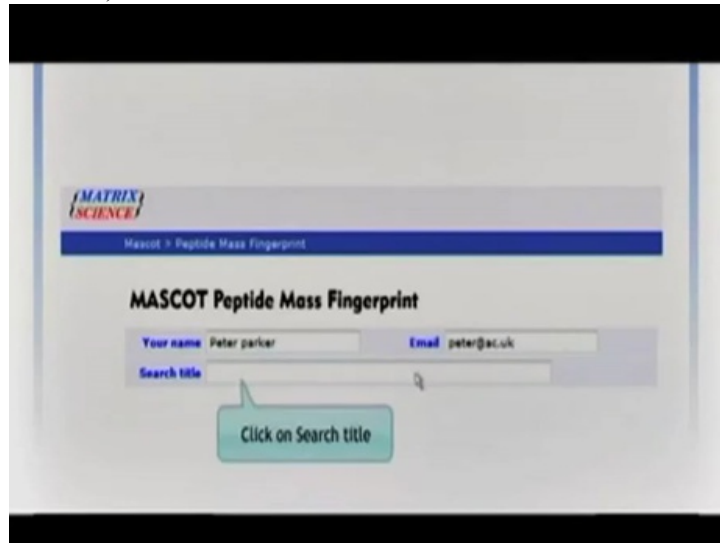
... User Id and details to acquire ...

(Refer Slide Time 21:52)



... information in case of ...

(Refer Slide Time 21:54)



... any network loss

(Refer Slide Time 21:56)



(Refer Slide Time 21:56)

MASCOT Peptide Mass Fingerprint

Your name: Peter Zeller Email: peter@zsl.ch

Search title: Serum protein from human sample

Database(s): Swiss Prot, NCBI, contaminants, UniProt, RefSeq

Enzyme: Trypsin

Allow up to: missed cleavages

Taxonomy: All entries

Fixed modifications: none selected

Variable modifications: none selected

Protein mass: kDa

Peptide tol.: kDa

Mass values: MH+ M MH-

Monoisotopic: Average

Data file: [Empty]

Query: [Empty]

Decoy:

Report top: A1/T0 = kDa

Start Search [button] Reset Form [button]

The following parameters should be selected.

(Refer Slide Time 22:03)

MASCOT Peptide Mass Fingerprint

Your name: Peter Zeller Email: peter@zsl.ch

Search title: Serum protein from human sample

Database(s): Swiss Prot, NCBI, contaminants, UniProt, RefSeq

Enzyme: Trypsin

Allow up to: missed cleavages

Taxonomy: All entries

Fixed modifications: none selected

Variable modifications: none selected

Protein mass: kDa

Peptide tol.: kDa

Mass values: MH+ M MH-

Monoisotopic: Average

Data file: [Empty]

Query: [Empty]

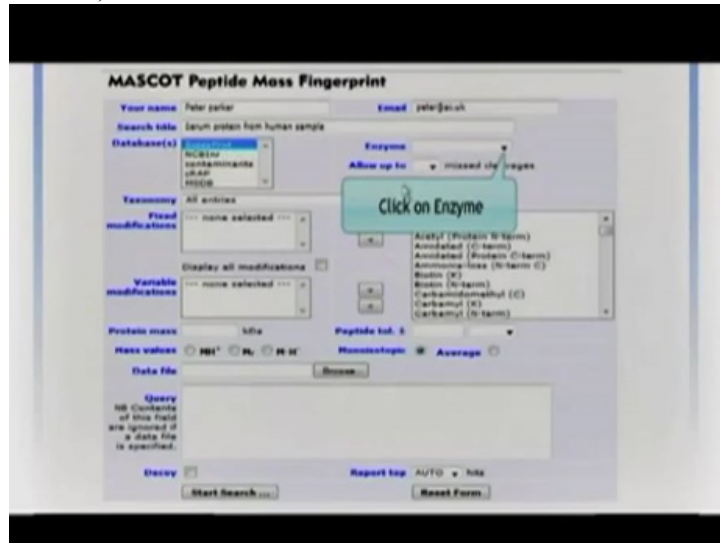
Decoy:

Report top: A1/T0 = kDa

Start Search [button] Reset Form [button]

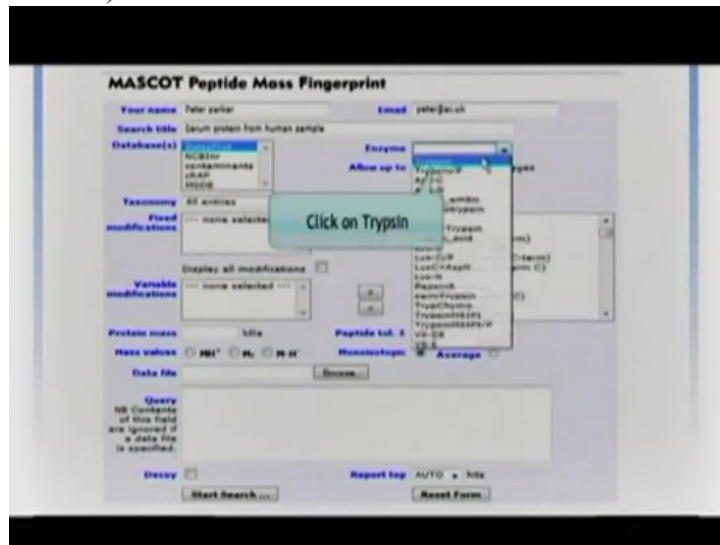
Database primary databases include SwissProt and NCBI. Select SwissProt database for data analysis.

(Refer Slide Time 22:12)



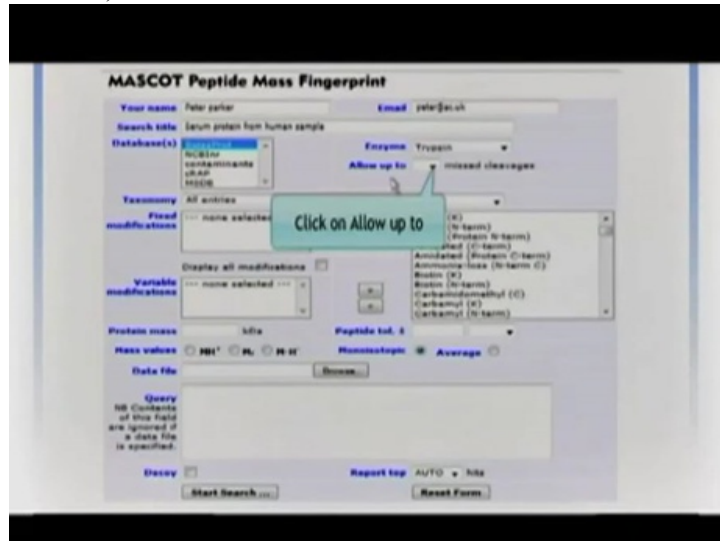
Enzyme, select enzyme ...

(Refer Slide Time 22:17)



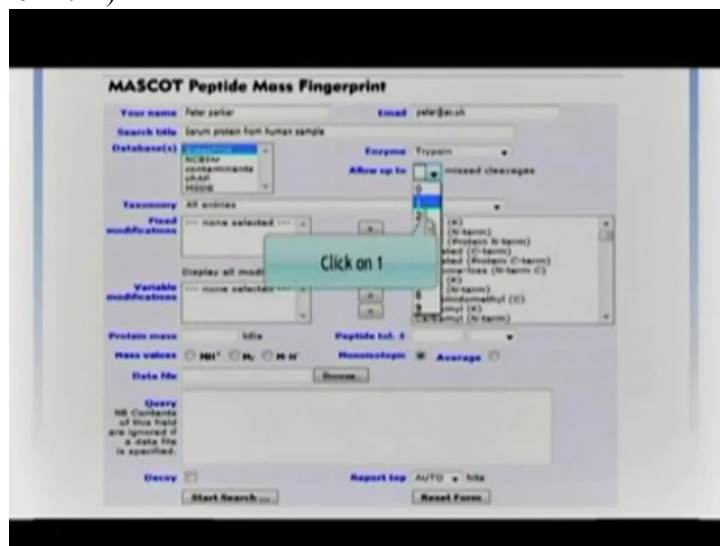
... as Trypsin.

(Refer Slide Time 22:18)

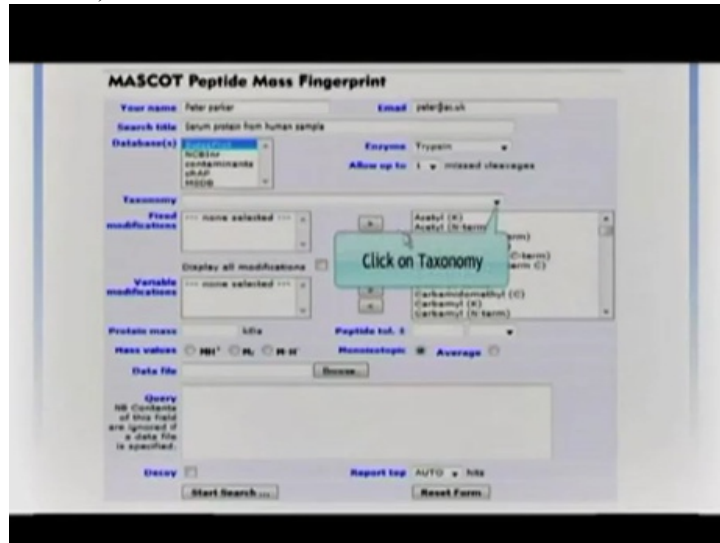


Missed Cleavages, missed cleavages are allowed up to 1

(Refer Slide Time 22:24)

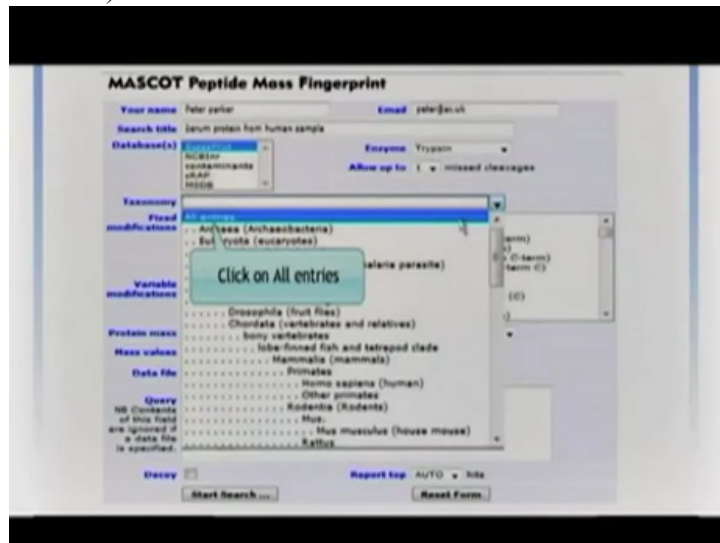


(Refer Slide Time 22:25)



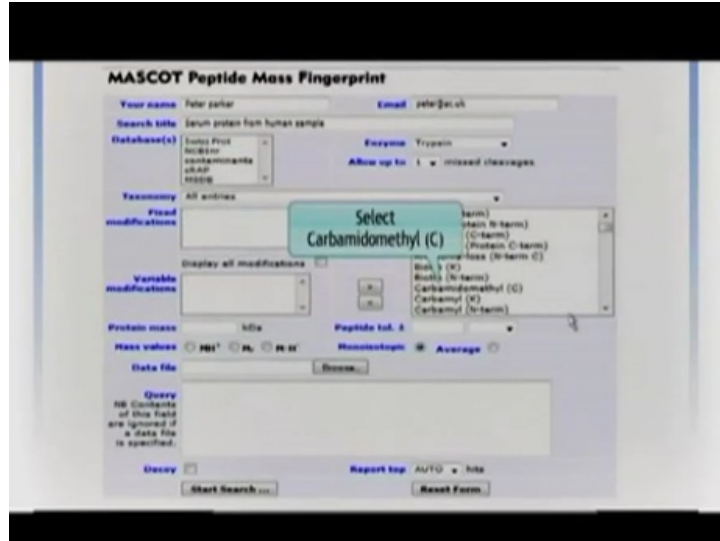
Next is taxonomy. The protein extracted from the biological specimen has to be assigned ...

(Refer Slide Time 22:30)



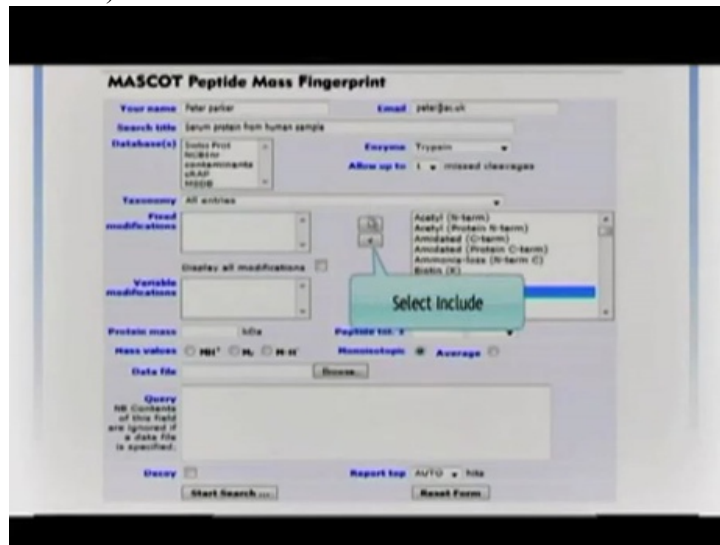
... to a particular species or a group of species to which the sample belongs. When you are not sure of organism select all entries.

(Refer Slide Time 22:41)



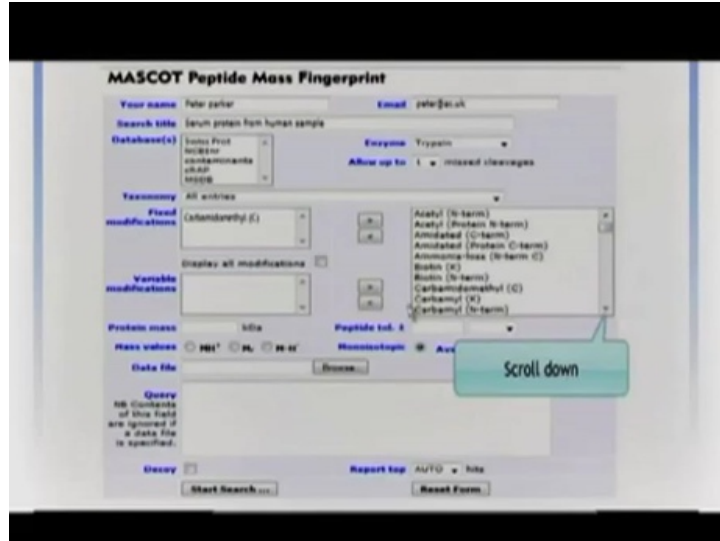
Fixed modification, select carbamidomethyl, fixed modifications are applied ...

(Refer Slide Time 22:48)



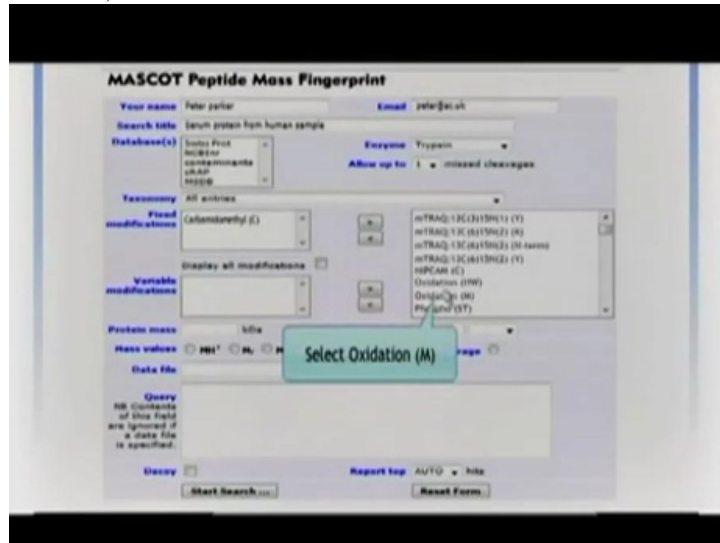
... collectively across the database to account for change in mass of specific residue

(Refer Slide Time 22:55)



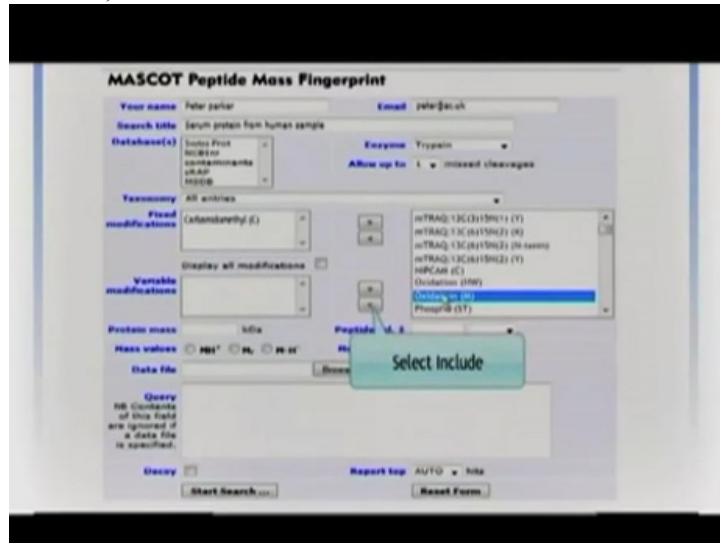
Now scroll down and select oxidation.

(Refer Slide Time 23:01)



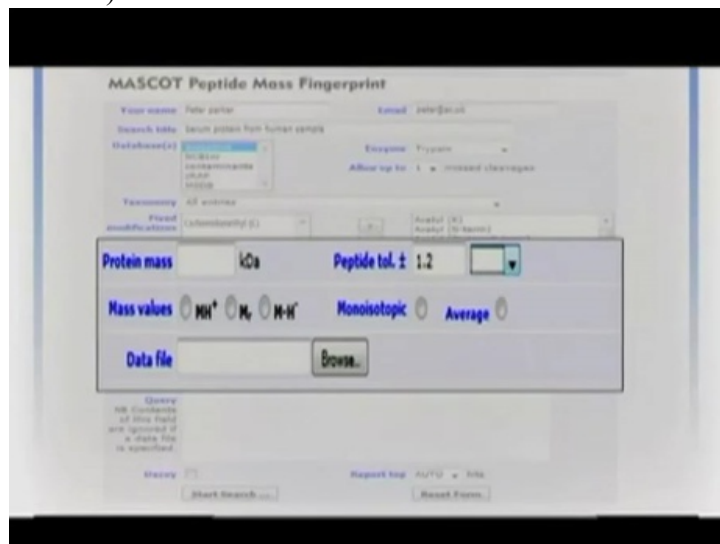
These are mass changes suspected ...

(Refer Slide Time 23:05)



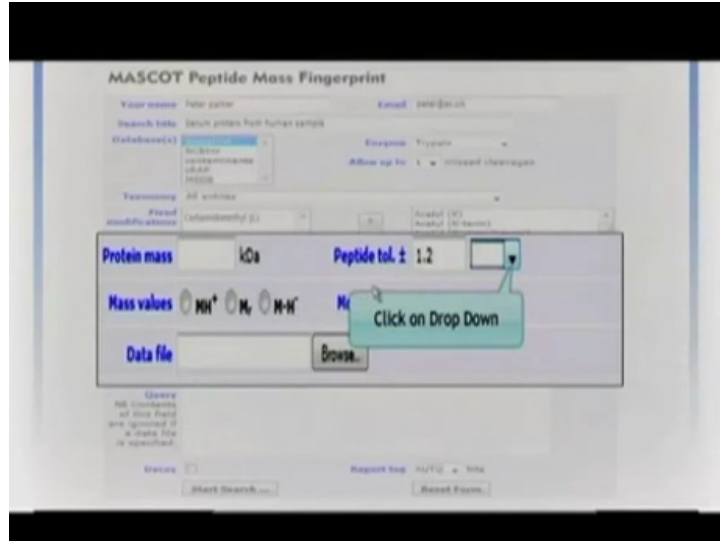
...to occur during sample handling and accounted for by increasing the number of primary sequences compared against experimental masses. Include it as variable modification.

(Refer Slide Time 23:19)



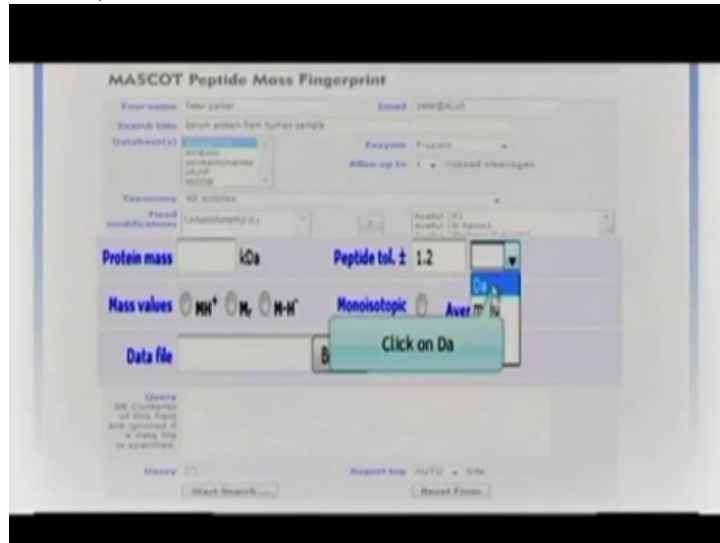
Depending upon user needs the parameters can be changed. Protein mass is the mass in that protein and is optional.

(Refer Slide Time 23:26)



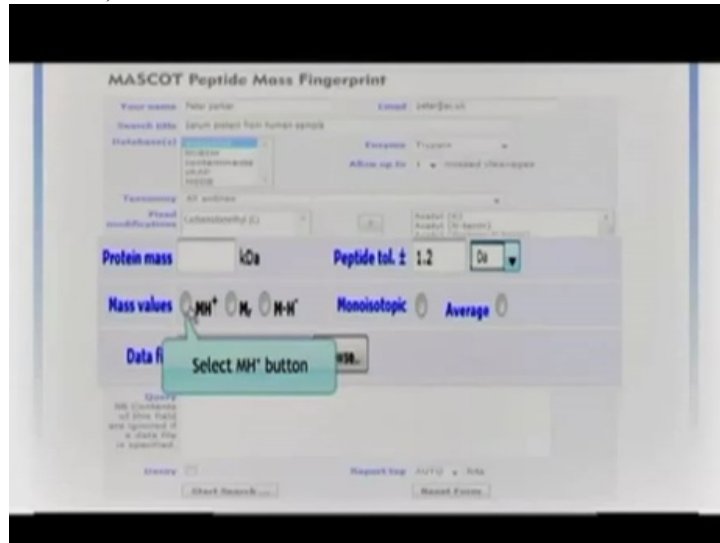
Set peptide tolerance as...

(Refer Slide Time 23:29)



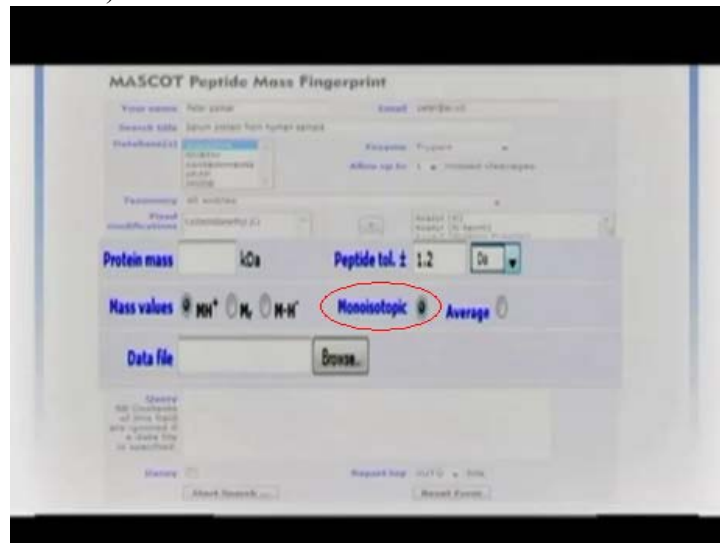
... +/- 1.2 Daltons. Mass values it specifies the type of charge to be examined.

(Refer Slide Time 23:38)



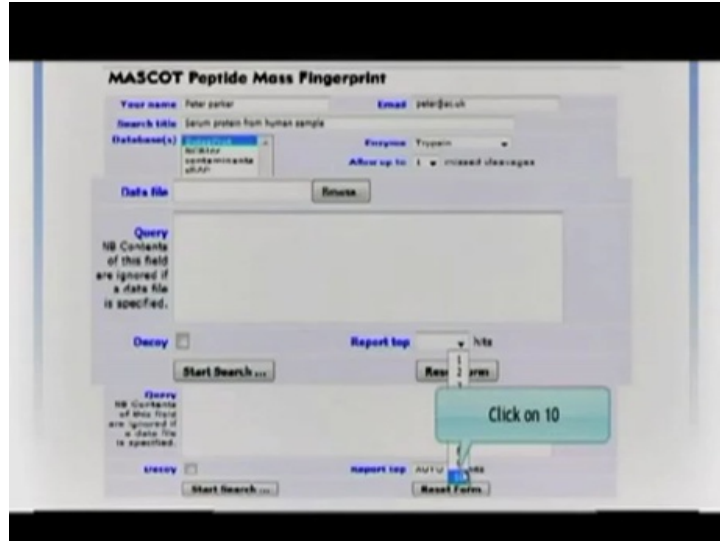
Select MH positive.

(Refer Slide Time 23:42)



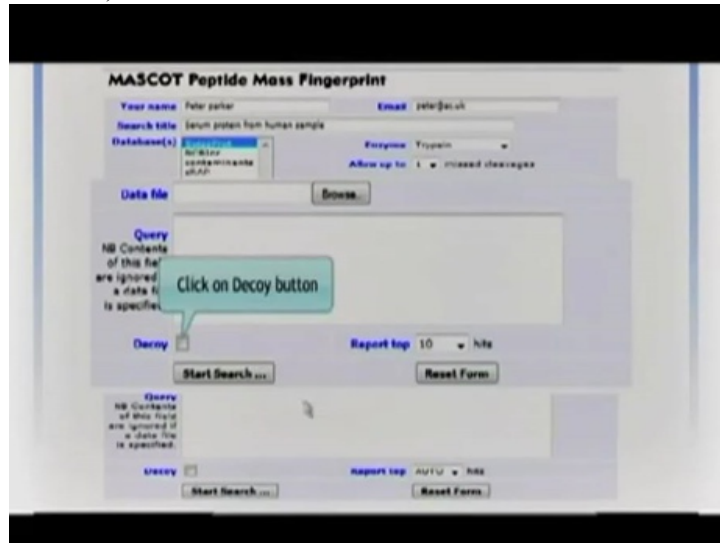
Select mono-isotopic.

(Refer Slide Time 23:45)



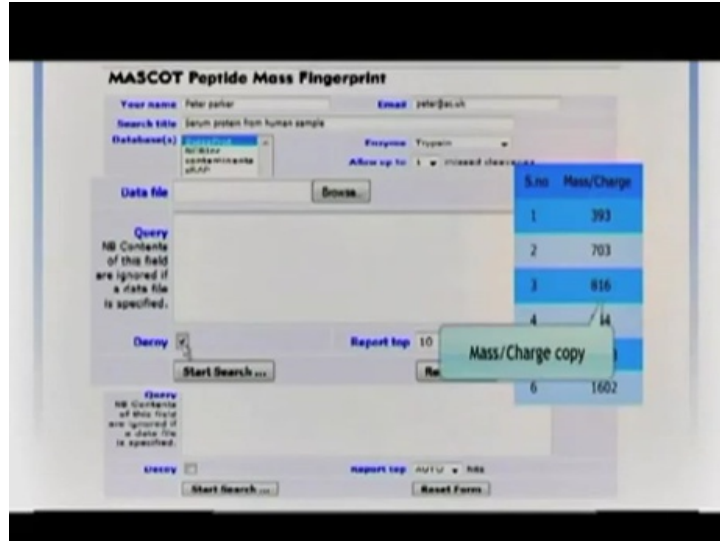
Report top 10 hits.

(Refer Slide Time 23:48)



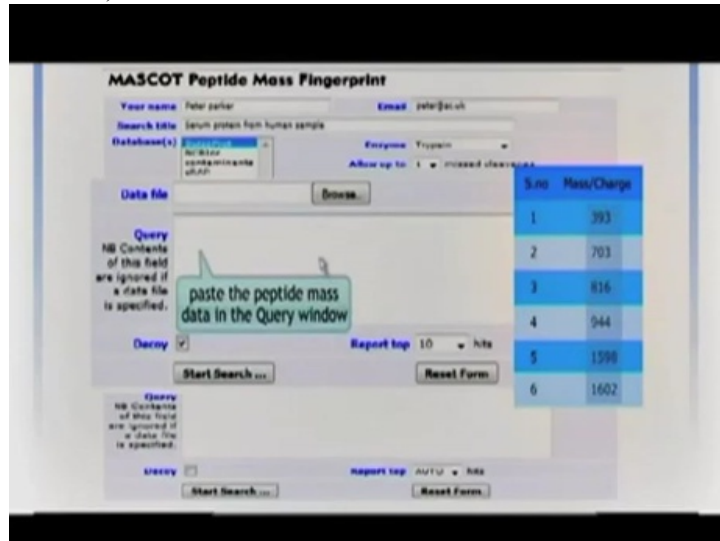
Select Decoy for statistical analysis

(Refer Slide Time 23:51)



Copy the m by z value ...

(Refer Slide Time 23:56)



... and paste in the selected box for Mascot search.

(Refer Slide Time 23:59)

S.no	Mass/Charge
1	793
2	703
3	816
4	944
5	1598
6	1602

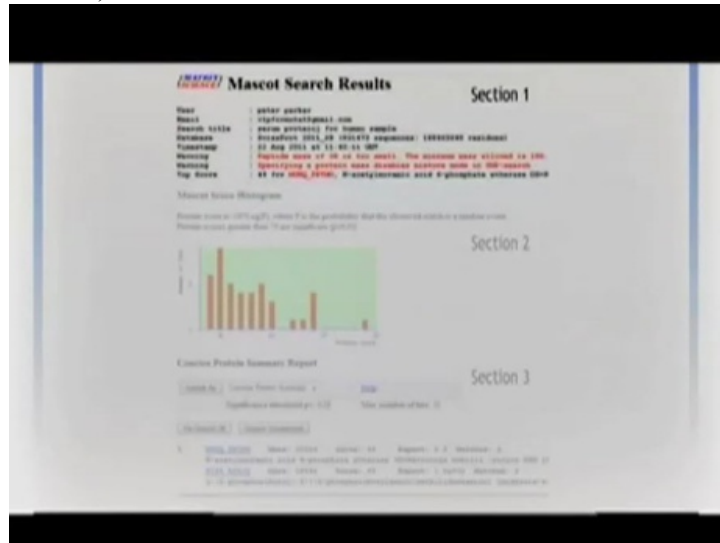
Click on search.

(Refer Slide Time 24:02)



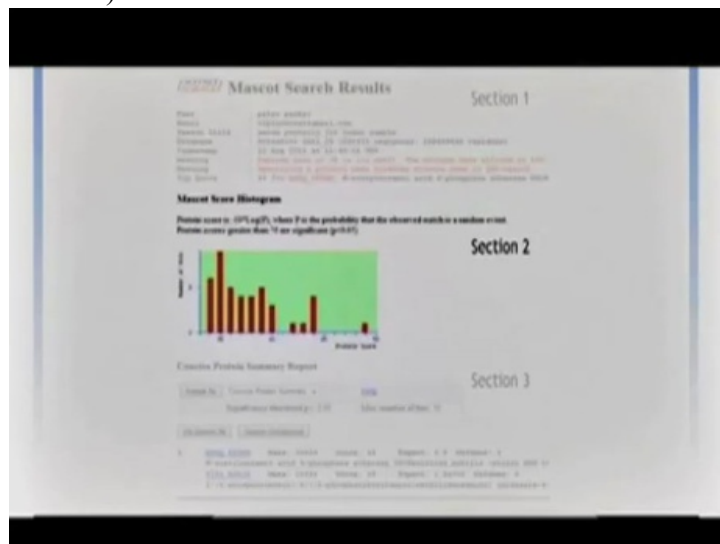
Data output

(Refer Slide Time 24:06)



The output can be seen in 3 sections. In section 1 the summary of spec parameters defined by user

(Refer Slide Time 24:14)



Section 2 Mascot's co-histogram the number of proteins with score is plotted along the graph

(Refer Slide Time 24:24)

The screenshot displays the Mascot Search Results interface, divided into three sections:

- Section 1:** Search Parameters. It lists search criteria such as 'Search Method: Mascot', 'Database: SwissProt', 'Enzyme: Trypsin', and 'Modification: Oxidation (M)'. It also shows the search date and time.
- Section 2:** Mass Score Histogram. A bar chart showing the distribution of mass scores for the search results.
- Section 3:** Concise Protein Summary Report. A table displaying search results with columns for 'Rank', 'Accession', 'Name', 'Score', 'E-Value', and 'Molecular Weight'. The top result is 'H19L_A0123' with a score of 10000 and an E-value of 1e-10.

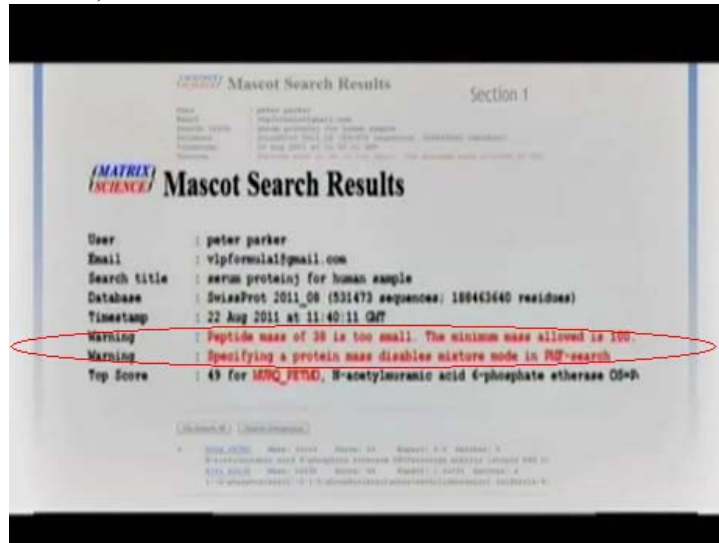
Section 3 summary report in which the matched protein from the database with the details of important parameters are displayed either in concise format protein format and the data can be exported too.

(Refer Slide Time 24:38)

The screenshot shows the 'Data Analysis' section of the Mascot search results interface. The text 'Data Analysis' is displayed in a blue font, centered on the page.

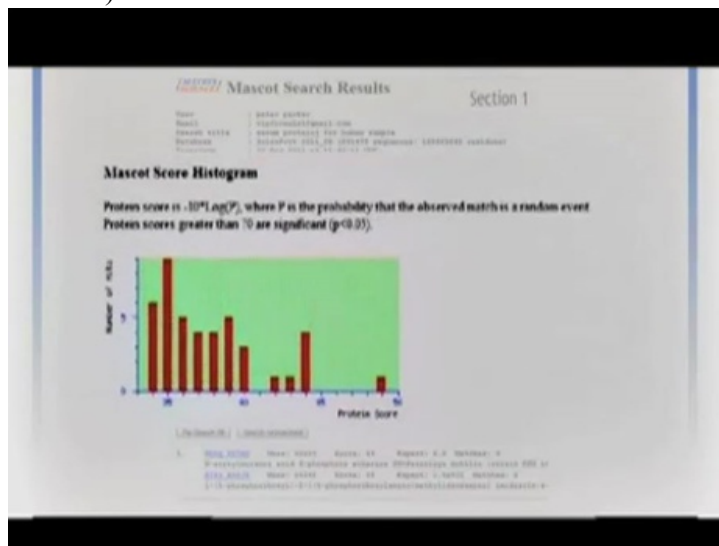
Data Analysis

(Refer Slide Time 24:43)



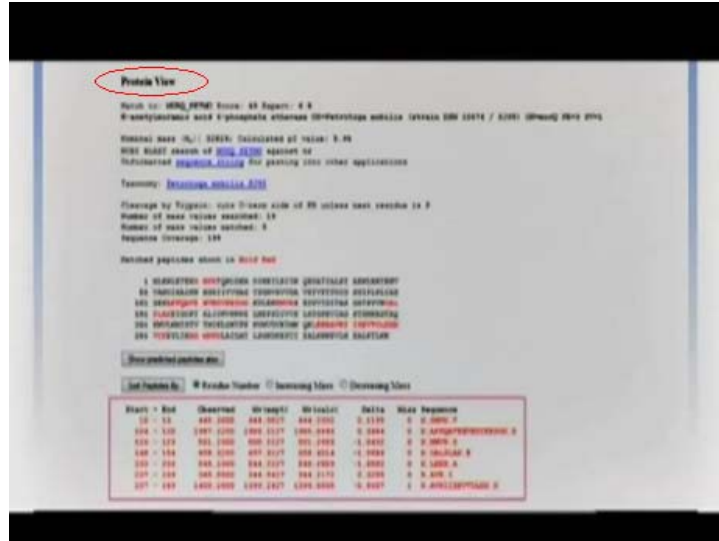
If the search parameters are not the best fit, the software generates the error message. Depending on the error message the user needs to change the parameter setting and do the search again

(Refer Slide Time 24:54)



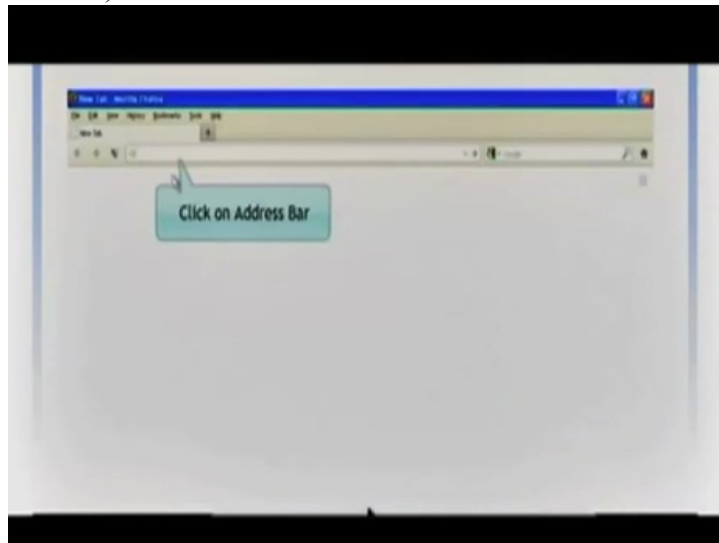
In section 2 the Mascot score histogram the number of protein hits and their score is displayed along the graph

(Refer Slide Time 25:03)



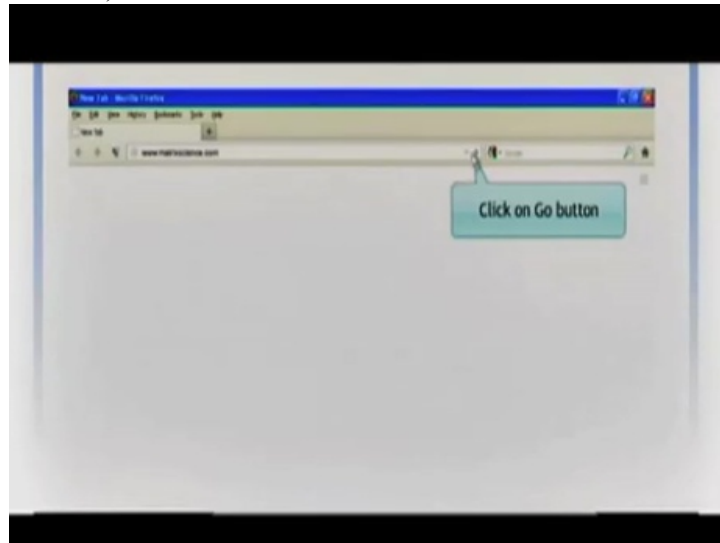
Protein view section displays matching of the query peptide to the protein sequence in the database. The sequence type, the matched region, what is the expected and the calculated value of the query peptide and the sequence details

(Refer Slide Time 25:17)



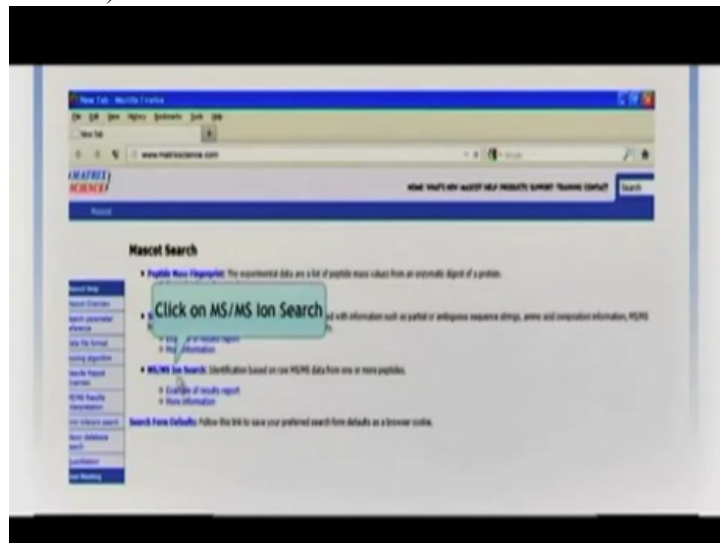
For better protein identification and to increase protein score CID of each peak generated ...

(Refer Slide Time 25:23)



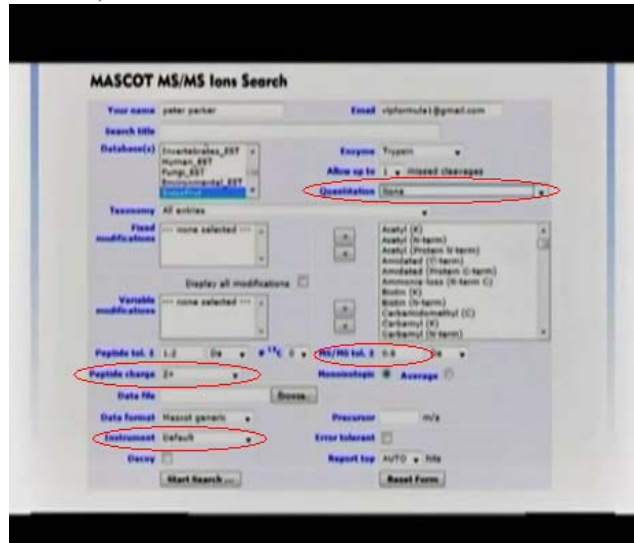
... is carried out to generate MS/MS data

(Refer Slide Time 25:27)



For such data analysis MS ion search option is selected from the Matrix Science browser window

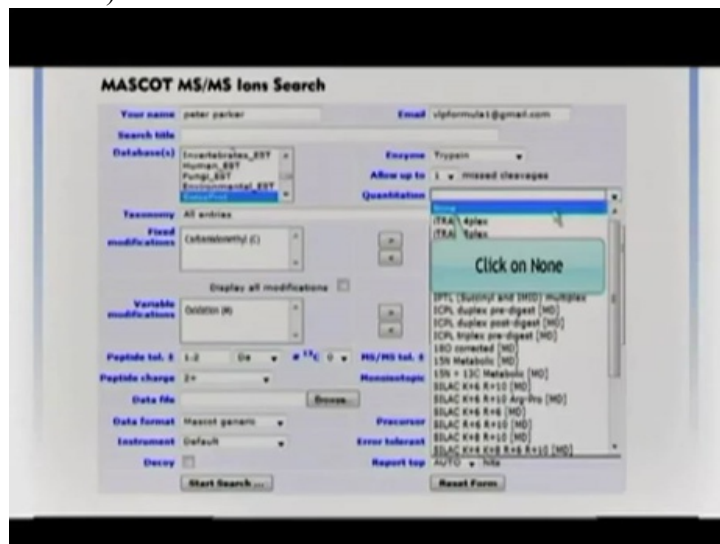
(Refer Slide Time 25:42)



In MS/MS search tool more input parameters like quantitation, MS/MS tolerance, peptide charge instrument etc in addition to fields for PMF and rest other parameters are similar to that of the peptide mass fingerprint

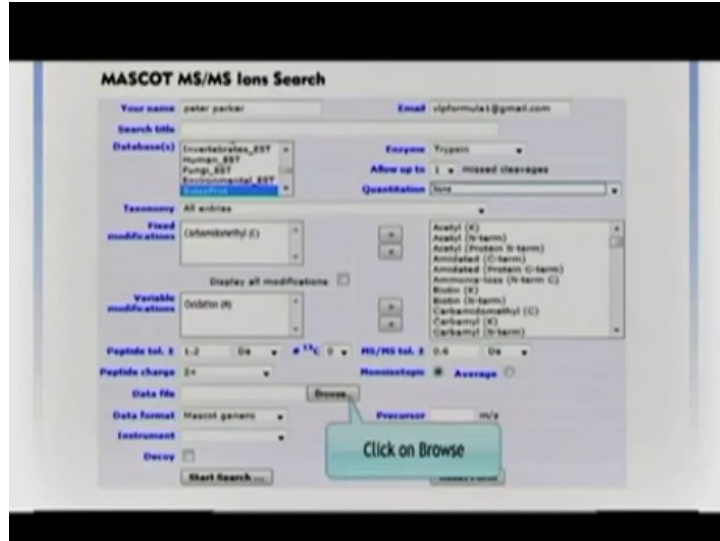
Depending upon the process carried out for data generation a selection in the quantitation must be made. In case of label--free quantitation ...

(Refer Slide Time 25:58)



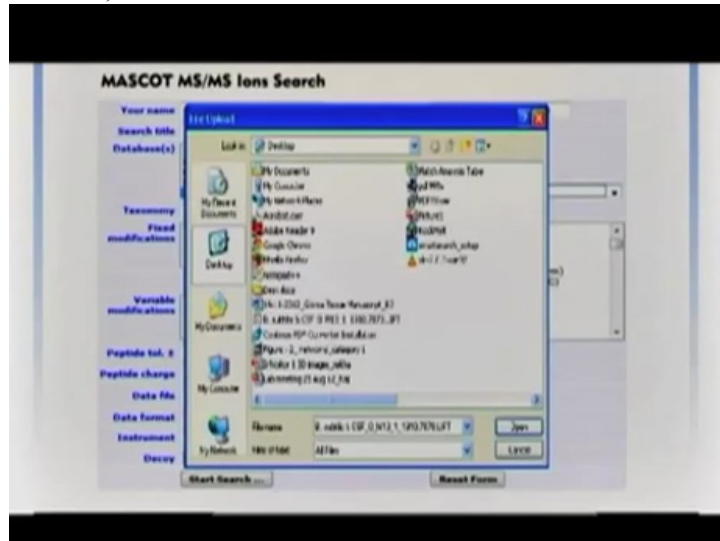
...select none in quantitation tab

(Refer Slide Time 26:02)



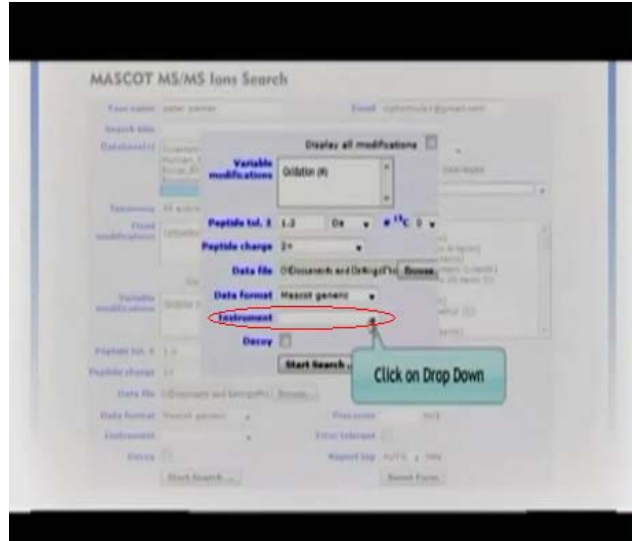
Remaining parameters remain same as PMF. Browse the MS/MS raw data file and ...

(Refer Slide Time 26:10)



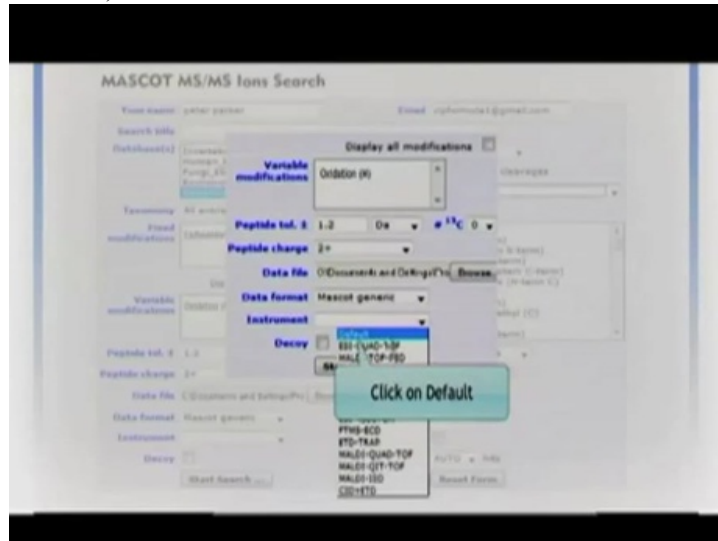
...search the results using Mascot

(Refer Slide Time 26:13)



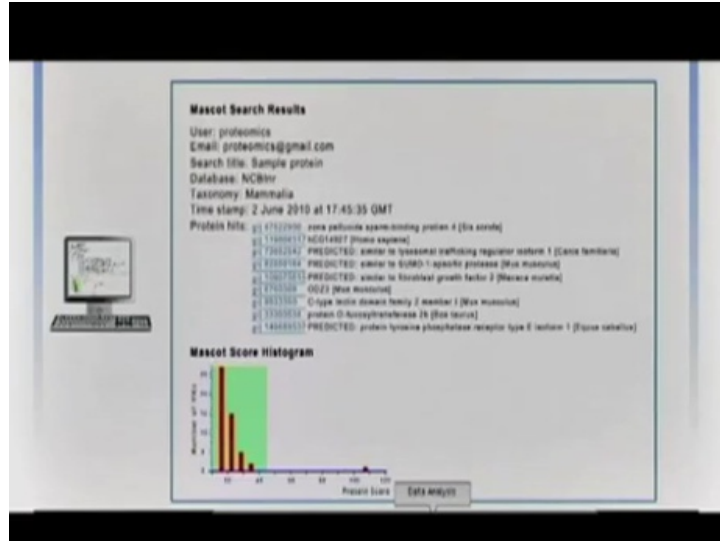
Define the instrument ...

(Refer Slide Time 26:15)



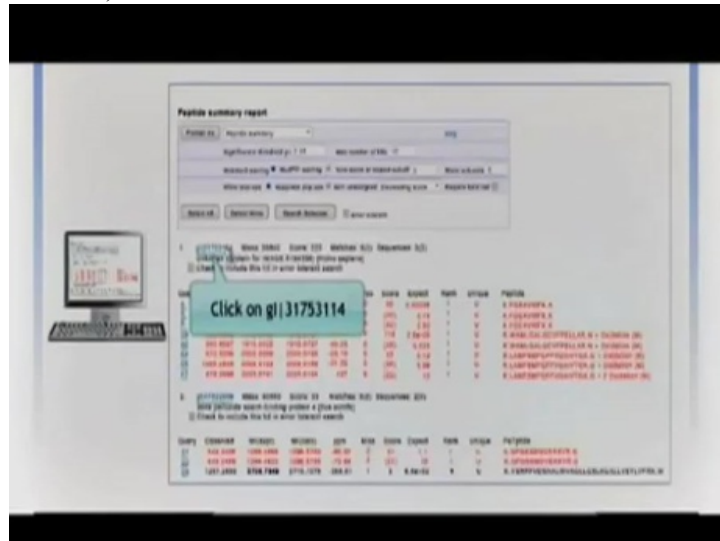
...that has been used to generate the raw data. When we don't know the name of the instrument select default.

(Refer Slide Time 26:27)



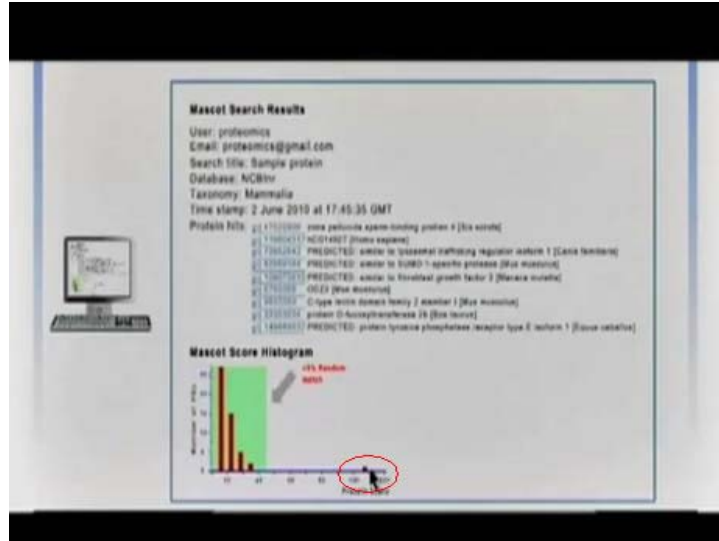
The result output generated is almost similar to PMF output.

(Refer Slide Time 26:29)



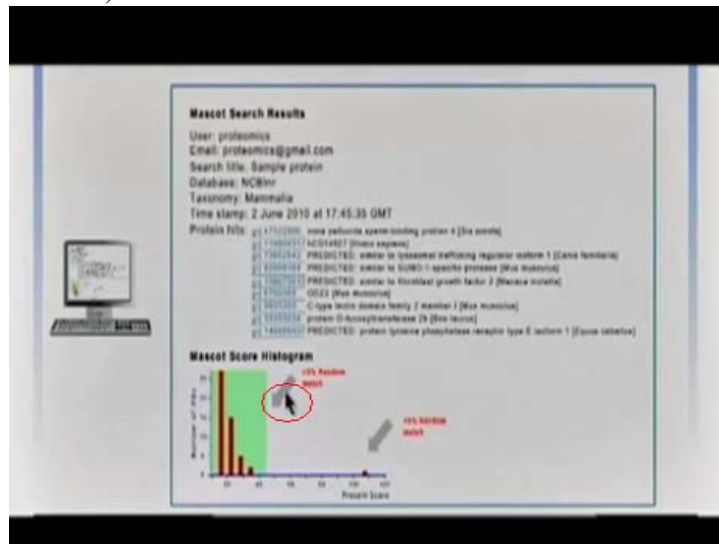
The accession ID indicates the protein information obtained from the database marked as green

(Refer Slide Time 26:37)



The protein hit outside the green box indicates the p value less than 0.05 which is statistically significant....

(Refer Slide Time 26:45)



... where as hits inside the green box indicates random matching

(Refer Slide Time 26:50)

Mascot search results

Protein view
Match to: p11791-14 Score: 201
Unimod search for: 00003194036 (none stated)
Found in search of: C:\Users\homer\Desktop\MSI_S1_11_01_01_data_analyse_files_data_files\mgf\Fraction147.mgf
Normal mass (Da): 20840; Calculated pI value: 4.82
MSI: Full search of: [p11791-14](#) against in:
Informatics: [pubmed.ncbi.nlm.nih.gov](#) for passing into other applications
Taxonomy: [uniprot.org](#)
Links to retrieve other entries containing this sequence from NCBI Entrez:
[p11791-14](#) [Pub](#) [Data](#) [Accession](#)
Fixed modification: Carbamidomethyl (C)
Variable modification: Oxidation (M)
Coverage by Trypsin: only C-term side of 88 unique next residue is P
Sequence Coverage: 14%

Matched peptides shown in [Data File](#)

1	SRVTFLEK	GRVTFLEK	AAVTFVAKL	PIVQAKRM	0.2744730
2	SRVTFVTK	GRVTFVSK	AVVDFVRL	GVVDFVPT	1.0277030
10	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
11	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
20	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
21	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012

Mascot search Results

(Refer Slide Time 26:56)

Mascot search results

Protein view
Match to: p11791-14 Score: 201
Unimod search for: 00003194036 (none stated)
Found in search of: C:\Users\homer\Desktop\MSI_S1_11_01_01_data_analyse_files_data_files\mgf\Fraction147.mgf
Normal mass (Da): 20840; Calculated pI value: 4.82
MSI: Full search of: [p11791-14](#) against in:
Informatics: [pubmed.ncbi.nlm.nih.gov](#) for passing into other applications
Taxonomy: [uniprot.org](#)
Links to retrieve other entries containing this sequence from NCBI Entrez:
[p11791-14](#) [Pub](#) [Data](#) [Accession](#)
Fixed modification: Carbamidomethyl (C)
Variable modification: Oxidation (M)
Coverage by Trypsin: only C-term side of 88 unique next residue is P
Sequence Coverage: 14%

Matched peptides shown in [Data File](#)

The protein score is a sum of the highest ion scores for each sequence, with duplicate matches being excluded. A score above 67 is considered significant.

1	SRVTFLEK	GRVTFLEK	AAVTFVAKL	PIVQAKRM	0.2744730
2	SRVTFVTK	GRVTFVSK	AVVDFVRL	GVVDFVPT	1.0277030
10	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
11	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
20	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012
21	SRVTFGLK	TVVDFVSK	AVVDFVRL	AVVDFVPT	0.8824012

Data Analysis

The score the peptide score is the sum of highest ion scores for each sequence with duplicate matches being excluded.

(Refer Slide Time 27:03)

Mascot search results

Protein view

Match to: p|I79314|Iscv: 225

Unknown protein for IMAGE: 519433a (Protein report)

Found in search of: C:\Users\hema\Desktop\MSI\LC-MS/MS data analysis\Raw data file\img\RawData\Raw1.mgf

Nominal mass (Da): 20440 Calculated pI value: 4.85

MS/MS search of p|I79314|Iscv: 225

Informations: **Predicted mass of the protein.** other applications

Links to retrieve other entries containing this sequence from NCBI Entrez: [ALL\(1\) \(0\) \(0\)](#) [View Data \(0\) \(0\)](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Charge by Trypsin: only C-term side of RR unless next residue is P

Sequence Coverage: 14%

Matched peptides shown in [bold face](#)

1	MSPTFLK	GRKTFLLK	AAATFRMLK	PIKALKRM	ILPISALPDK
2	ARVTRNTAL	AAKPTSDK	WYSDKPLK	GLSDPPK	LTGDFPKS
10	WYKALGK	ETANRSLK	WIKRRAKLS	ALSDPPK	AKNRPISL
15	WYKALGK	ISGLDRLK	PLIKRQK	AKNRPISL	GAEDPKS
20	SPLEDEPK	YKGLDFPK	LADEPK	LVKRNK	LAETAPDK
25	YKATYK	LEKALSLK	PKKAKK	TKPKK	

Nominal mass it is predicted mass of the protein

(Refer Slide Time 27:09)

Mascot search results

Protein view

Match to: p|I79314|Iscv: 225

Unknown protein for IMAGE: 519433a (Protein report)

Found in search of: C:\Users\hema\Desktop\MSI\LC-MS/MS data analysis\Raw data file\img\RawData\Raw1.mgf

Nominal mass (Da): 20440 **Calculated pI value: 4.85**

MS/MS search of p|I79314|Iscv: 225

Informations: **Predicted isoelectric point of the protein.**

Links to retrieve other entries containing this sequence from NCBI Entrez: [ALL\(1\) \(0\) \(0\)](#) [View Data \(0\) \(0\)](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Charge by Trypsin: only C-term side of RR unless next residue is P

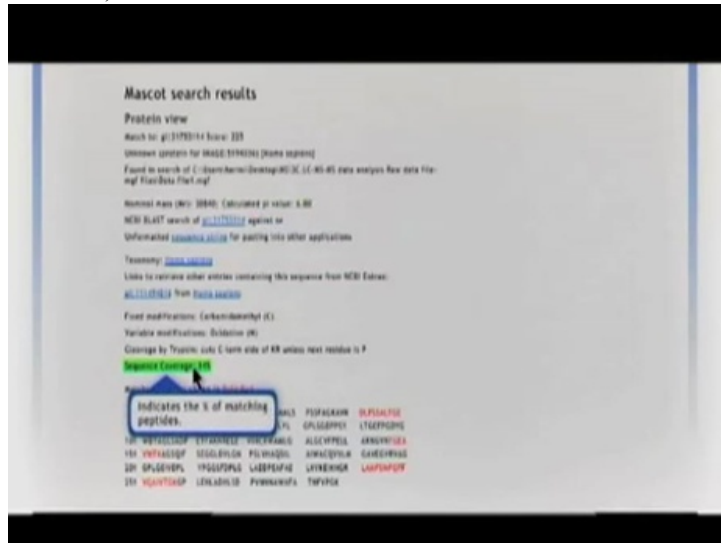
Sequence Coverage: 14%

Matched peptides shown in [bold face](#)

1	MSPTFLK	GRKTFLLK	AAATFRMLK	PIKALKRM	ILPISALPDK
2	ARVTRNTAL	AAKPTSDK	WYSDKPLK	GLSDPPK	LTGDFPKS
10	WYKALGK	ETANRSLK	WIKRRAKLS	ALSDPPK	AKNRPISL
15	WYKALGK	ISGLDRLK	PLIKRQK	AKNRPISL	GAEDPKS
20	SPLEDEPK	YKGLDFPK	LADEPK	LVKRNK	LAETAPDK
25	YKATYK	LEKALSLK	PKKAKK	TKPKK	

Calculated pI value, predicted isoelectric point of the protein

(Refer Slide Time 27:17)



Mascot search results

Protein view
Match to: p11701-14 (score: 333)
Unknown protein for M04255 (Swiss-Prot)
Found in search of: C:\chemdata\development\MSI\11-03-05\data\analysis\raw_data\filemgf\F100001.mgf

Molecular mass (kDa): 38.940; Calculated pI: 4.80
NCBI BLAST search of p11701-14 against nr
Unformatted: [sequence viewer](#) for putting into other applications

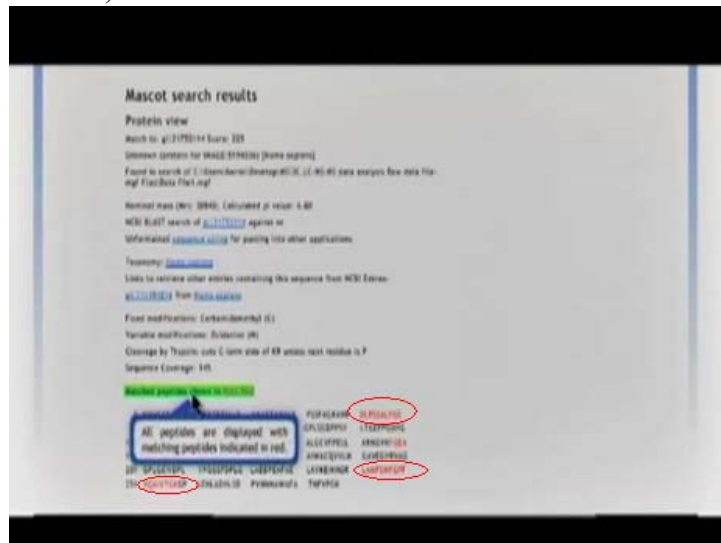
Taxonomy: [View taxonomy](#)
Links to retrieve other entries containing this sequence from NCBI Entrez:
[p11701-14](#) from [Swiss-Prot](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Coverage by Trypsin: only C-term side of KR within next residue is F
Sequence Coverage: 100%

Sequence: **MDLS** PFKRGRKRM **RLPKLPLD**
 FL **GLLGGPPEL** **LTGPPGQMS**
101 **NYEYASDIDF** **ETVDSNDEE** **ISGSLVAGLS** **ALGCVPLQL** **AKKQWYLSA**
104 **YNYASDIDF** **ISGSLVAGLS** **FLVWAGD** **AKKQWYLSA** **GAKKQWYLSA**
105 **SPALSDVPL** **YNYASDIDF** **LADPEKFAI** **LYIKRQKQR** **LADPEKFAI**
108 **YQATYKQDP** **LEKASDLSL** **PKKAKWFA** **TPKPKFA**

Sequence coverage indicates the percent of matching peptide

(Refer Slide Time 27:24)



Mascot search results

Protein view
Match to: p11701-14 (score: 333)
Unknown protein for M04255 (Swiss-Prot)
Found in search of: C:\chemdata\development\MSI\11-03-05\data\analysis\raw_data\filemgf\F100001.mgf

Molecular mass (kDa): 38.940; Calculated pI: 4.80
NCBI BLAST search of p11701-14 against nr
Unformatted: [sequence viewer](#) for putting into other applications

Taxonomy: [View taxonomy](#)
Links to retrieve other entries containing this sequence from NCBI Entrez:
[p11701-14](#) from [Swiss-Prot](#)

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Coverage by Trypsin: only C-term side of KR within next residue is F
Sequence Coverage: 100%

Matched peptides shown in red:

All peptides are displayed with matching peptides indicated in red.

Sequence: **MDLS** PFKRGRKRM **RLPKLPLD**
 FL **GLLGGPPEL** **LTGPPGQMS**
101 **NYEYASDIDF** **ETVDSNDEE** **ISGSLVAGLS** **ALGCVPLQL** **AKKQWYLSA**
104 **YNYASDIDF** **ISGSLVAGLS** **FLVWAGD** **AKKQWYLSA** **GAKKQWYLSA**
105 **SPALSDVPL** **YNYASDIDF** **LADPEKFAI** **LYIKRQKQR** **LADPEKFAI**
108 **YQATYKQDP** **LEKASDLSL** **PKKAKWFA** **TPKPKFA**

All the peptides are displayed with matching peptides indicated in red

(Refer Slide Time 27:32)

Mascot search results

Protein view

Show predicted peptides also

Sort Peptides By: Residue number (Increasing mass, Decreasing mass)

Start	End	Observed	M(isopt)	M(iscalc)	Delta	Miss	Sequence
126	142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE: 112)
126	142	960.4587	1918.9079	1918.9797	40	0	R.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE: 85)
147	154	492.2200	982.4254	982.4913	67	0	K.FGLAVYFK.A (100% SCORE: 55)
147	154	492.2305	982.4464	982.4913	46	0	K.FGLAVYFK.A (100% SCORE: 50)
147	154	492.2348	982.4511	982.4913	37	0	K.FGLAVYFK.A (100% SCORE: 43)
241	238	670.6395	2008.8966	2009.0155	59	0	R.LAMPSPGFYVQAVYTK.G Oxidation (M) (100% SCORE: 42)
241	238	1009.4435	2008.9124	2009.0155	91	0	R.LAMPSPGFYVQAVYTK.G Oxidation (M) (100% SCORE: 33)
241	238	676.2996	2025.8741	2025.9104	427	0	R.LAMPSPGFYVQAVYTK.G 2 Oxidation (M) (100% SCORE: 22)

The protein view obtained on selecting the particular protein link is very similar to protein view observed in PMF. It provides detailed information

(Refer Slide Time 27:41)

Mascot search results

Protein view

Show predicted peptides also

Indicates beginning & end of each peptide.

Sort Peptides By: Residue number (Increasing mass, Decreasing mass)

Start	End	Observed	M(isopt)	M(iscalc)	Delta	Miss	Sequence
126	142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE: 112)
126	142	960.4587	1918.9079	1918.9797	40	0	R.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE: 85)
147	154	492.2200	982.4254	982.4913	67	0	K.FGLAVYFK.A (100% SCORE: 55)
147	154	492.2305	982.4464	982.4913	46	0	K.FGLAVYFK.A (100% SCORE: 50)
147	154	492.2348	982.4511	982.4913	37	0	K.FGLAVYFK.A (100% SCORE: 43)
241	238	670.6395	2008.8966	2009.0155	59	0	R.LAMPSPGFYVQAVYTK.G Oxidation (M) (100% SCORE: 42)
241	238	1009.4435	2008.9124	2009.0155	91	0	R.LAMPSPGFYVQAVYTK.G Oxidation (M) (100% SCORE: 33)
241	238	676.2996	2025.8741	2025.9104	427	0	R.LAMPSPGFYVQAVYTK.G 2 Oxidation (M) (100% SCORE: 22)

...about each of the matched peptide displayed The start ...

(Refer Slide Time 27:46)

Mascot search results
Protein view

Show predicted peptides also

Observed molecular weight number Increasing Mass Decreasing Mass

Start	End	Observed	M(iso)pt	M(iso)c	Delta	Miss	Sequence
126	142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE 112)
126	142	960.4587	1918.9029	1918.9797	-40	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE 88)
147	154	492.2200	982.4254	982.4913	-67	0	K.FGLAYWFK.A (100% SCORE 64)
147	154	492.2305	982.4464	982.4913	-46	0	K.FGLAYWFK.A (100% SCORE 60)
147	154	492.2348	982.4951	982.4913	37	0	K.FGLAYWFK.A (100% SCORE 33)
241	258	670.6395	2008.8946	2009.0155	-59	0	R.LANFSPGFYQAVYTK.G Oxidation (M) (100% SCORE 42)
241	258	1005.4635	2008.9124	2009.0155	-51	0	R.LANFSPGFYQAVYTK.G Oxidation (M) (100% SCORE 35)
241	258	676.2986	2023.8741	2023.0104	-427	0	R.LANFSPGFYQAVYTK.G 2 Oxidation (M) (100% SCORE 22)

... and end position of amino acids ...

(Refer Slide Time 27:50)

Mascot search results
Protein view

Show predicted peptides also

Sort Peptides By Calculated molecular weights Decreasing Mass

Start	End	Observed	M(iso)pt	M(iso)c	Delta	Miss	Sequence
126	142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE 112)
126	142	960.4587	1918.9029	1918.9797	-40	0	K.WANGALGCVPELLAR.N Oxidation (M) (100% SCORE 88)
147	154	492.2200	982.4254	982.4913	-67	0	K.FGLAYWFK.A (100% SCORE 64)
147	154	492.2305	982.4464	982.4913	-46	0	K.FGLAYWFK.A (100% SCORE 60)
147	154	492.2348	982.4951	982.4913	37	0	K.FGLAYWFK.A (100% SCORE 33)
241	258	670.6395	2008.8946	2009.0155	-59	0	R.LANFSPGFYQAVYTK.G Oxidation (M) (100% SCORE 42)
241	258	1005.4635	2008.9124	2009.0155	-51	0	R.LANFSPGFYQAVYTK.G Oxidation (M) (100% SCORE 35)
241	258	676.2986	2023.8741	2023.0104	-427	0	R.LANFSPGFYQAVYTK.G 2 Oxidation (M) (100% SCORE 22)

... calculated and ...

(Refer Slide Time 27:51)

Mascot search results
Protein view

Show predicted peptides also

Sort Peptides Experimental molecular weight Using Mass Decreasing mass

Start - End	Observed	Miscalls	Miscalc	Delta	Miss	Sequence
126 - 142	960.4446	1918.8746	1918.9797	55	0	R.WANIGALGCVPELLAR.N Oxidation (M) (100% SCORE 112)
126 - 142	960.4587	1918.9029	1918.9797	40	0	R.WANIGALGCVPELLAR.N Oxidation (M) (100% SCORE 48)
147 - 154	492.2200	982.4254	982.4913	67	0	K.FGLEAYWPK.A (100% SCORE 85)
147 - 154	492.2305	982.4464	982.4913	-46	0	K.FGLEAYWPK.A (100% SCORE 69)
147 - 154	492.2348	982.4931	982.4913	37	0	K.FGLEAYWPK.A (100% SCORE 3)
241 - 258	670.6395	2008.8966	2009.0155	-59	0	R.LANFIMFCFFVQAVYTK.G Oxidation (M) (100% SCORE 42)
241 - 258	1005.4435	2008.9124	2009.0155	91	0	R.LANFIMFCFFVQAVYTK.G Oxidation (M) (100% SCORE 33)
241 - 258	676.2986	2025.8741	2025.9104	427	0	R.LANFIMFCFFVQAVYTK.G 2 Oxidation (M) (100% SCORE 22)

... experimental molecular weights...

(Refer Slide Time 27:55)

Mascot search results
Protein view

Show predicted peptides also

Sort Peptides By Calculated molecular weight Decreasing mass

Start - End	Observed	Miscalls	Miscalc	Delta	Miss	Sequence
126 - 142	960.4446	1918.8746	1918.9797	55	0	R.WANIGALGCVPELLAR.N Oxidation (M) (100% SCORE 112)
126 - 142	960.4587	1918.9029	1918.9797	40	0	R.WANIGALGCVPELLAR.N Oxidation (M) (100% SCORE 48)
147 - 154	492.2200	982.4254	982.4913	67	0	K.FGLEAYWPK.A (100% SCORE 85)
147 - 154	492.2305	982.4464	982.4913	-46	0	K.FGLEAYWPK.A (100% SCORE 69)
147 - 154	492.2348	982.4931	982.4913	37	0	K.FGLEAYWPK.A (100% SCORE 3)
241 - 258	670.6395	2008.8966	2009.0155	-59	0	R.LANFIMFCFFVQAVYTK.G Oxidation (M) (100% SCORE 42)
241 - 258	1005.4435	2008.9124	2009.0155	91	0	R.LANFIMFCFFVQAVYTK.G Oxidation (M) (100% SCORE 33)
241 - 258	676.2986	2025.8741	2025.9104	427	0	R.LANFIMFCFFVQAVYTK.G 2 Oxidation (M) (100% SCORE 22)

... number of missed tryptic cleavages,

(Refer Slide Time 27:56)

Mascot search results
Protein view

Show predicted peptides also

Sort Peptides By: Residue Number Increasing Mass Decreasing Mass

Start - End	Observed	M(expt)	M(calc)	Delta	Miss	Sequence
126 - 142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (IONS SCORE 118)
126 - 142	960.4587	1918.9029	1918.9797	-40	0	R.WANGALGCVPELLAR.N Oxidation (M) (IONS SCORE 45)
147 - 154	492.2200	982.4254	982.4913	-67	0	K.FGLEAVYFK.A (IONS SCORE 85)
147 - 154	492.2305	982.4464	982.4913	-46	0	K.FGLEAVYFK.A (IONS SCORE 80)
147 - 154	492.2348	982.4551	982.4913	-37	0	K.FGLEAVYFK.A (IONS SCORE 73)
241 - 258	670.6390	2008.8966	2009.0135	-59	0	R.LANFIMFGFFVQAVYGR.G Oxidation (M) (IONS SCORE 62)
241 - 258	1005.4435	2008.9124	2009.0195	-51	0	R.LANFIMFGFFVQAVYGR.G Oxidation (M) (IONS SCORE 33)
241 - 258	676.2986	2023.8741	2023.0104	427	0	R.LANFIMFGFFVQAVYGR.G 2 Oxidation (M) (IONS SCORE 22)

... sequence of each peptide segment and their corresponding ion scores are shown

(Refer Slide Time 28:02)

Mascot search results
Protein view

Show predicted peptides also

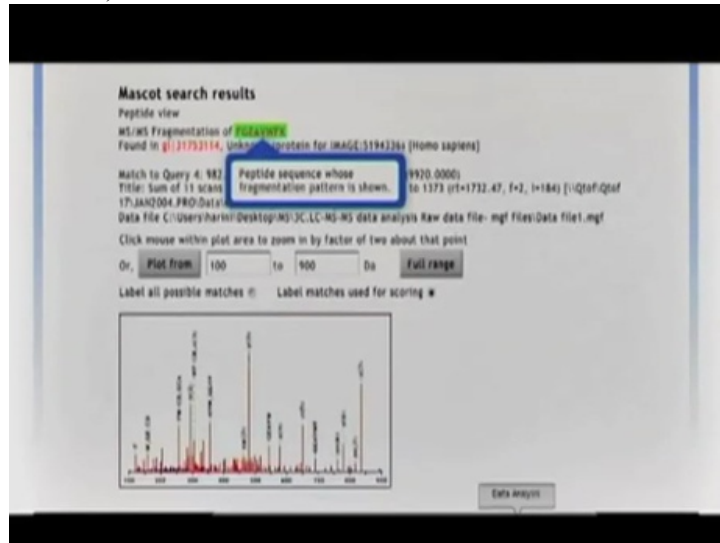
Sort Peptides By: Residue Number Increasing Mass Decreasing Mass

Indicates score of each ion fragment, used for calculation of the protein score.

Start - End	Observed	M(expt)	M(calc)	Delta	Miss	Sequence
126 - 142	960.4446	1918.8746	1918.9797	55	0	K.WANGALGCVPELLAR.N Oxidation (M) (IONS SCORE 118)
126 - 142	960.4587	1918.9029	1918.9797	-40	0	R.WANGALGCVPELLAR.N Oxidation (M) (IONS SCORE 45)
147 - 154	492.2200	982.4254	982.4913	-67	0	K.FGLEAVYFK.A (IONS SCORE 85)
147 - 154	492.2305	982.4464	982.4913	-46	0	K.FGLEAVYFK.A (IONS SCORE 80)
147 - 154	492.2348	982.4551	982.4913	-37	0	K.FGLEAVYFK.A (IONS SCORE 73)
241 - 258	670.6390	2008.8966	2009.0135	-59	0	R.LANFIMFGFFVQAVYGR.G Oxidation (M) (IONS SCORE 62)
241 - 258	1005.4435	2008.9124	2009.0195	-51	0	R.LANFIMFGFFVQAVYGR.G Oxidation (M) (IONS SCORE 33)
241 - 258	676.2986	2023.8741	2023.0104	427	0	R.LANFIMFGFFVQAVYGR.G 2 Oxidation (M) (IONS SCORE 22)

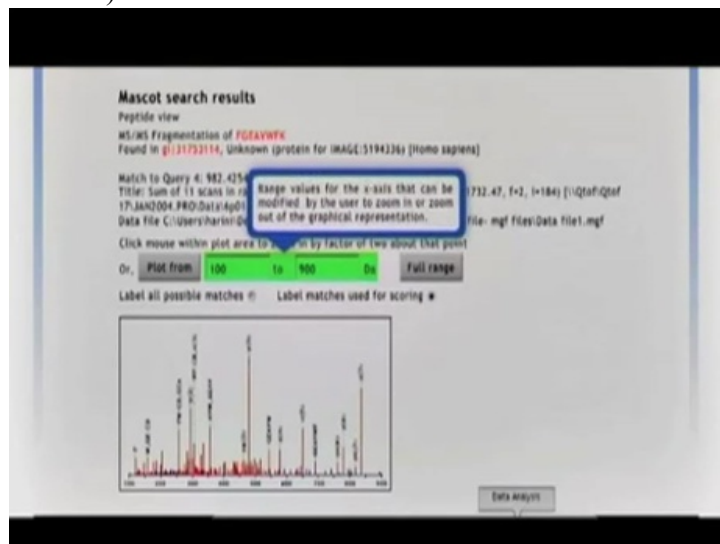
The highest ion scores are used for computing the final protein score

(Refer Slide Time 28:07)



Each peptide in Tandem MS/MS undergoes through second round of fragmentation when it passes through the second mass analyzer before it reaches the detector

(Refer Slide Time 28:16)



This provides significantly larger amount of information regarding each peptide segment which can be viewed by clicking on the peptide link provided in the summary report. The fragmentation pattern is displayed graphically ...

(Refer Slide Time 28:35)

Mascot search results
Peptide view

Monoisotopic mass of neutral peptide M(m/z): 982.4913

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ion Score: 66 Expect: 0.00036

Matches: 23/78 fragment ions using 16 most intense peaks (b/y)

#	Ion	a	a'	b	b'	Seq	y	y'	y''	#
1	120.0908	120.0908		148.0757		F				8
2	30.0338	177.1023		205.0972		G	836.4301	819.4036	818.4196	7
3	102.0550	306.1448	288.1343	334.1397	316.1292	I	779.4087	762.3821	761.3981	6
1	44.0495	377.1819	359.1714	405.1769	387.1663	A	650.3661	633.3395		5
5	72.0908	476.2504	458.2398	504.2453	486.2347	V	579.3289	562.3024		4
4	159.0917	642.3297	624.3191	650.3246	632.3140	W	480.2605	463.2340		3
7	120.0908	809.3981	791.3875	837.3930	819.3824	F	294.1812	277.1547		2
8	101.1073					K	147.1128	130.0863		1

...which can be zoomed into as per the requirement

(Refer Slide Time 28:50)

Points to Ponder

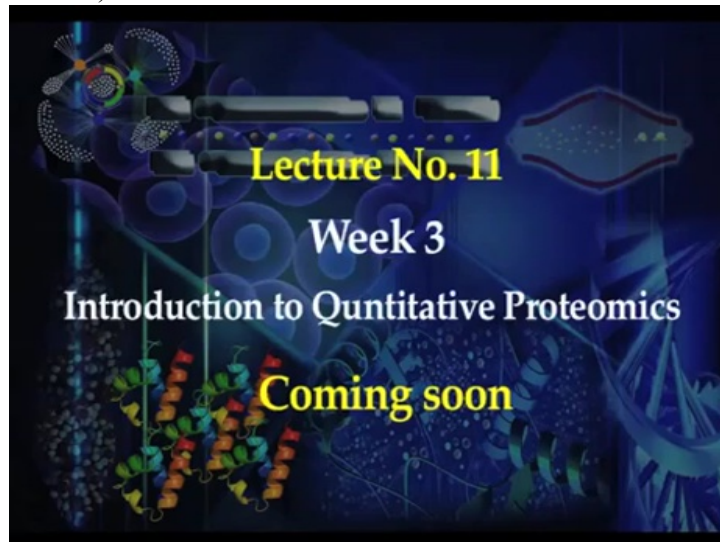
- # Higher the score better the hit
- # The hit should have at least two peptides
- # Higher the sequence coverage better the hit
- # y and b ions should be matched with MS/MS spectrum

(Refer Slide Time 29:01)

Summary

- # Basics of MALDI-TOF MS
- # An overview of proteomic experiment using 2DE gel & MALDI-TOF was demonstrated
- # MALDI Experiment:
 - Sample preparation discussed
 - MALDI-TOF instrument demonstrated
 - PMF and MS/MS analysis was discussed

(Refer Slide Time 29:09)



Lecture No. 11
Week 3
Introduction to Quantitative Proteomics
Coming soon

The slide features a dark blue background with a complex, abstract design. It includes a 3D molecular model of a protein structure on the left, a central horizontal bar with a grid pattern, and various other molecular and cellular motifs. The text is centered and uses a mix of white and yellow colors for emphasis.