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

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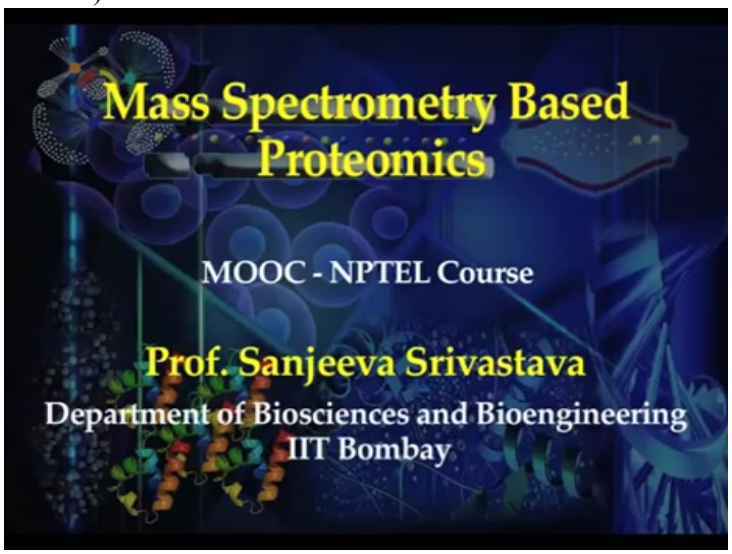

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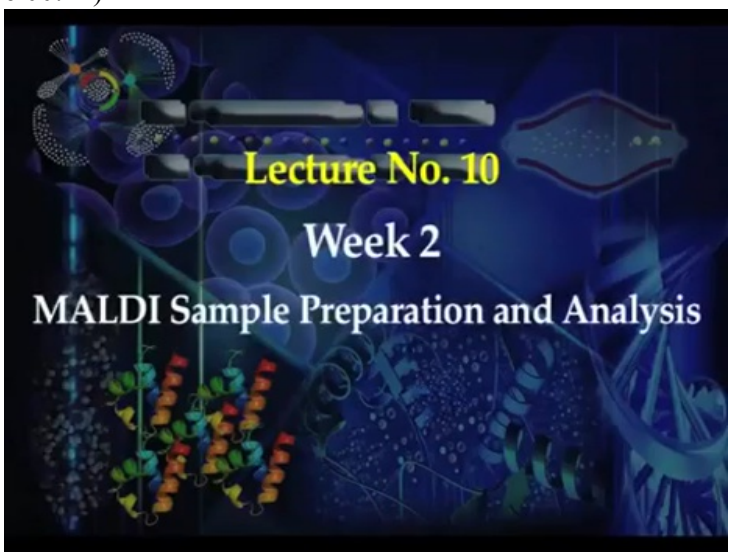

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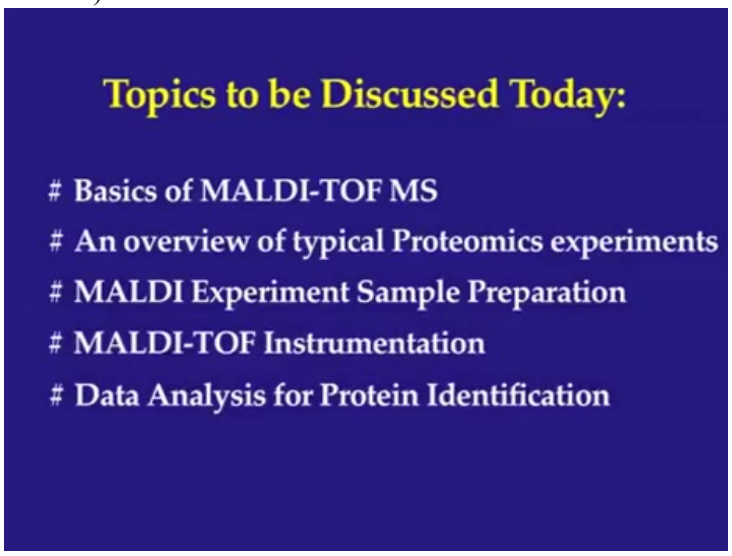

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Welcome to the proteomics course, today we will talk about …

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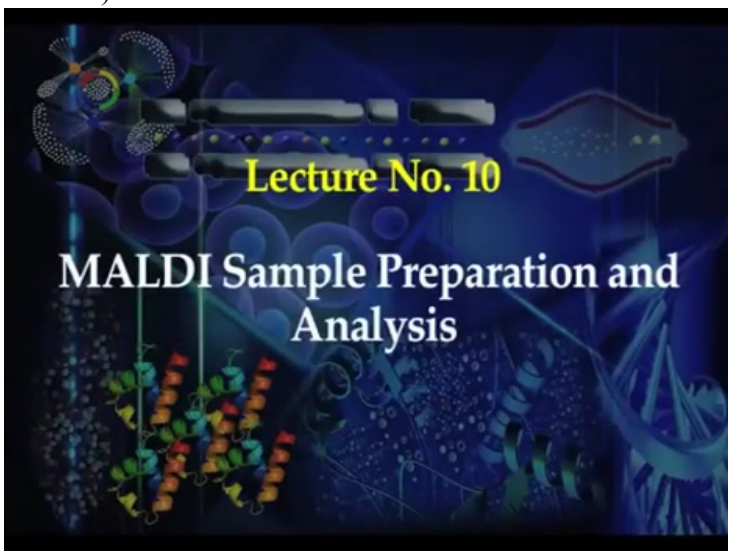

Matrix Assisted Laser Desorption Ionization Time Of Flight MALDI TOF.

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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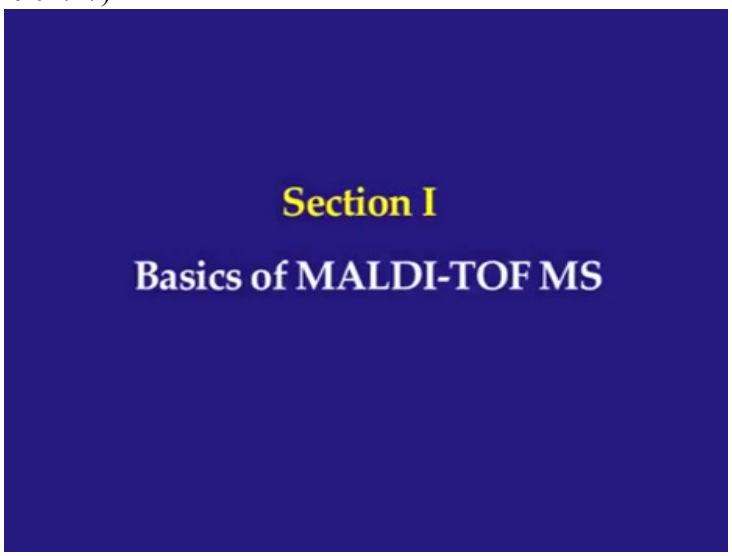

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Let us first start about basics of MALDI-TOF. So, MALDI is an efficient process for generating gas phase ions of peptides and proteins for mass spectrometric detection. MALDI is one of the most widely used ionization technique, currently applicable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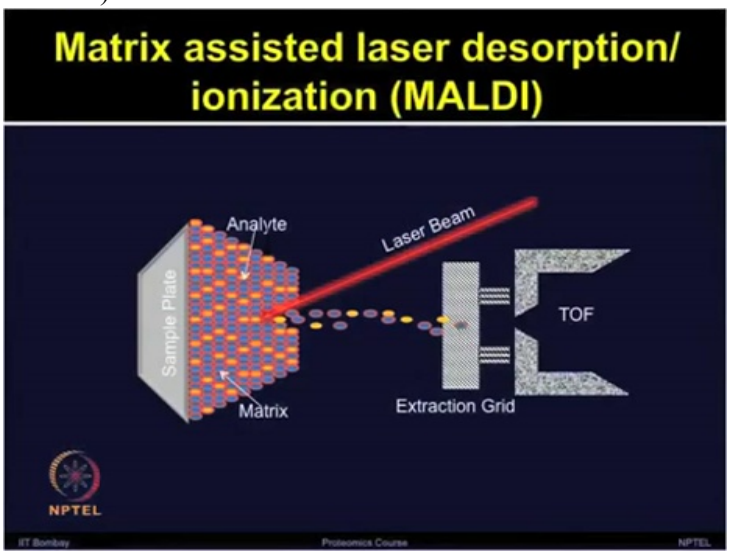

Let's first talk about Matrix Assisted Laser Desorption Ionization or MALDI. So analyte or the proteins of interest are mixed with matrix which is usually an aromatic compound.

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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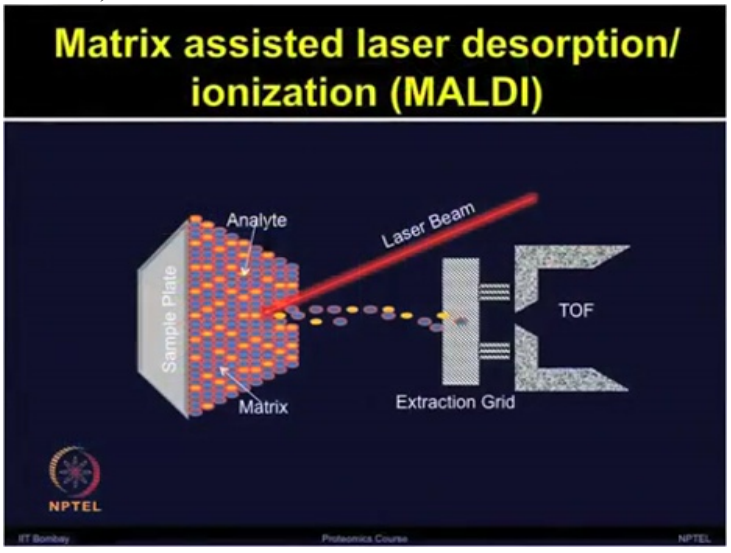

Once you have selected a matrix for the experiment then analyte and matrix can be dissolved in an organic solvent after which then it can be placed on a metallic target.

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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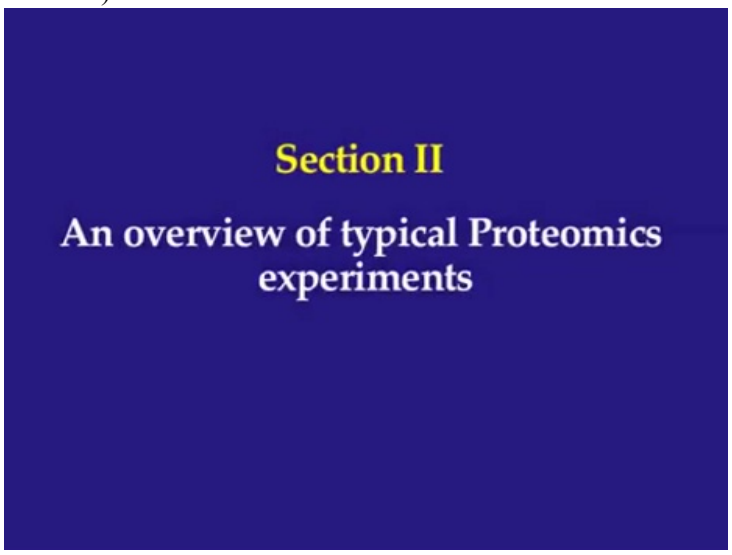

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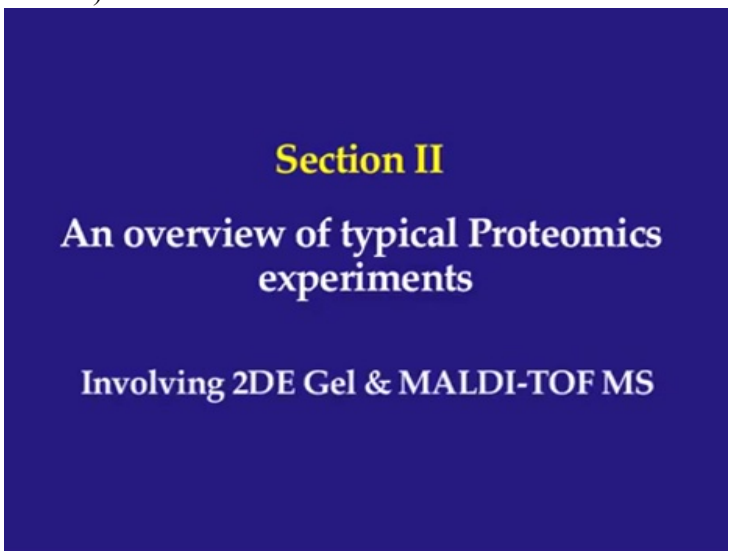

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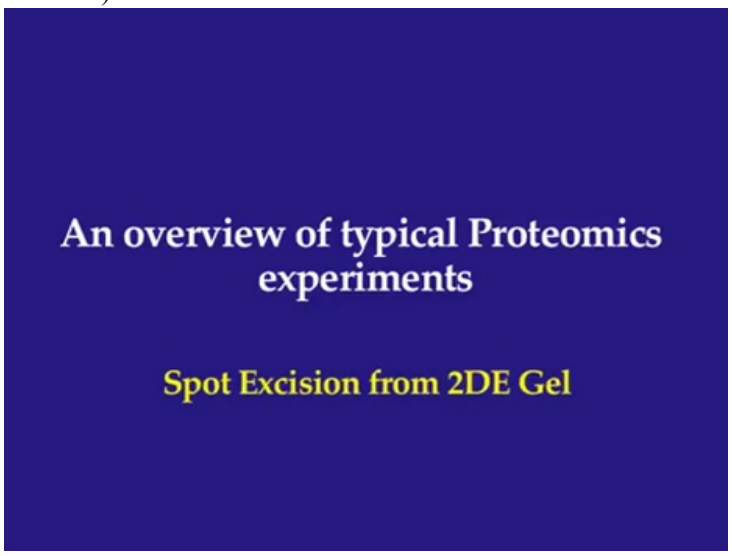

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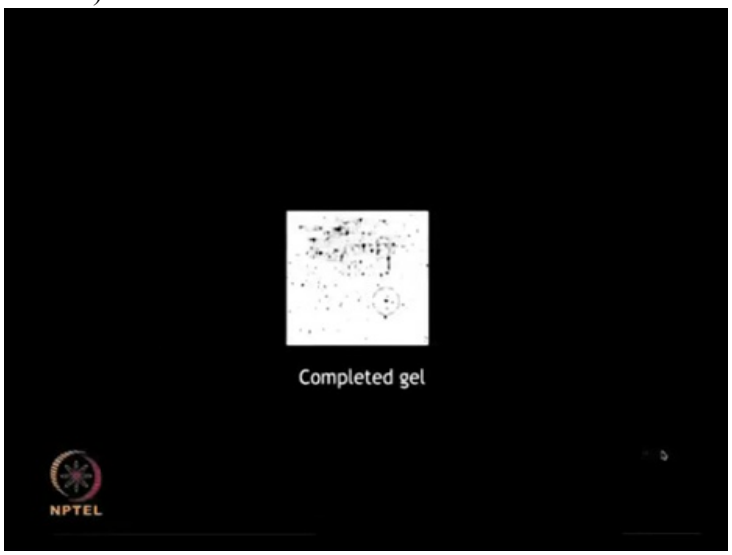

Sample preparation

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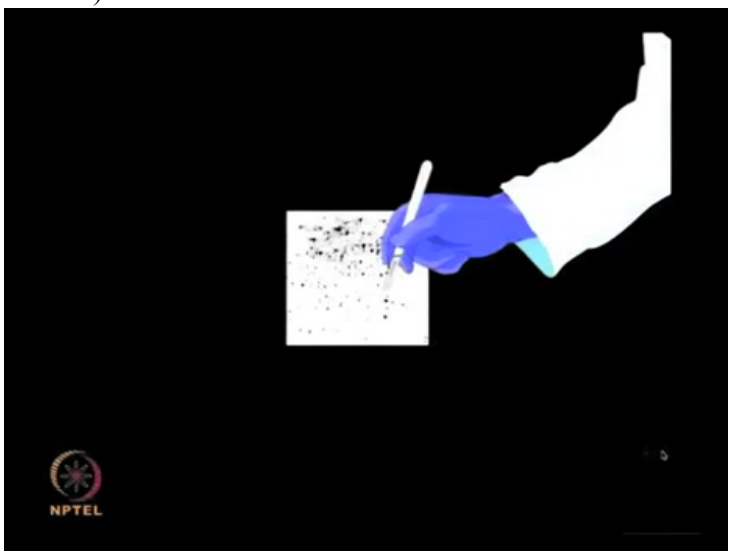

… and spotting

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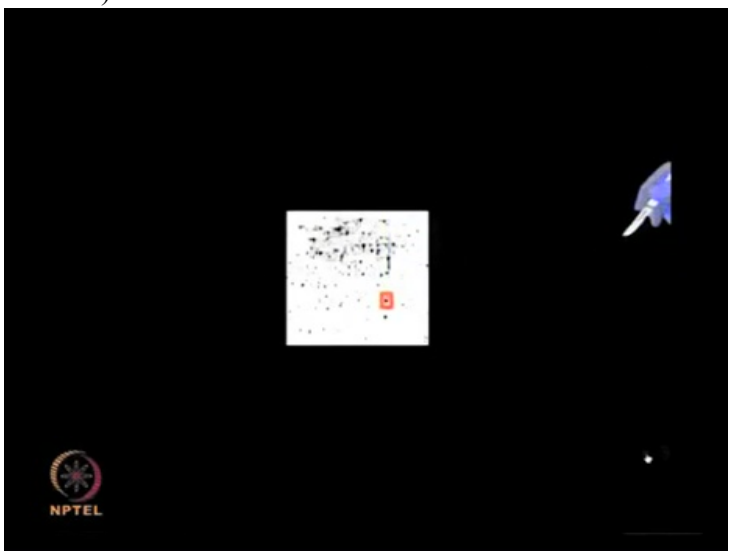

The protein sample must be prepared suitably before it can be analyzed by mass the spectrometer.

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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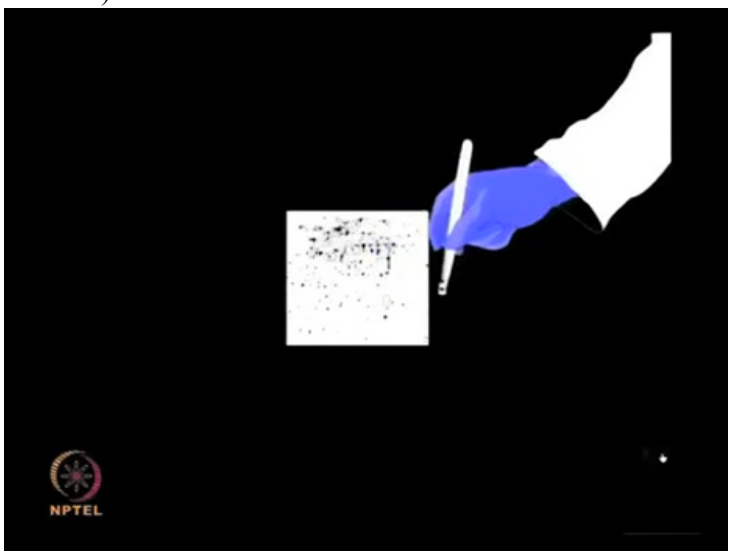

Spot can be dissolved …

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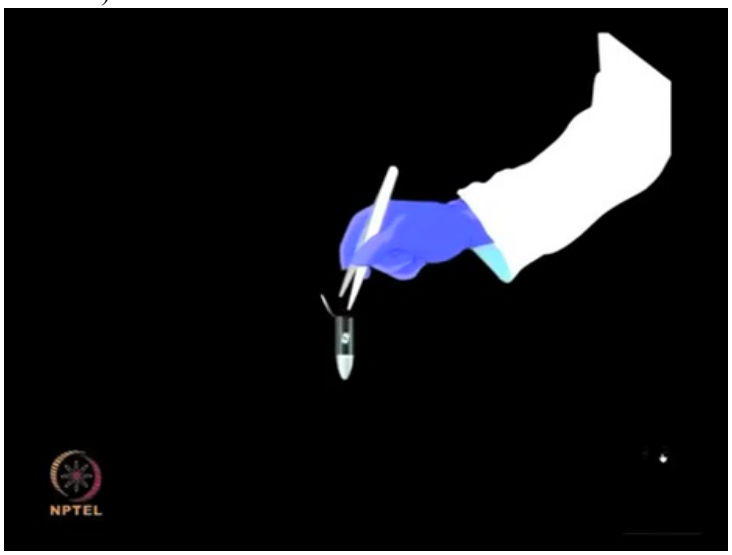

… in the suitable buffer

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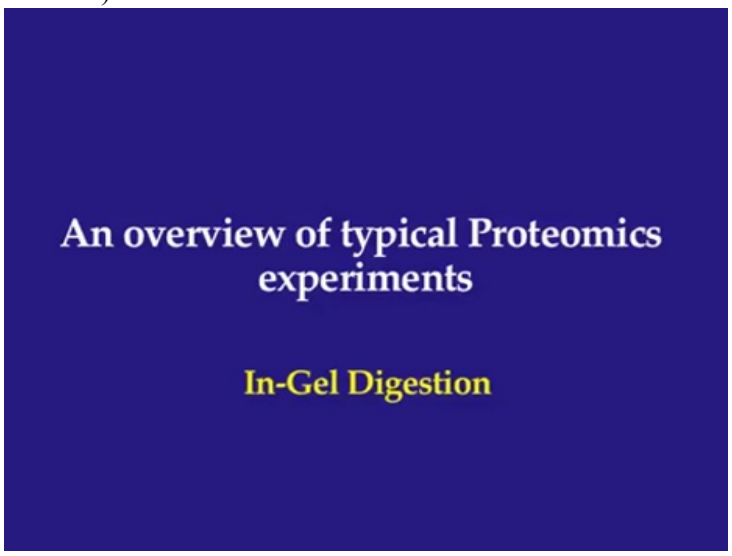

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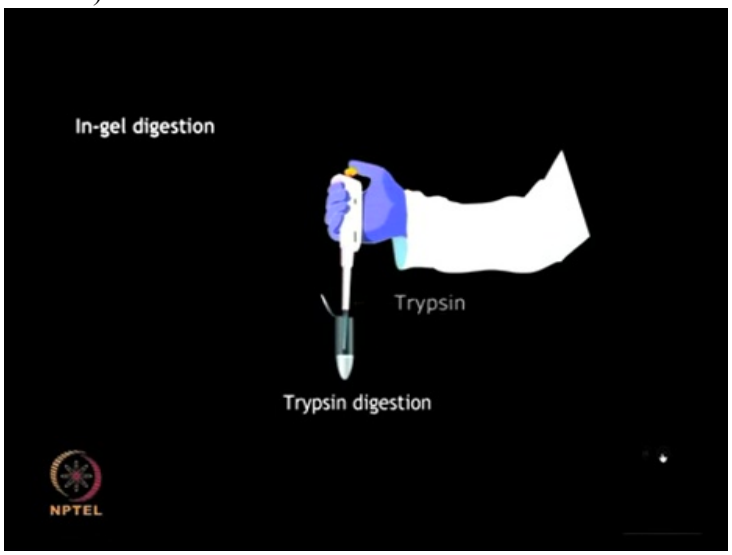

Trypsin is then added to this mixture in order to carry out...

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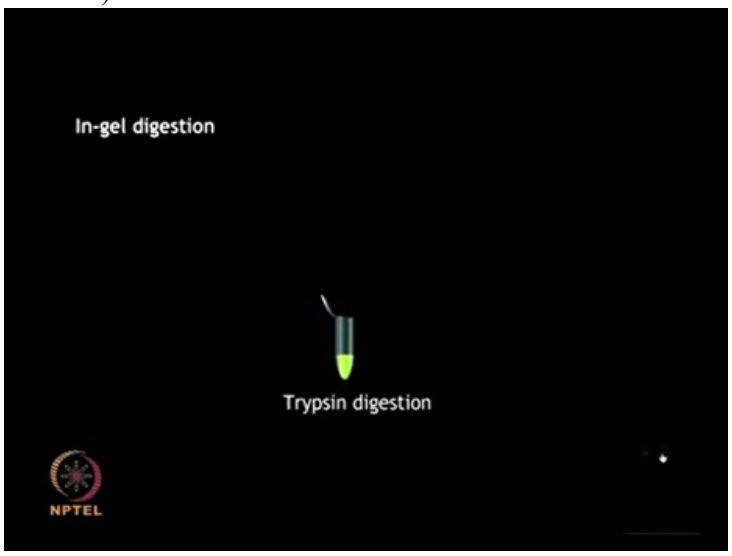

…digestion of the protein

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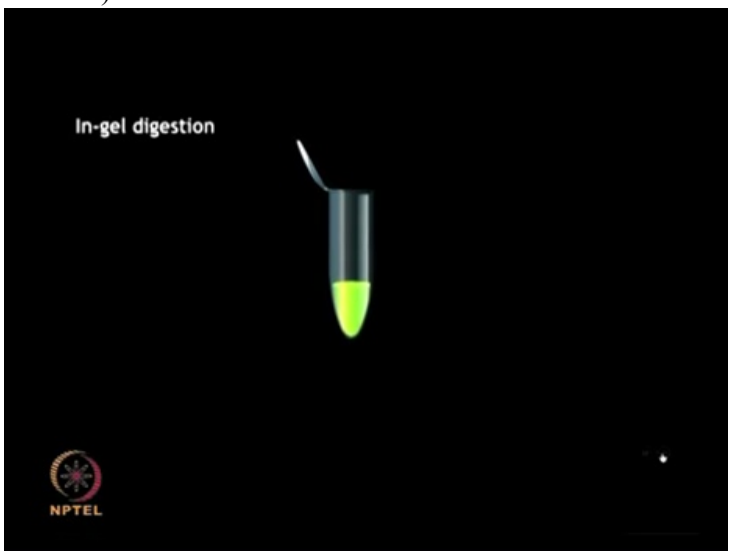

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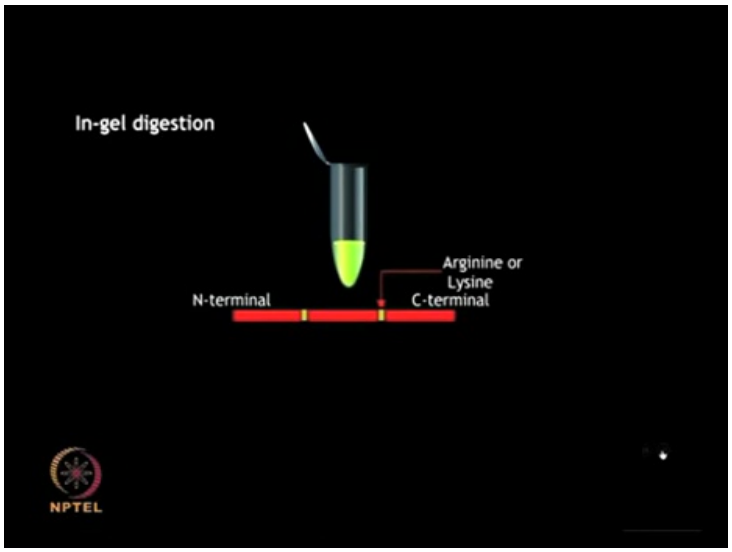

Trypsin cleaves the protein at the C-terminal of its arginine and lysine residues. But that is not always universal. If you have a proline present immediately after …

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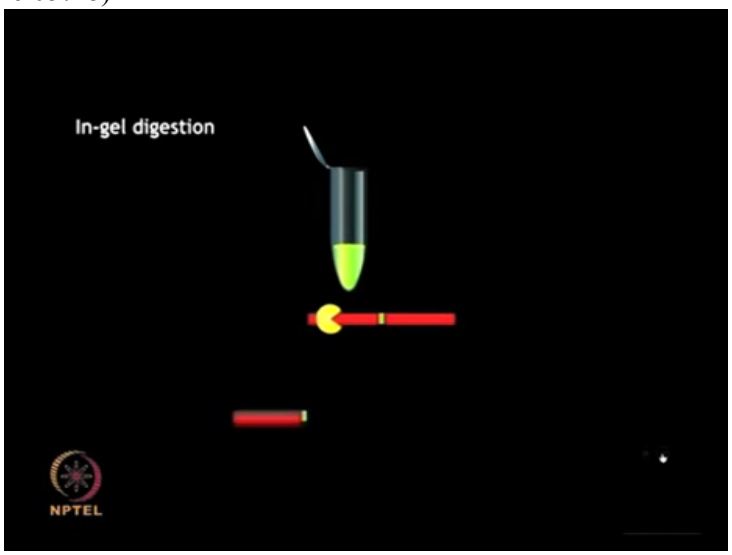

… then it will hinder that.

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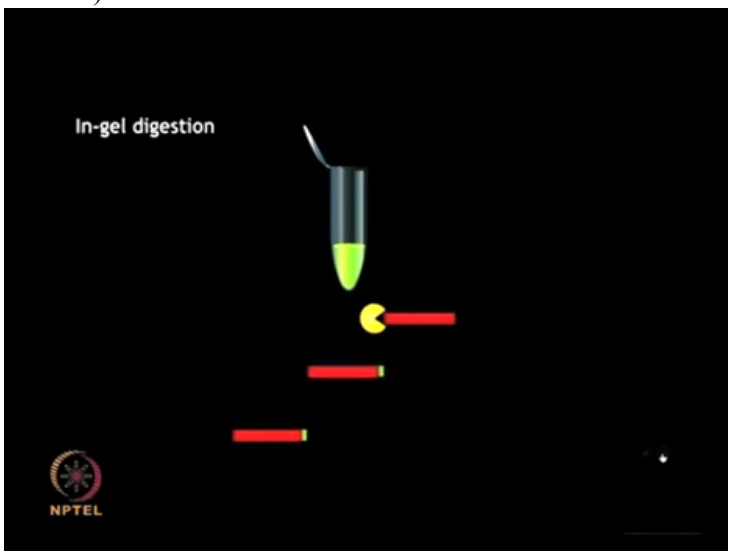

But overall, the protein is ….

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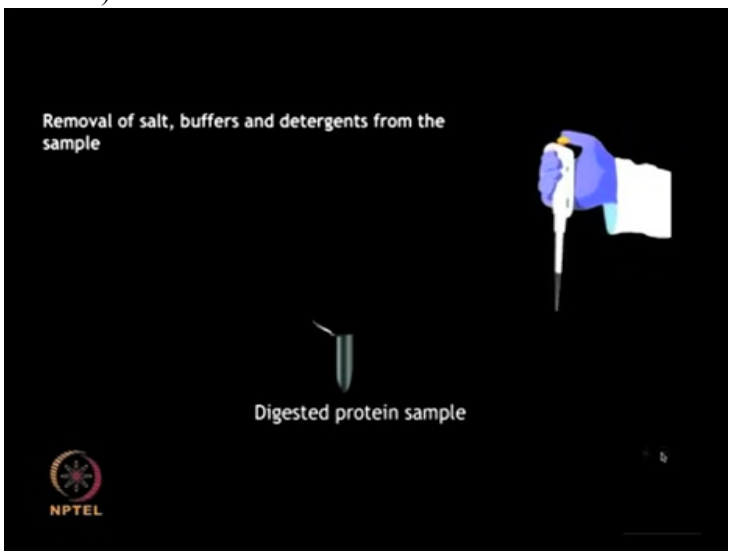

… digested into smaller fragments …

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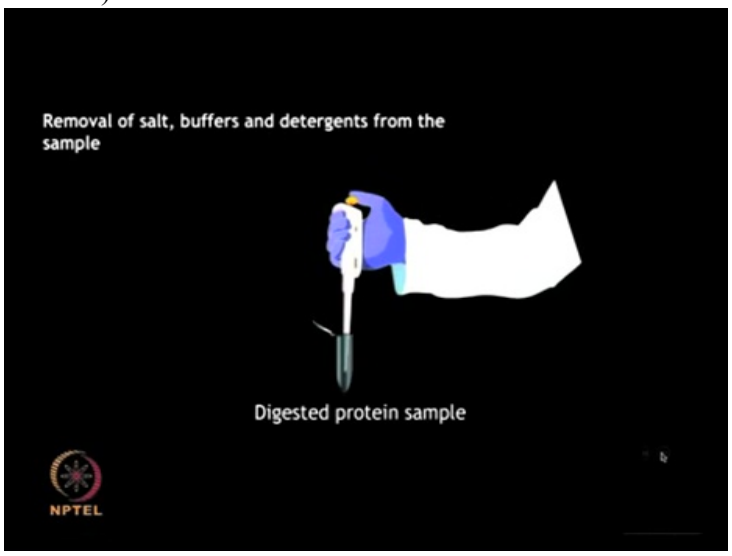

… of manageable size.

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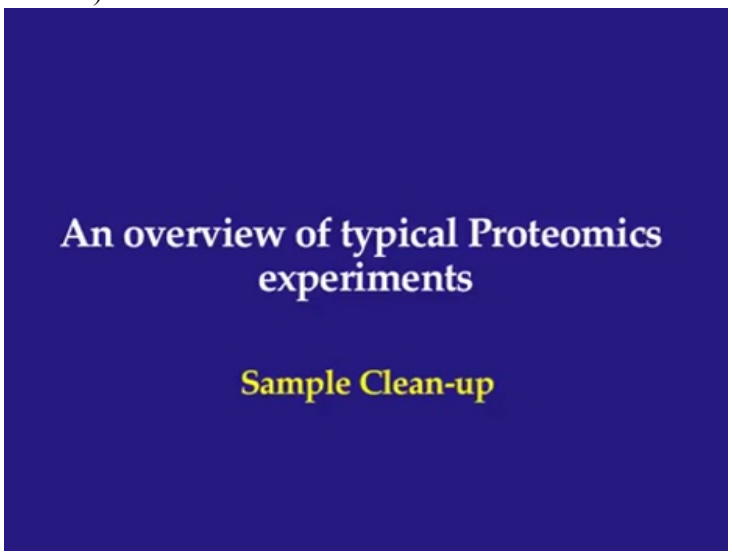

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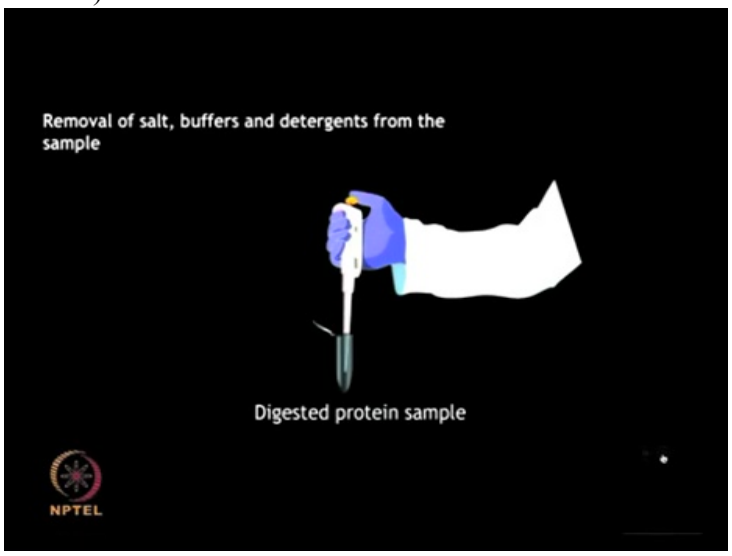

Once the protein sample has been digested, all the salt, buffers and any detergents must be removed from the sample. After doing in-gel digestion…

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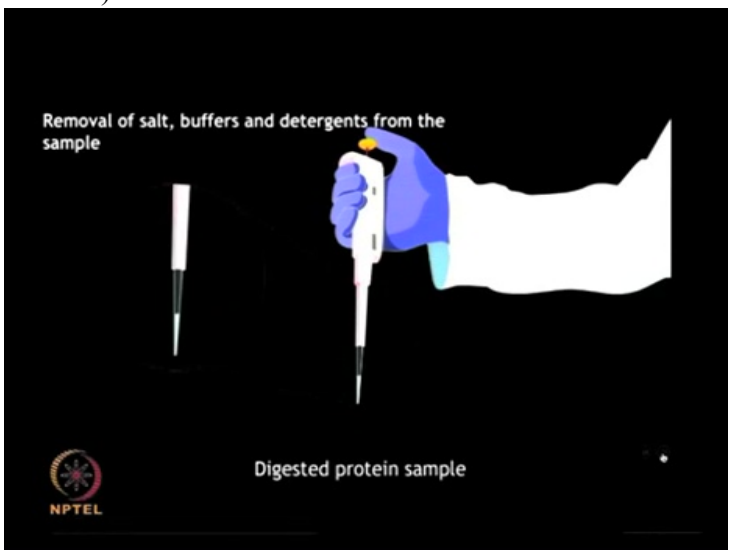

…and before proceeding for the mass spectrometry analysis, in between an efficient step is to use some filters…

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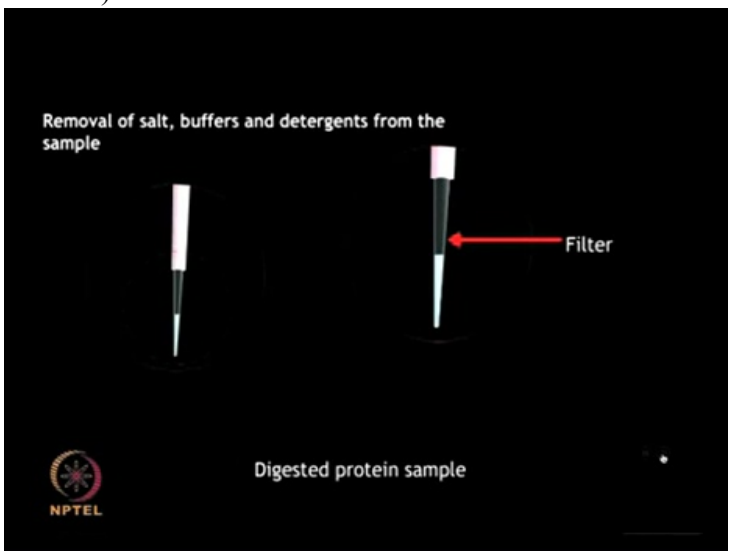

… or ZipTip which can eliminate some of these contaminants and salts. It offers several advantages such as quick verification, sample enrichment and ensures that there is no contamination.

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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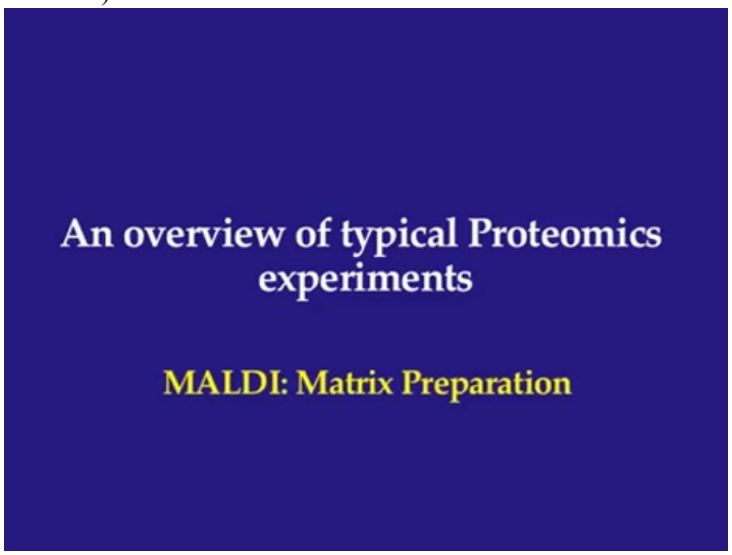

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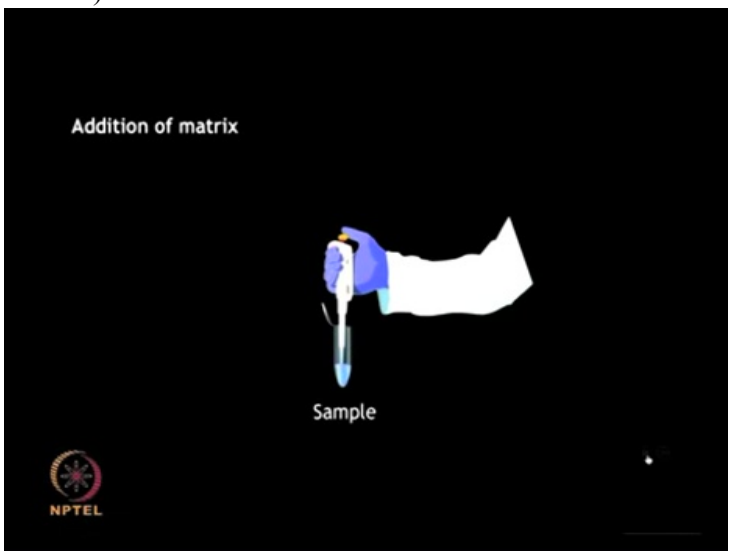

The purified protein sample can be mixed with an aromatic matrix compound such as …

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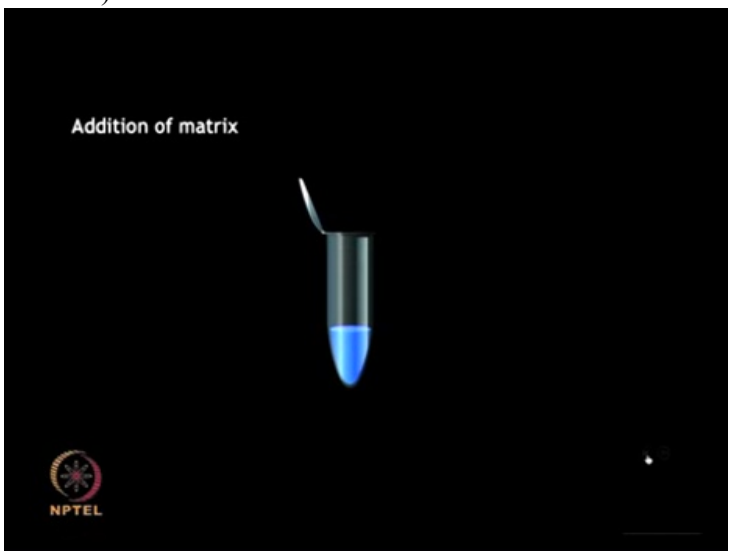

… alpha-cyano-4-Hydroxycinnamic acid or Sinapinic acid in the presence of an organic solvent.

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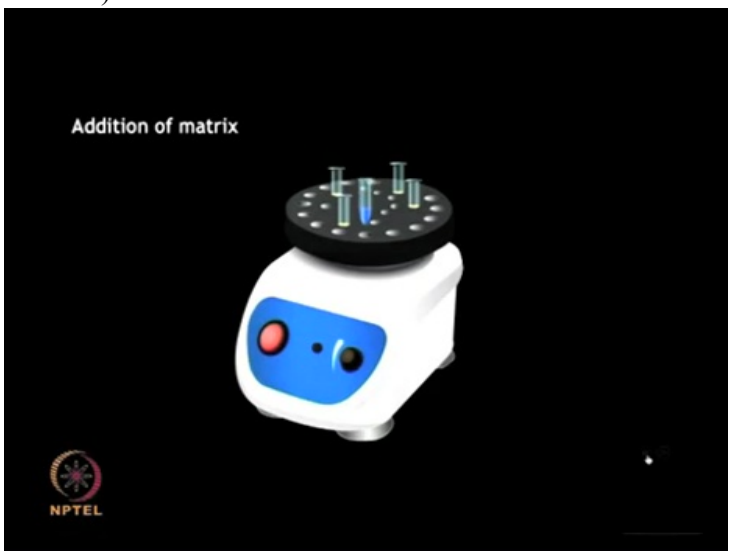

The components are mixed thoroughly.

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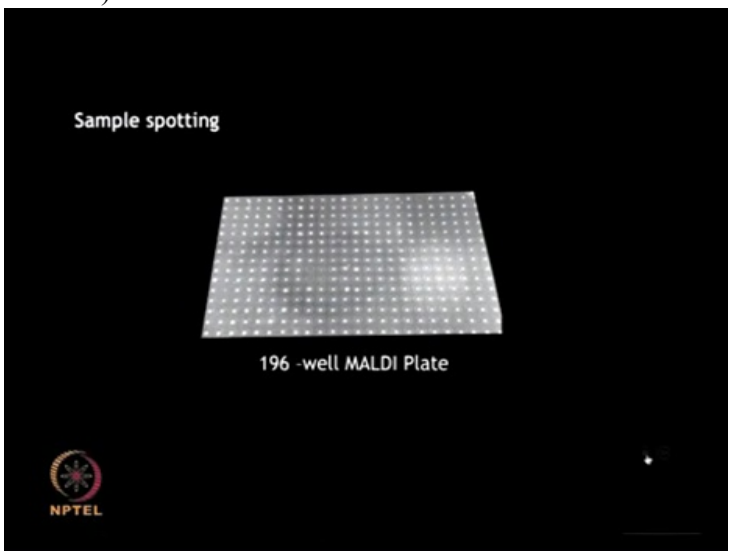

And then the solution containing the organic matrix with the embedded analyte of interest can be spotted on to a metallic MALDI sample plate.

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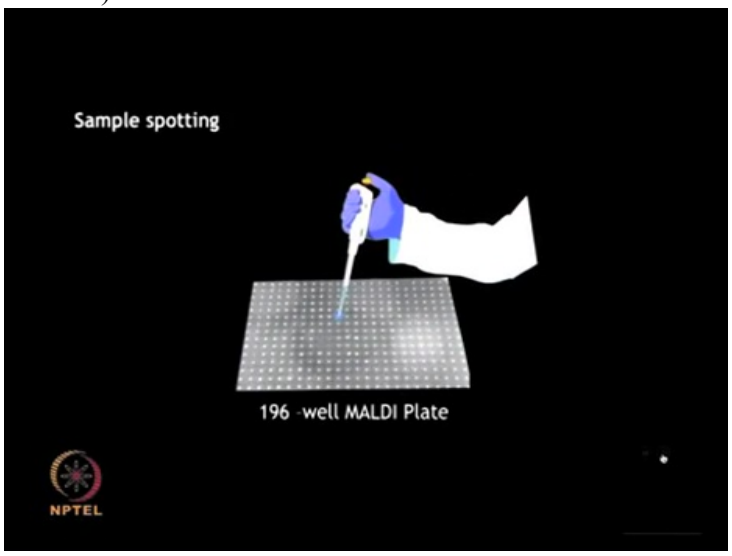

MALDI gives you an opportunity to analyze large number of samples …

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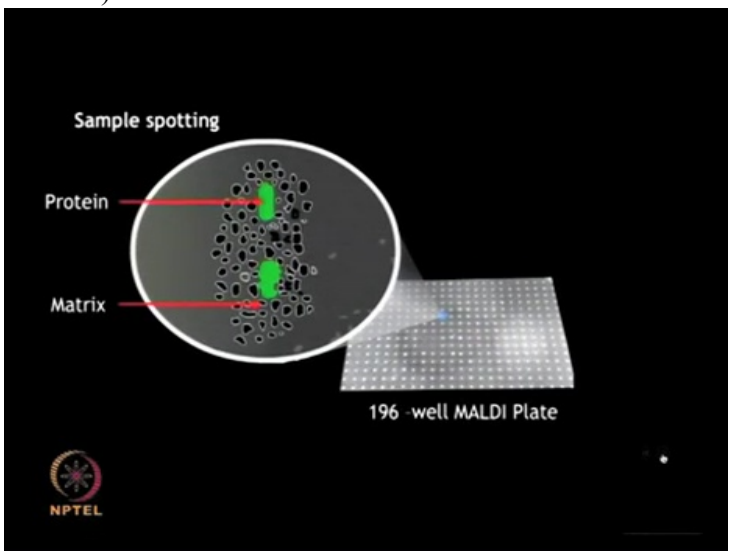

…in a high-throughput fashion.

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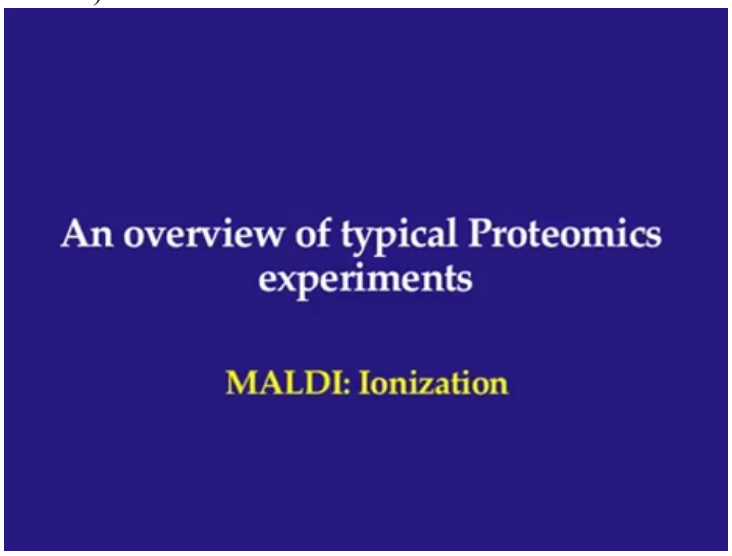

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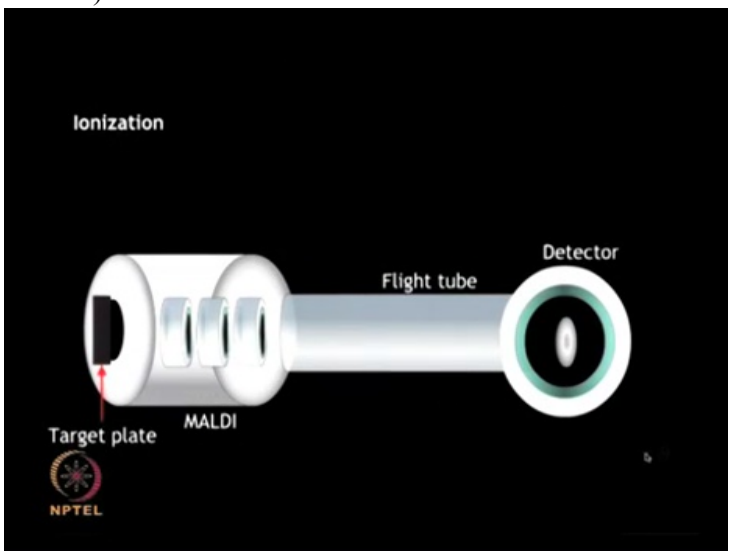

The target plate containing the spotted matrix and analyte can be further placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy …

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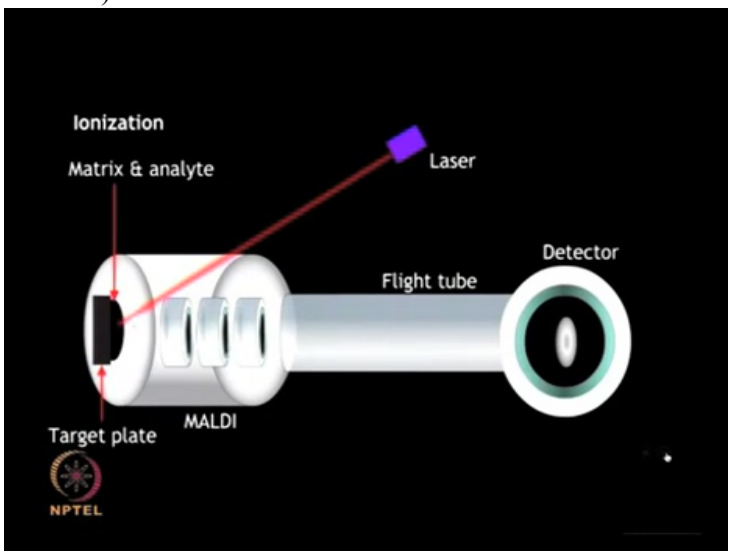

…gets absorbed by the matrix and is transferred to the analyte molecules …

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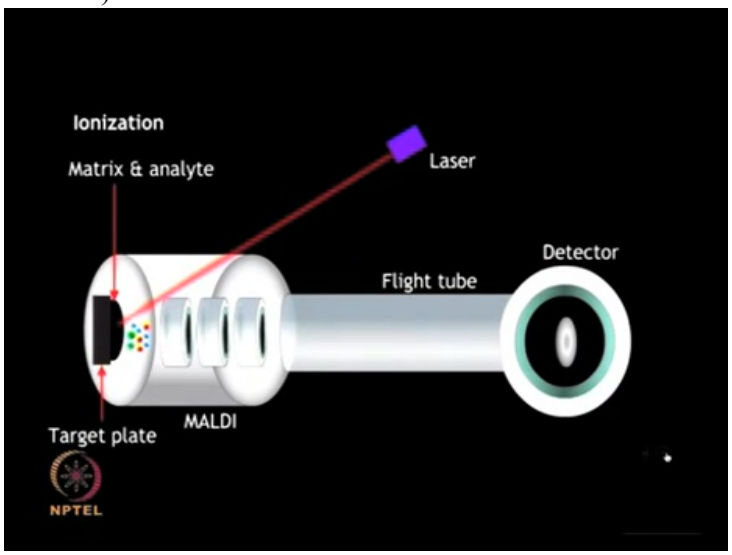

… which undergo rapid sublimation resulting in gas phase ions

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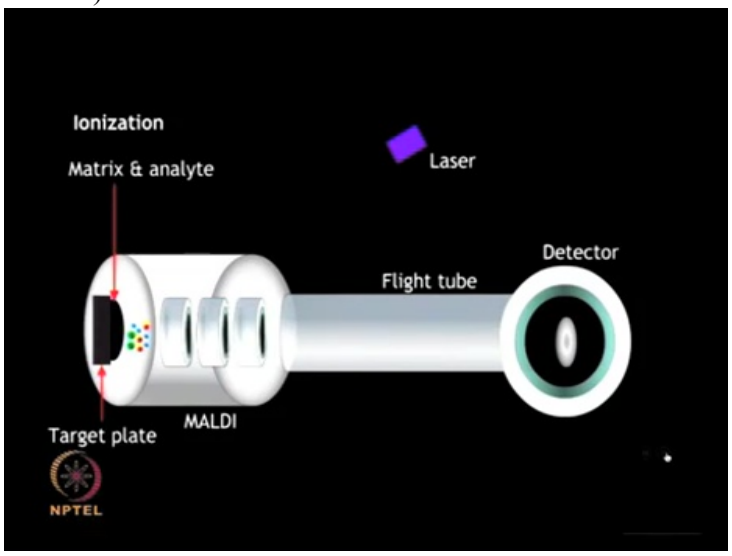

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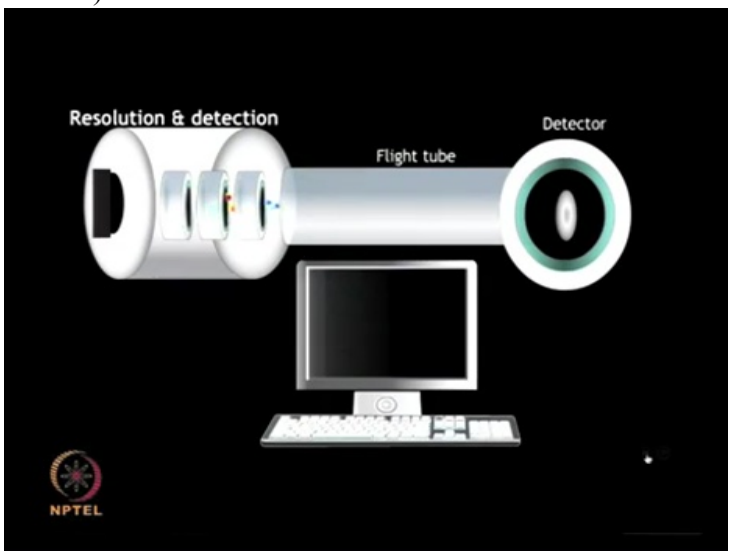

The gas phase ions generated are accelerated and travel through the flight tube at different rates.

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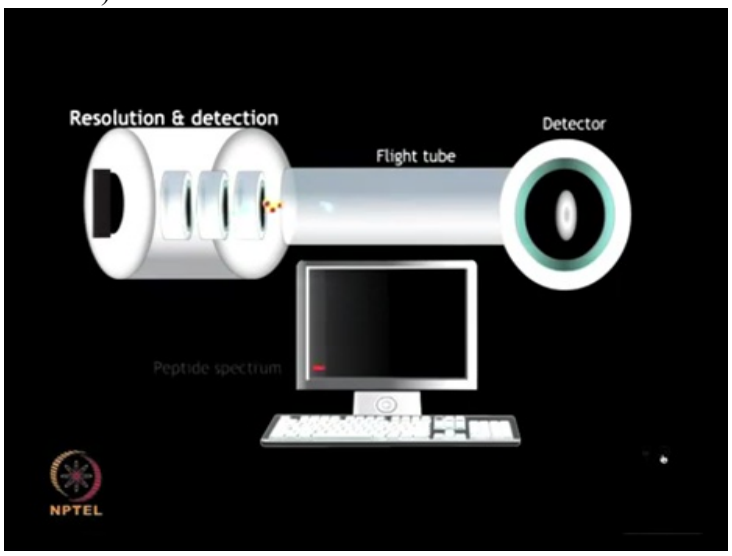

The lighter ion moves rapidly …

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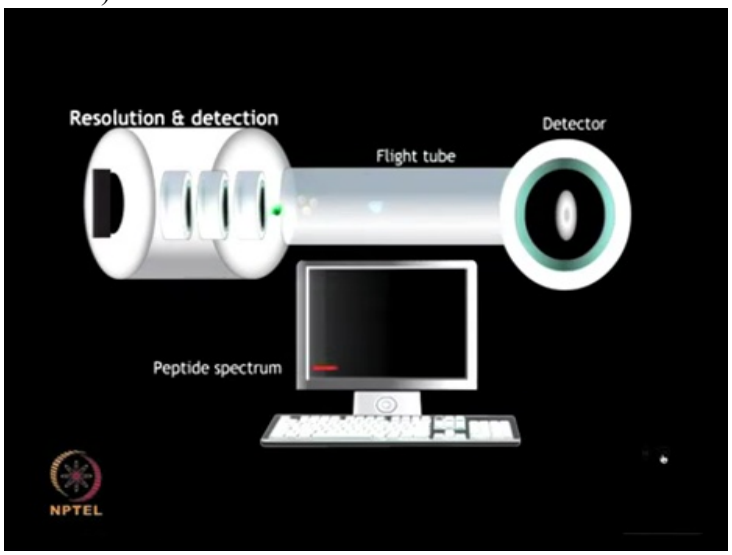

…and reaches the detector first while the heavier ions …

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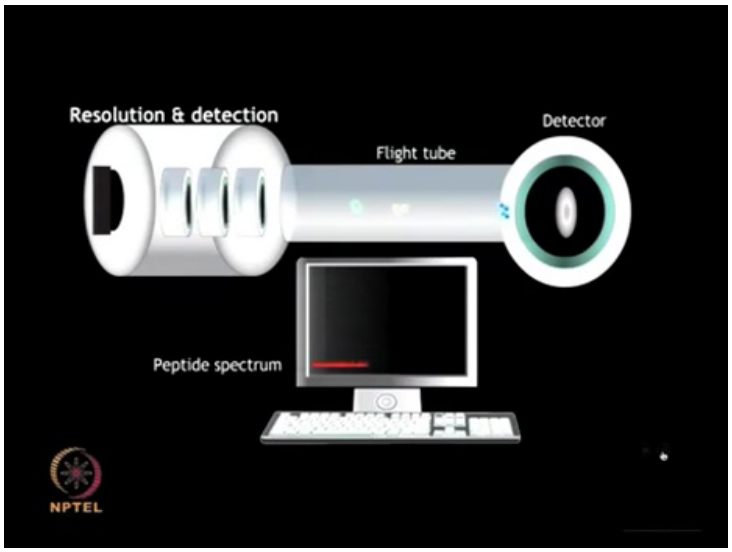

…migrate slowly. These ions are resolved and detected …

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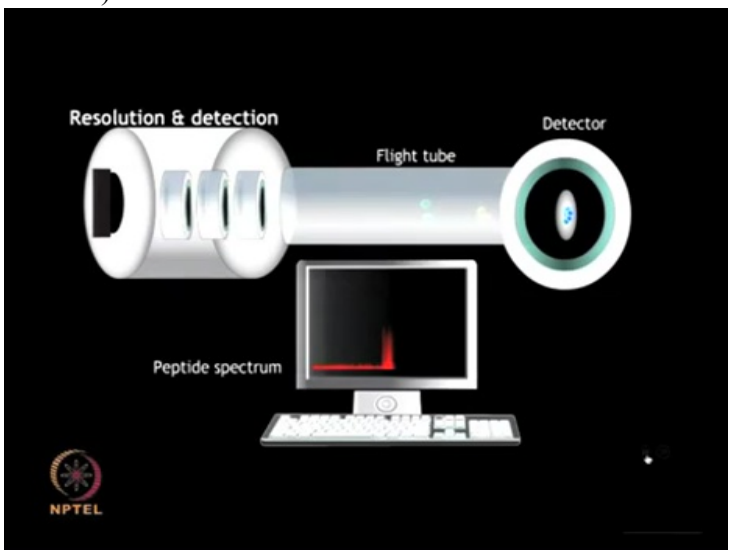

…on the basis of their mass to charge ratio and a mass spectrum is generated.

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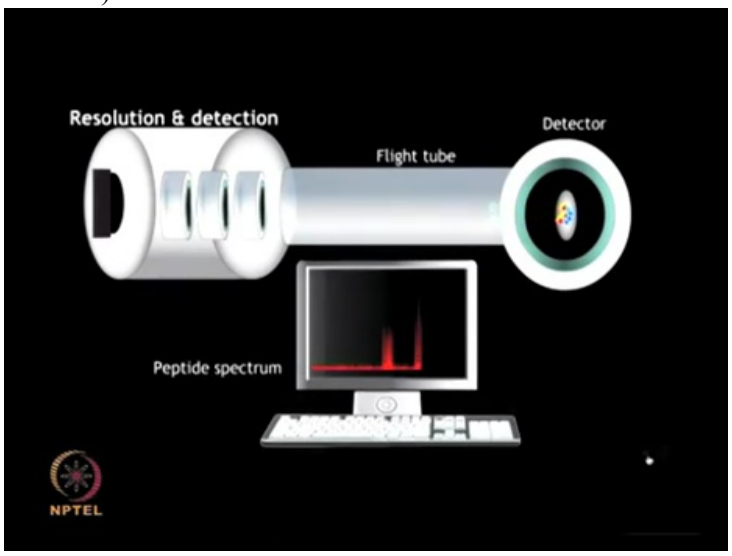

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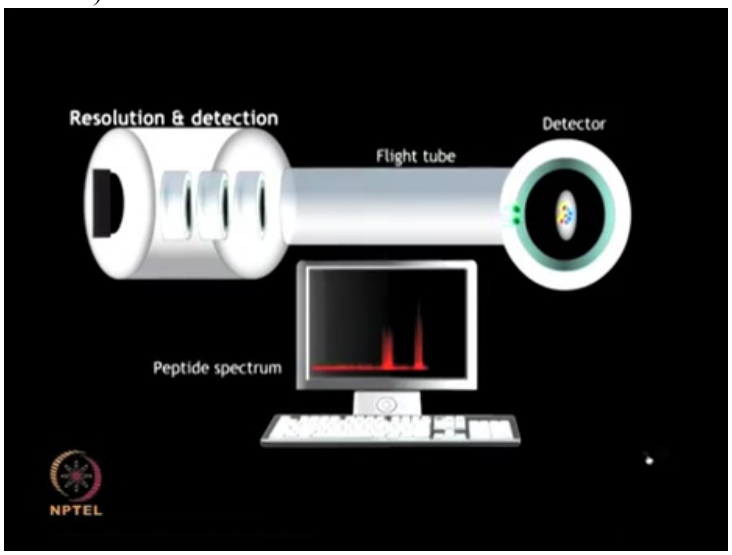

Parameters such as geometric design….

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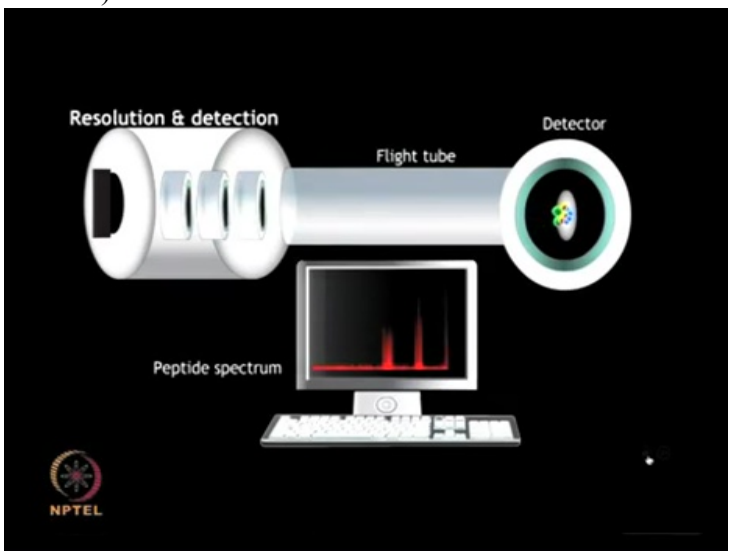
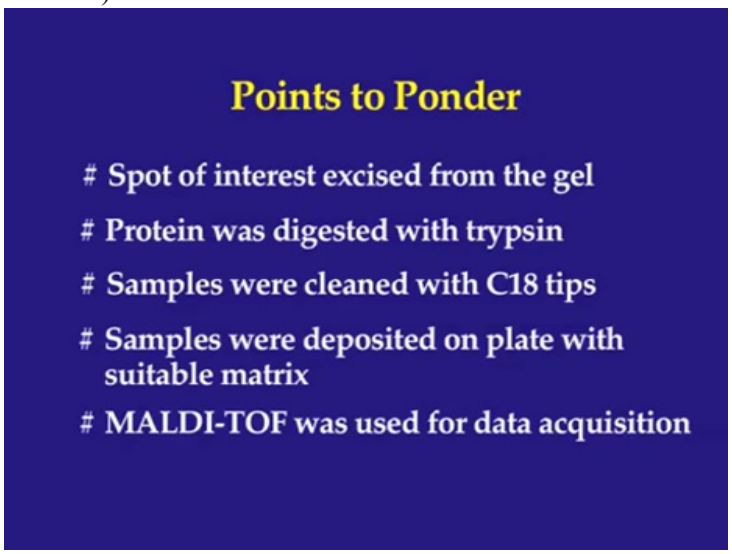


… power supply quality, calibration method, sample morphology, ion beam velocity etc. all of these factors affect the accuracy of mass detection.

(Refer Slide Time 10:10)



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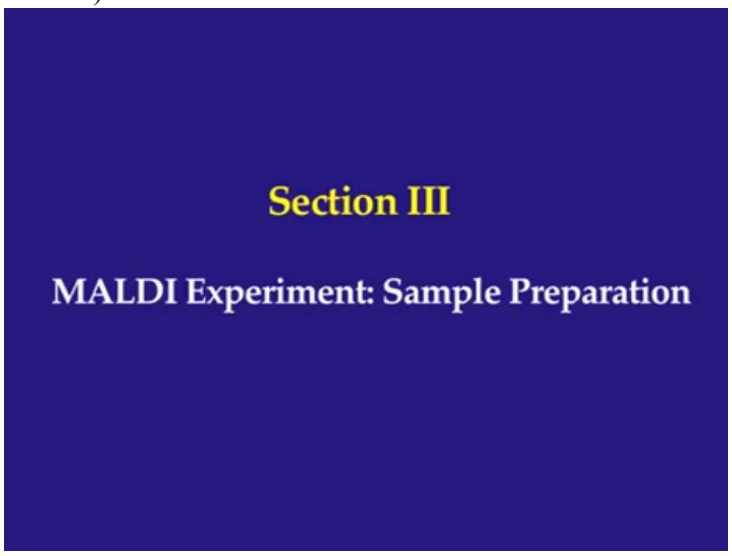

So now you know how to perform the cleaning step by using ZipTips. Now you have the sample ready and you have selected the matrix. So now let me show you these various steps involved before you can actually start the MALDI experiment

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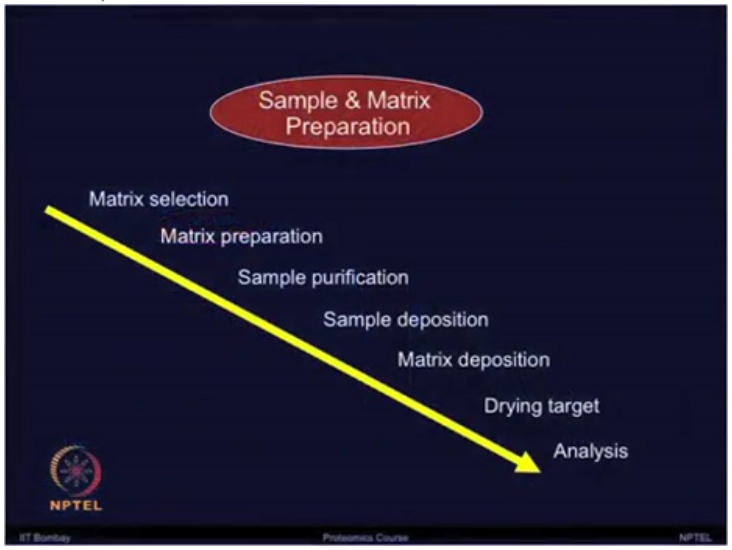

So, you need to select the matrix, you need to prepare the matrix. You have already done the sample purification.

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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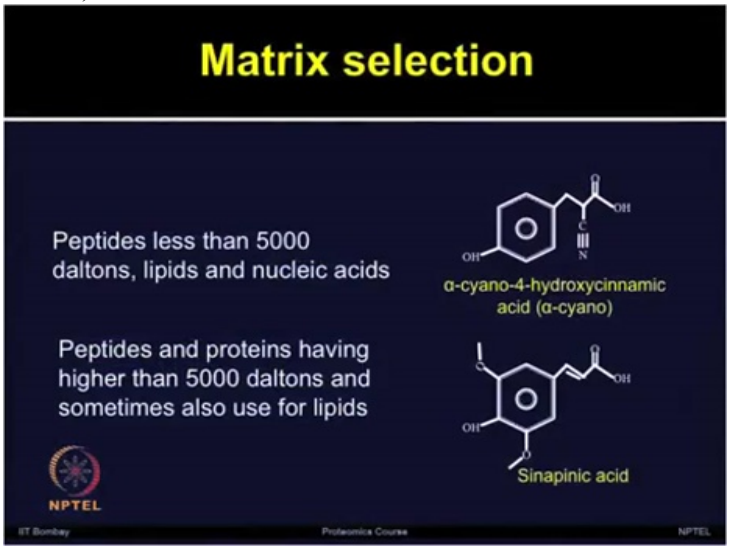

So, the one is alpha-cyano-4- hydroycinnamic acid. When you have peptides less than 5000 Daltons or lipids and nucleic acids one can use this matrix. One can also use Sinapinic acid if peptides and proteins are having more than 5000 Daltons and it can also be used sometimes for the lipids.

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

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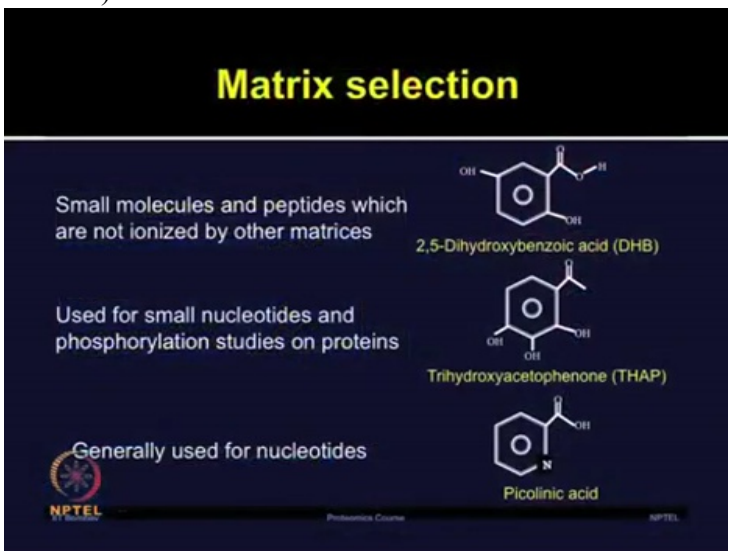
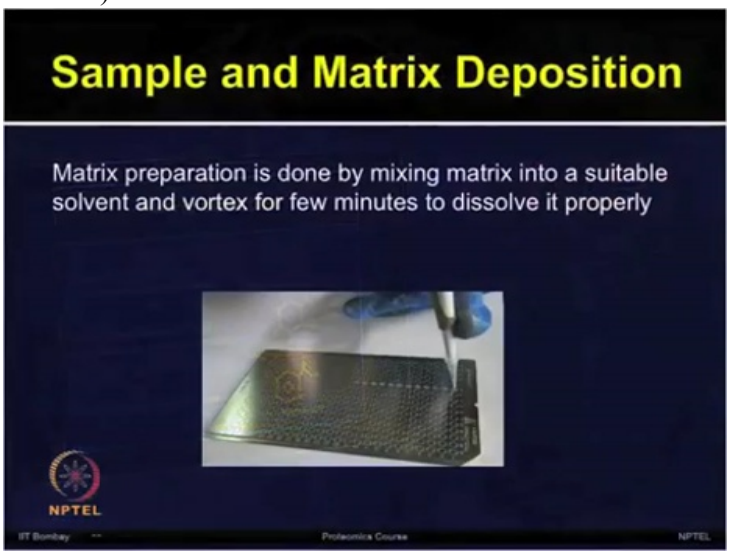


…small molecules and peptides which are not ionized by the other molecules can be analyzed by using this matrix. TriHydroxyAcetoPhenone, THAP, this is used for small nucleotides and also used for phosphorylation and specialized applications.

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.

(Refer Slide Time 13:57)



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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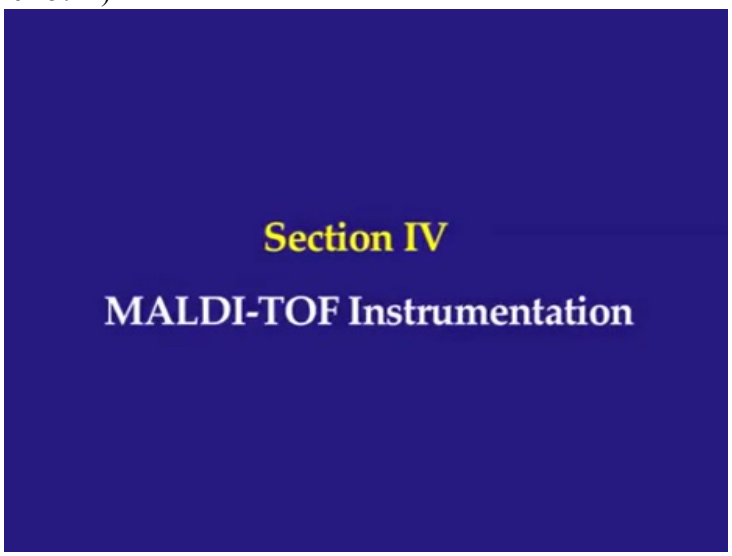

After spotting is done and the MALDI plate is dried almost 30 minutes, then the instrument can be turned on and MS analysis can be performed.

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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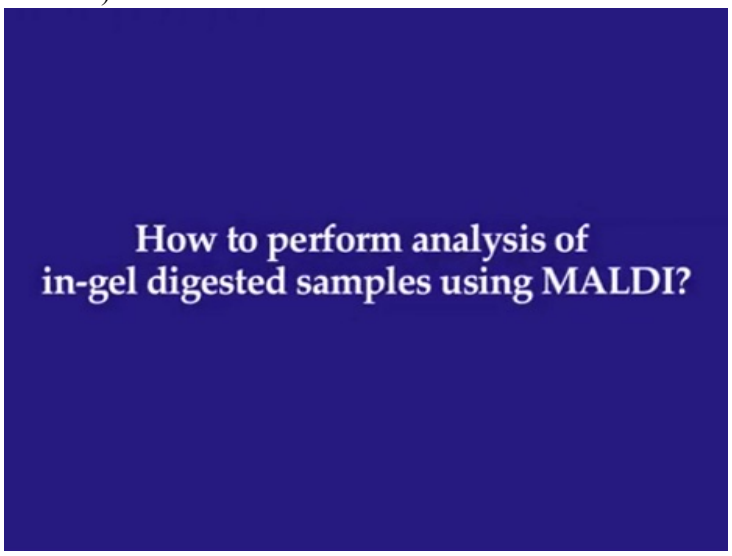

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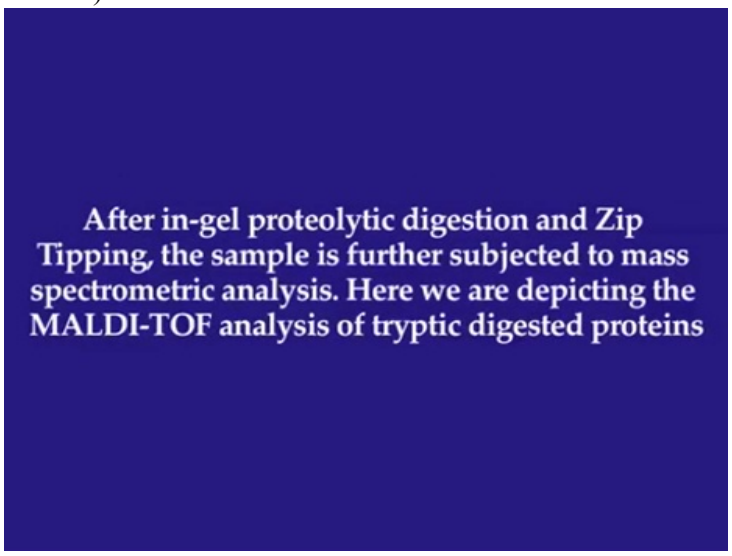
(Refer Slide Time 16:33)



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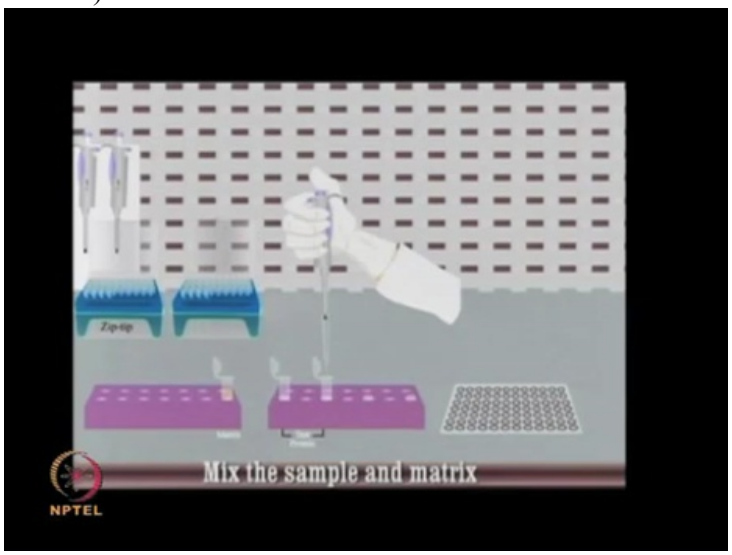

MALDI is performed in two steps. In first step, the compound for the analysis should be dissolved in a solvent containing small organic molecules, known as matrix.

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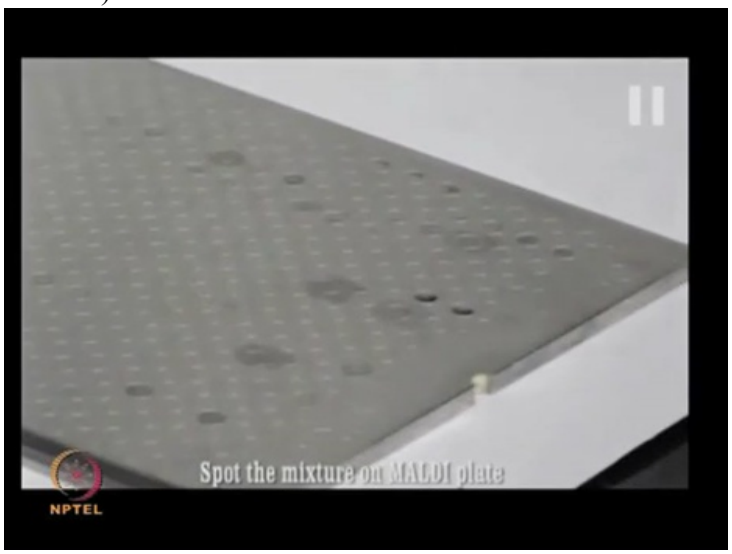
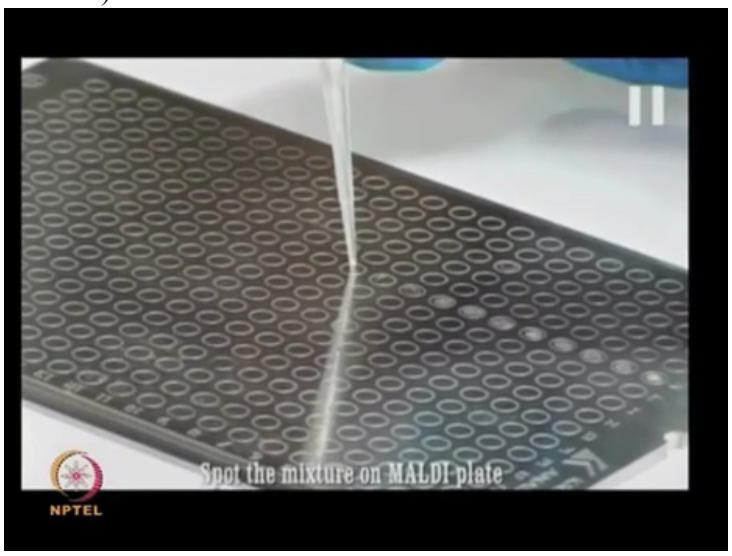
This mixture is dried before analysis and liquid solvent used in the preparation of the solution is removed.

(Refer Slide Time 17:11)



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.

(Refer Slide Time 17:33)



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

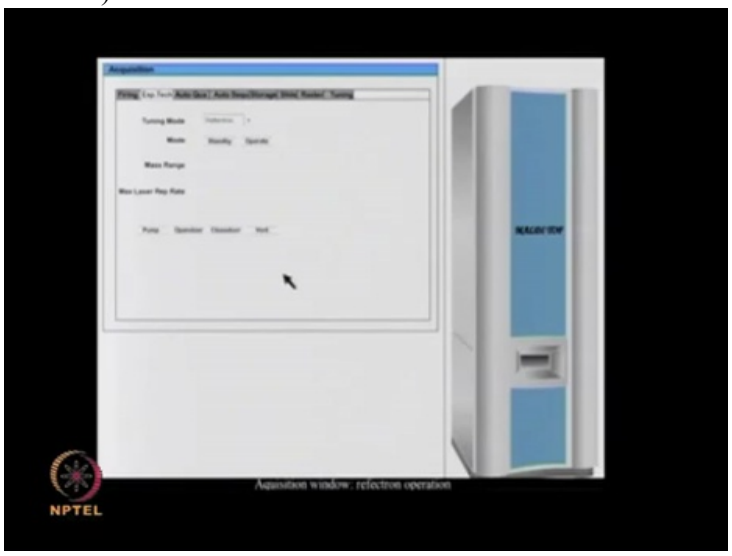
Completed on the MALDI plate ….

(Refer Slide Time 17:54)

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”.

(Refer Slide Time 18:29)

Insert MALDI target plate …

(Refer Slide Time 18:32)

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

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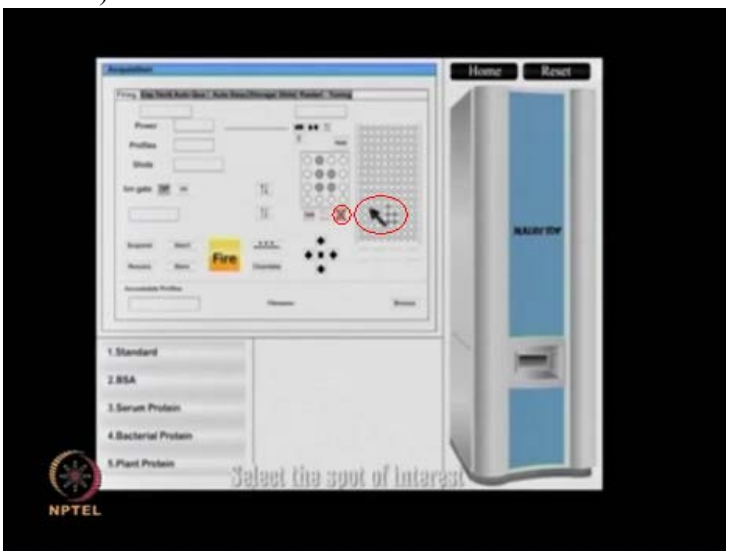
And now by using the software, close the door.

(Refer Slide Time 18:49)

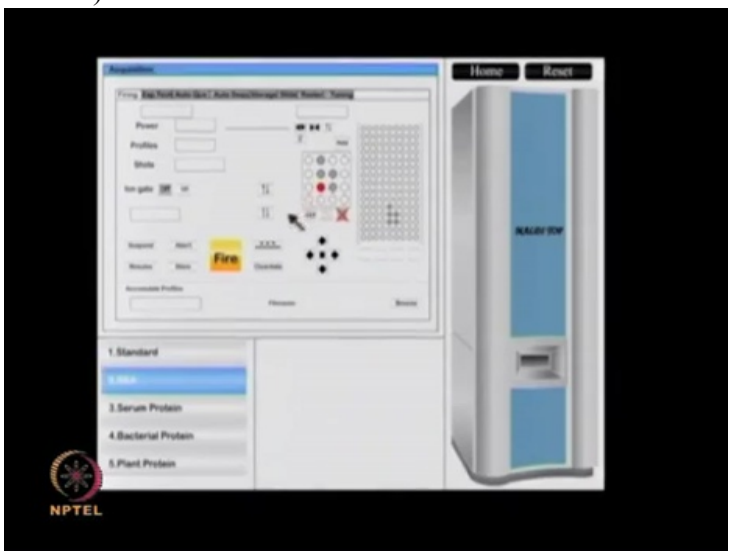
The door of the insertion chamber is now closed.

(Refer Slide Time 18:55)



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 …

(Refer Slide Time 19:10)



select “go to the location”.

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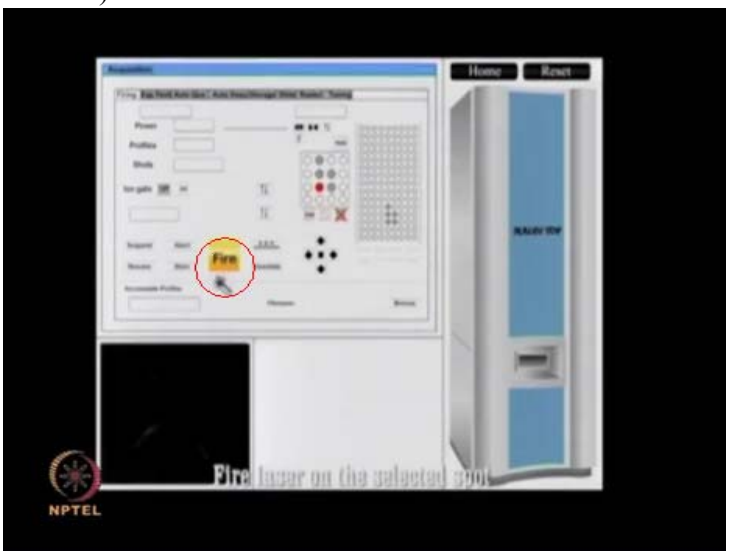
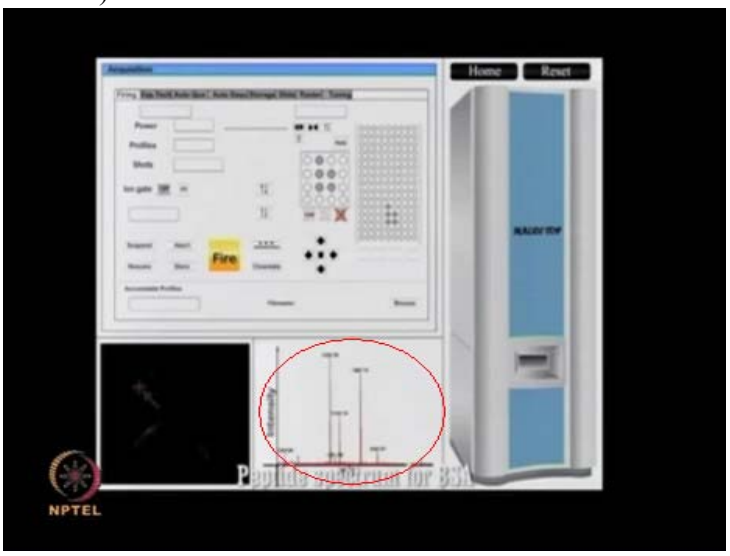


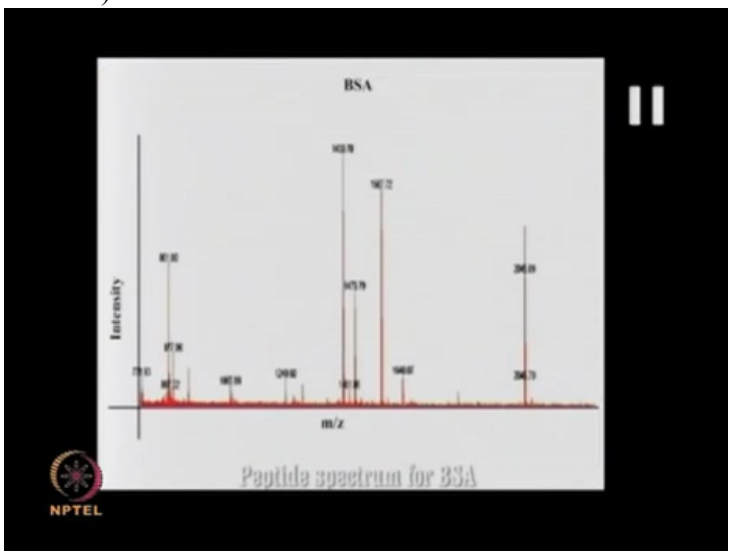
You can now do the laser bombarding and ….

(Refer Slide Time 19:15)



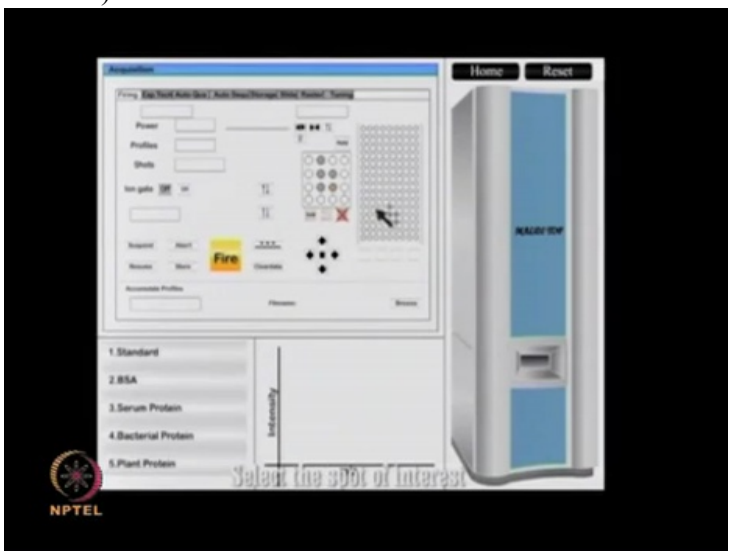
… peptide spectrum is generated.

(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)



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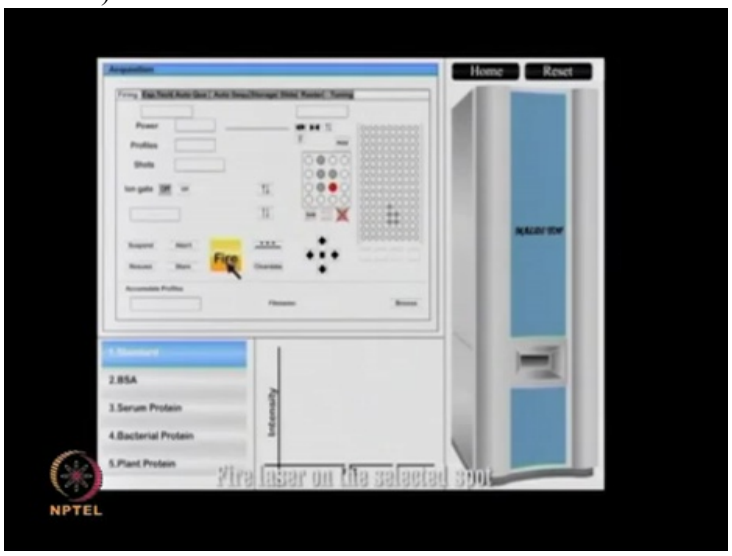
Same process can be performed for …

(Refer Slide Time 19:42)



….different spots and different regions

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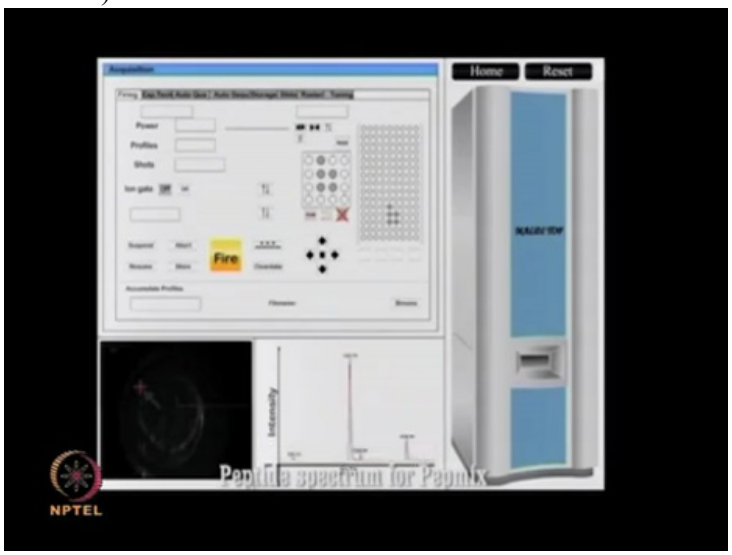
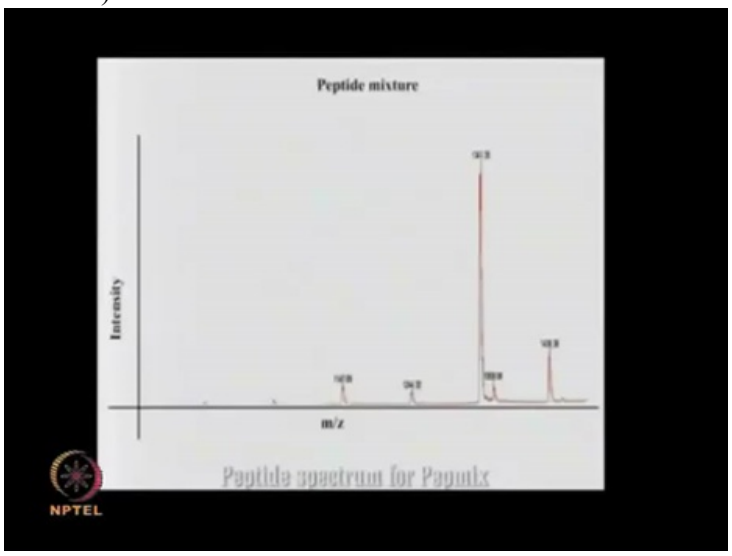


Now we have shown here …

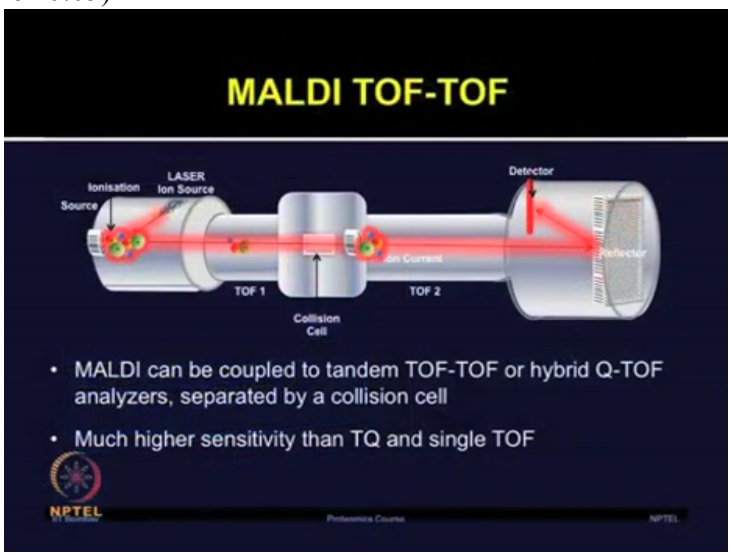
(Refer Slide Time 19:51)



…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 Quadrapole 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.

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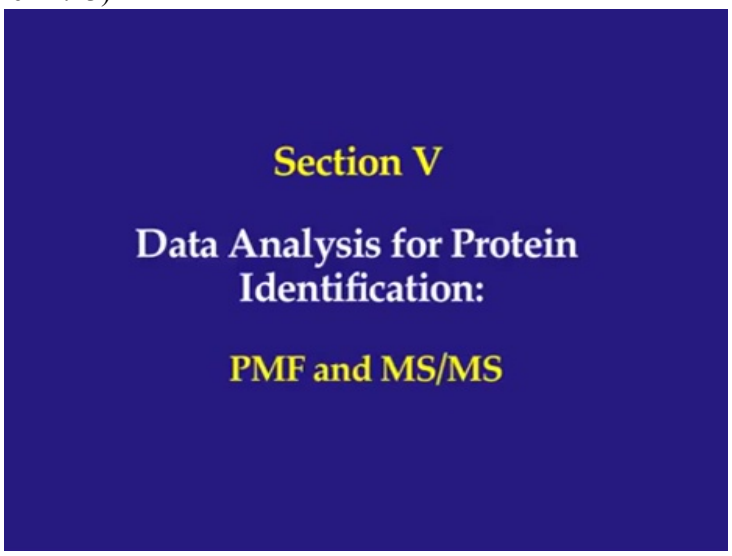

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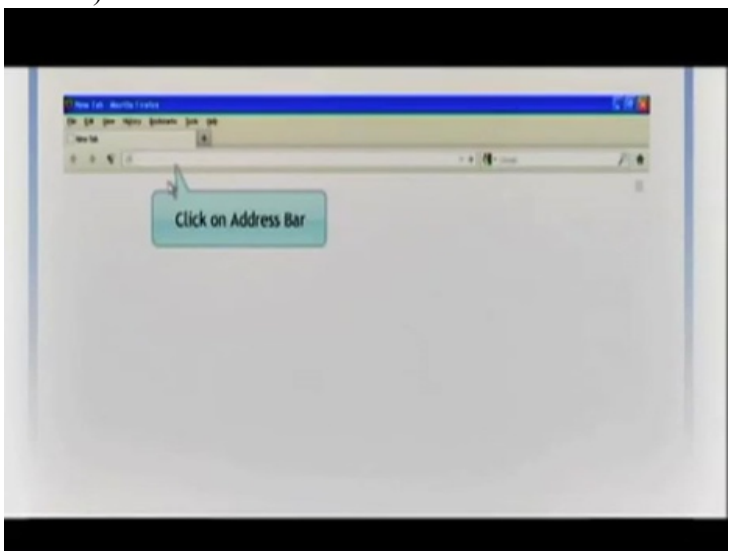
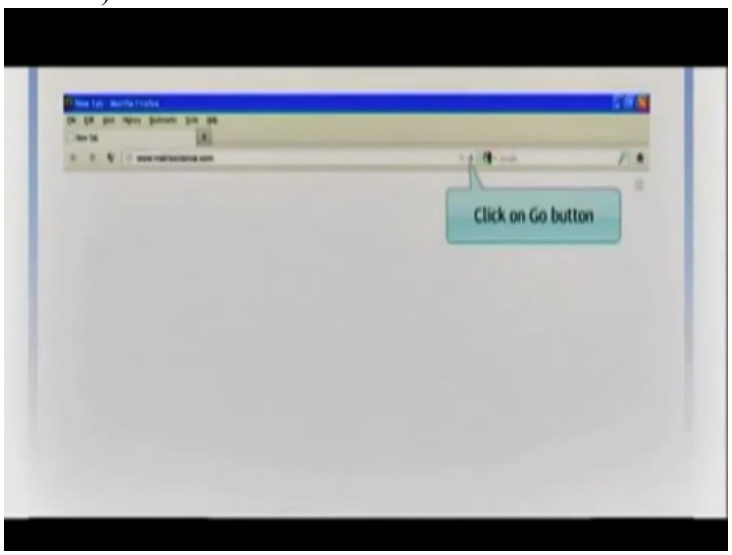


Open the Matrix Science browser window to carry out online data analysis.

(Refer Slide Time 21:37)



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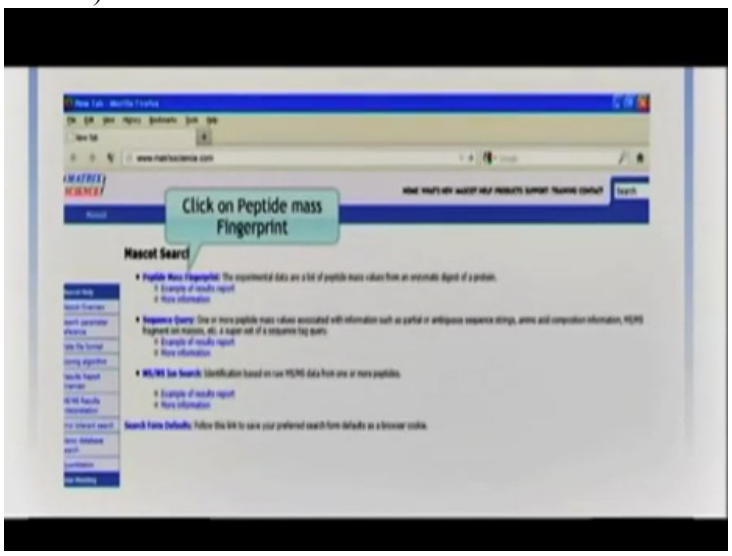

For peptide analysis, click on peptide mass fingerprinting.

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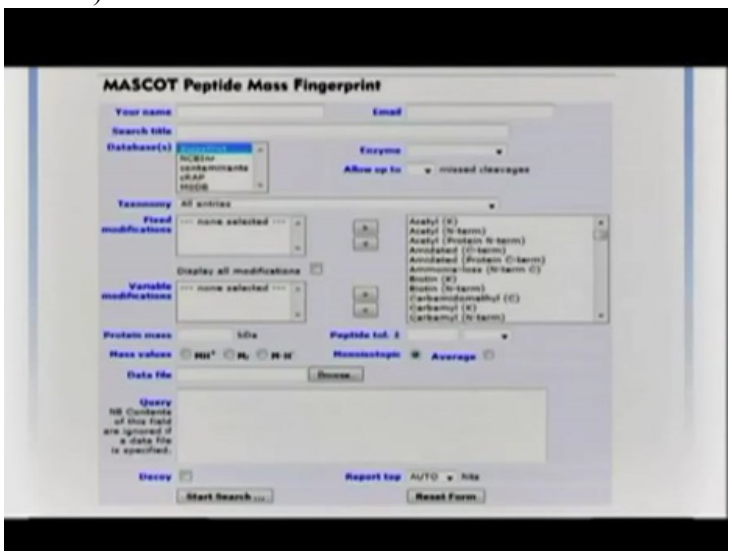

Mascot peptide mass fingerprint. Please enter the …

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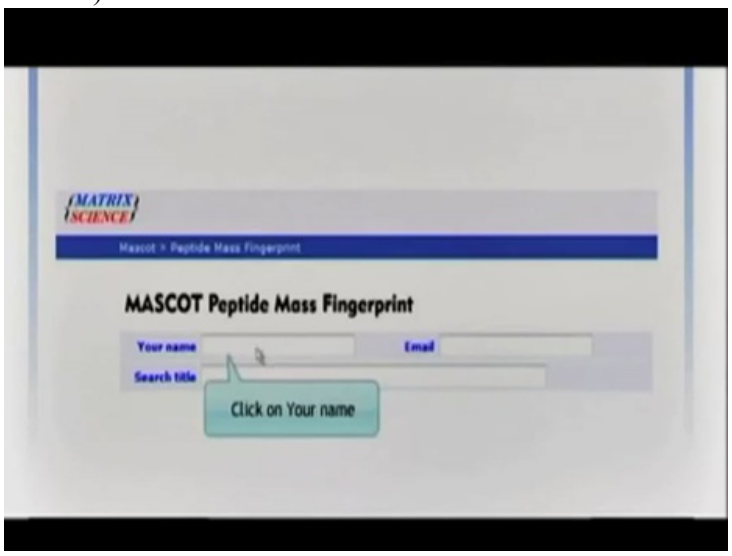

…User Id and details to acquire …

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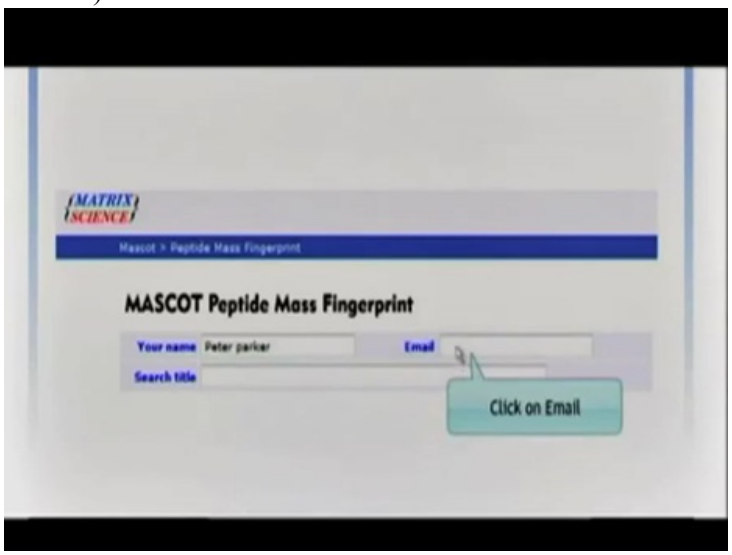

… information in case of …

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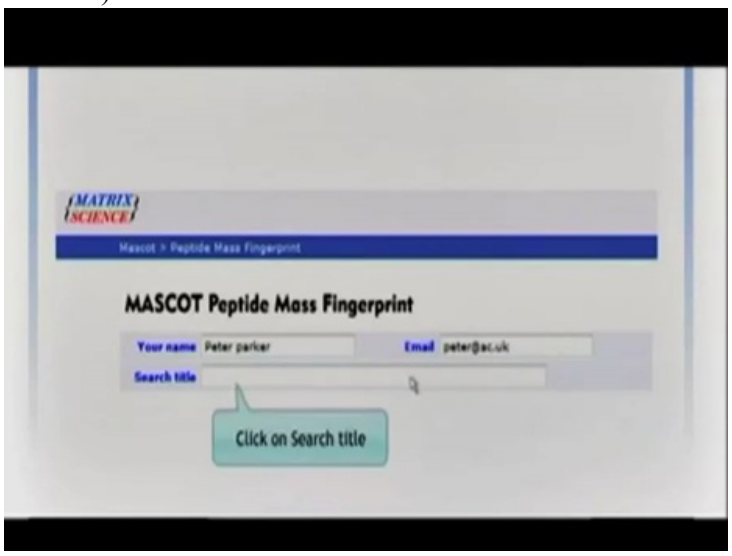

… any network loss

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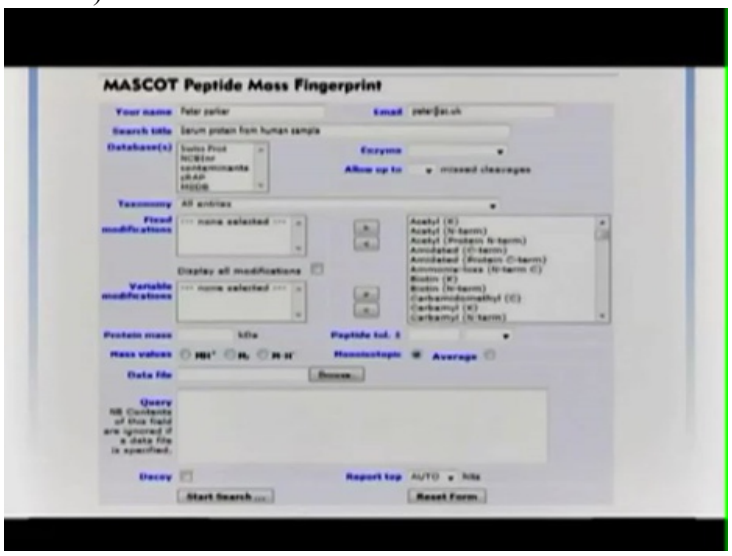
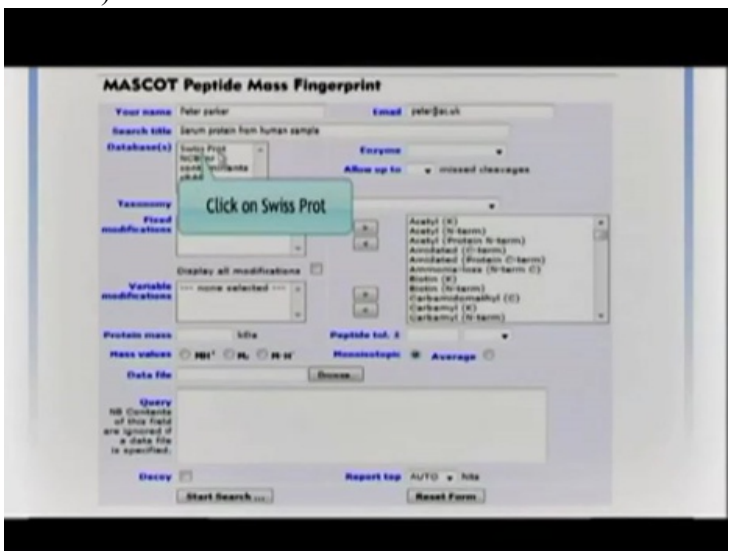
(Refer Slide Time 21:56)



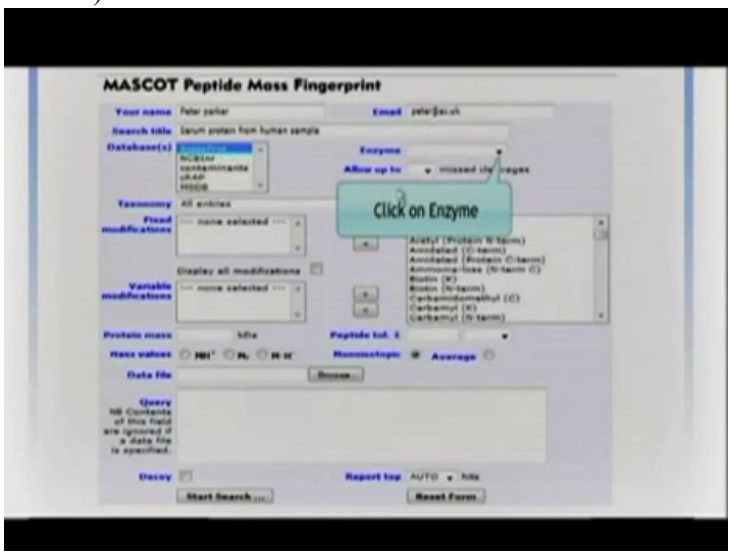
The following parameters should be selected.

(Refer Slide Time 22:03)



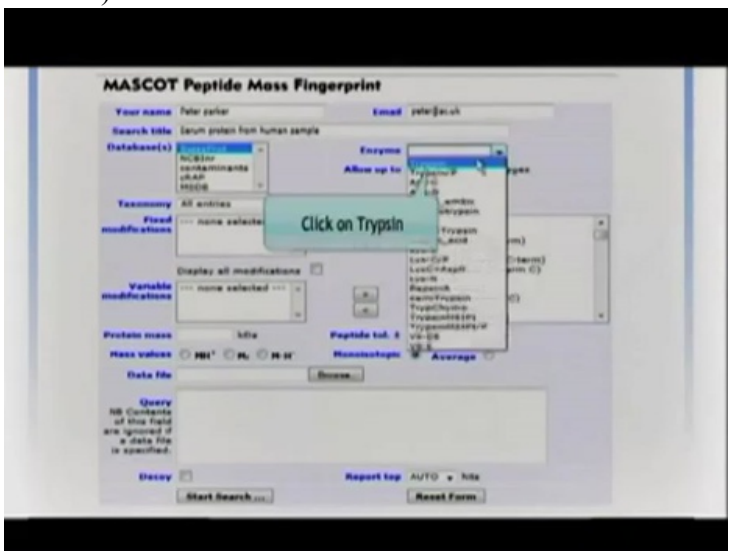
Database primary databases include SwissProt and NCBI. Select SwissProt database for data analysis.

(Refer Slide Time 22:12)



Enzyme, select enzyme …

(Refer Slide Time 22:17)



… as Trypsin.

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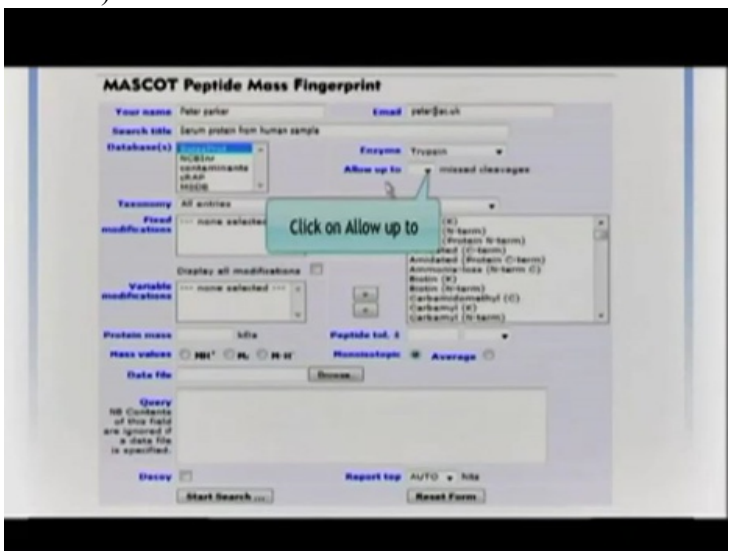
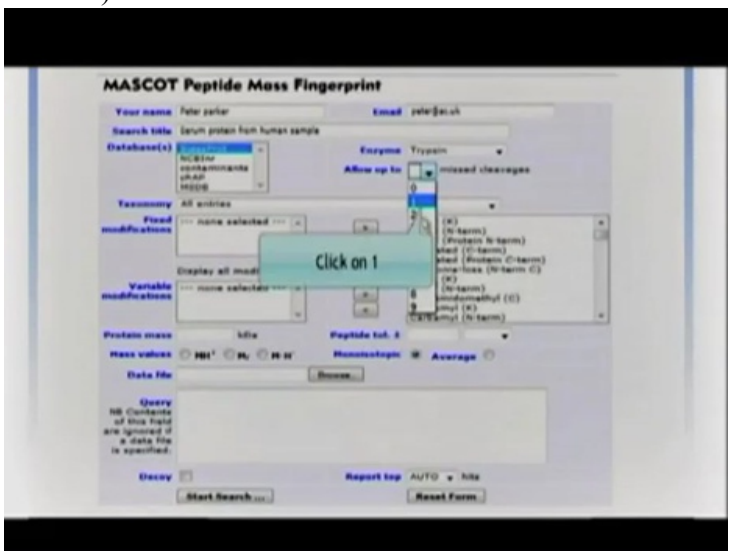
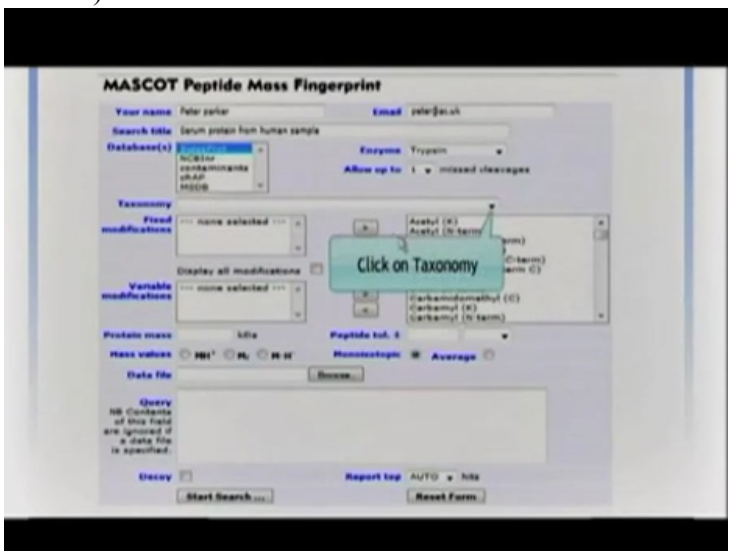


Missed Cleavages, missed cleavages are allowed up to 1

(Refer Slide Time 22:24)

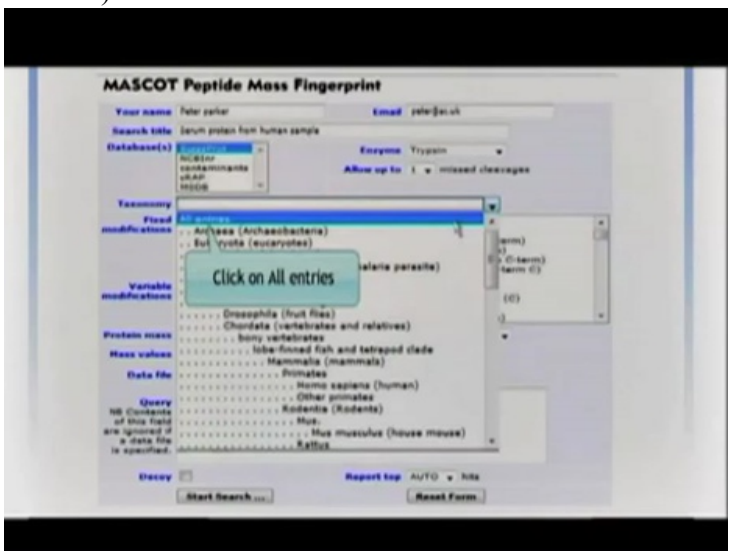


(Refer Slide Time 22:25)



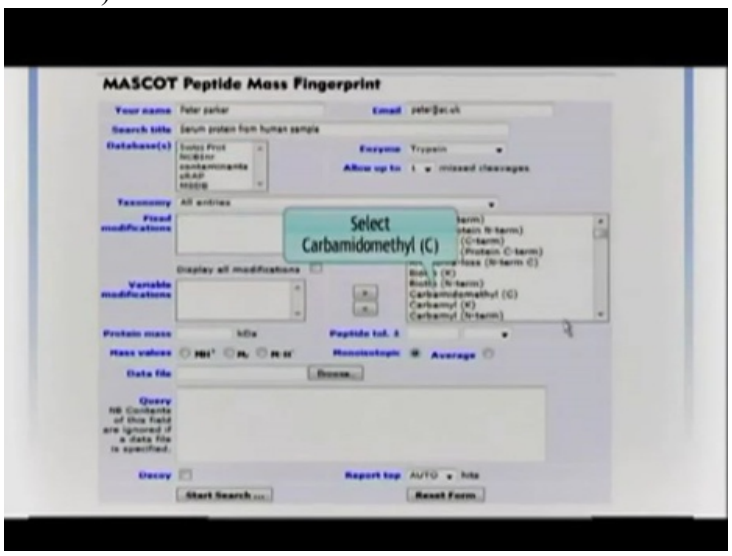
Next is taxonomy. The protein extracted from the biological specimen has to be assigned …

(Refer Slide Time 22:30)



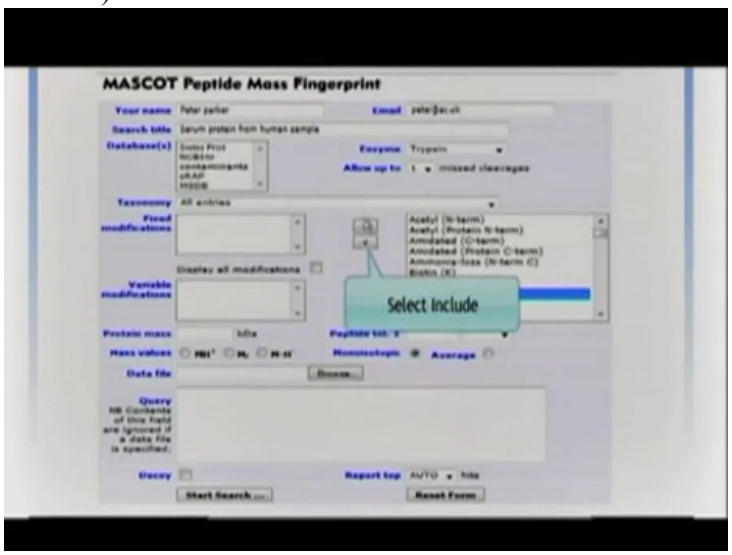
… to a particular species or a group of species to which the sample belongs. When you are not sure of organism select all entries.

(Refer Slide Time 22:41)



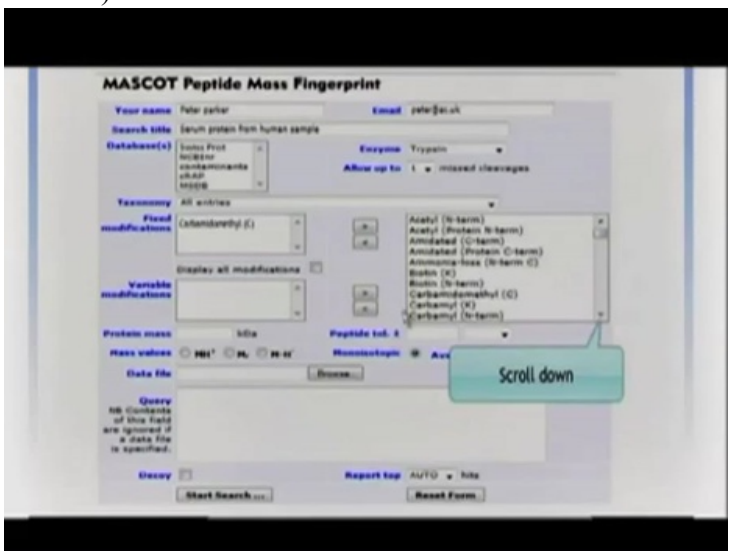
Fixed modification, select carbamidomethyl, fixed modifications are applied …

(Refer Slide Time 22:48)



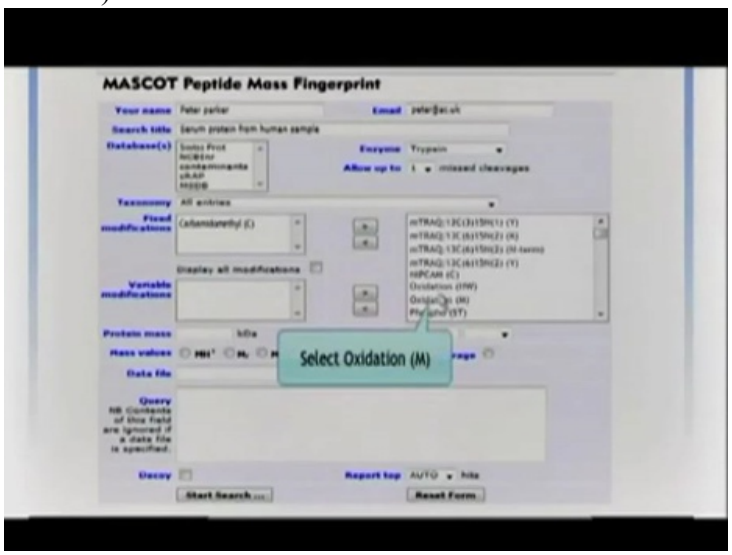
… collectively across the database to account for change in mass of specific residue ….

(Refer Slide Time 22:55)



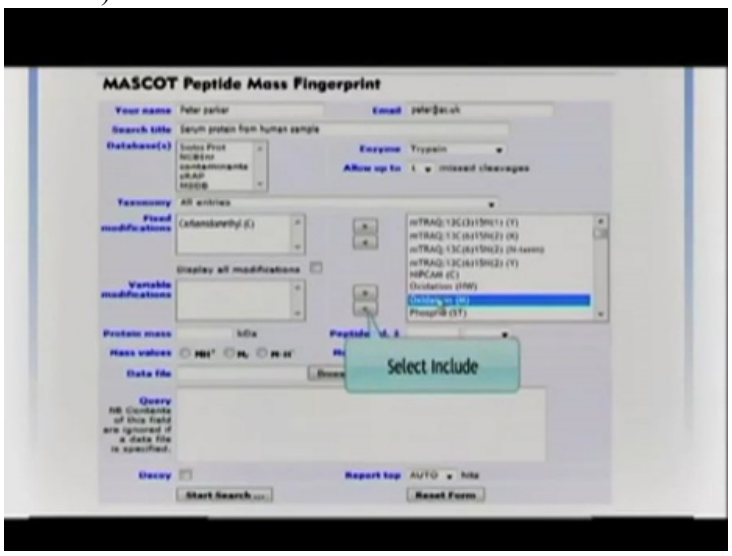
Now scroll down and select oxidation.

(Refer Slide Time 23:01)



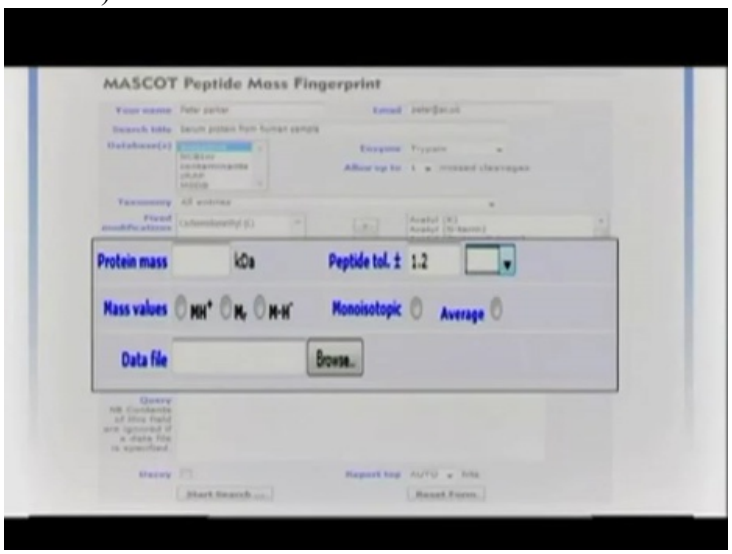
These are mass changes suspected …

(Refer Slide Time 23:05)



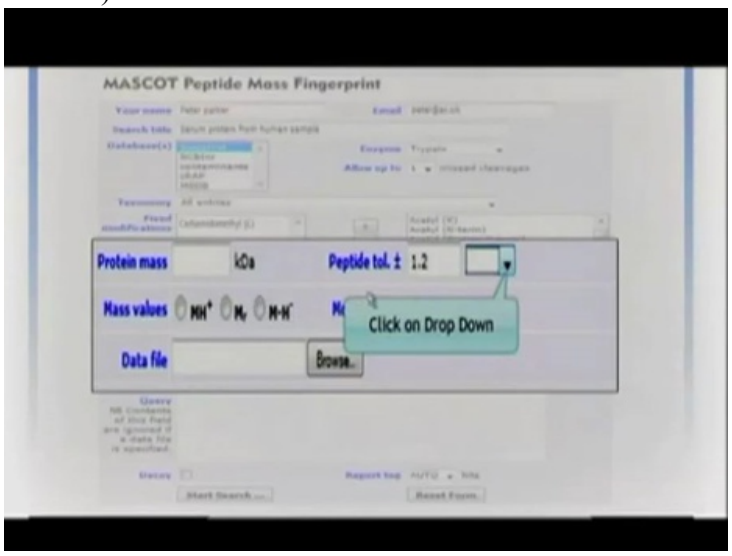
…to occur during sample handling and accounted for by increasing the number of primary sequences compared against experimental masses. Include it as variable modification.

(Refer Slide Time 23:19)



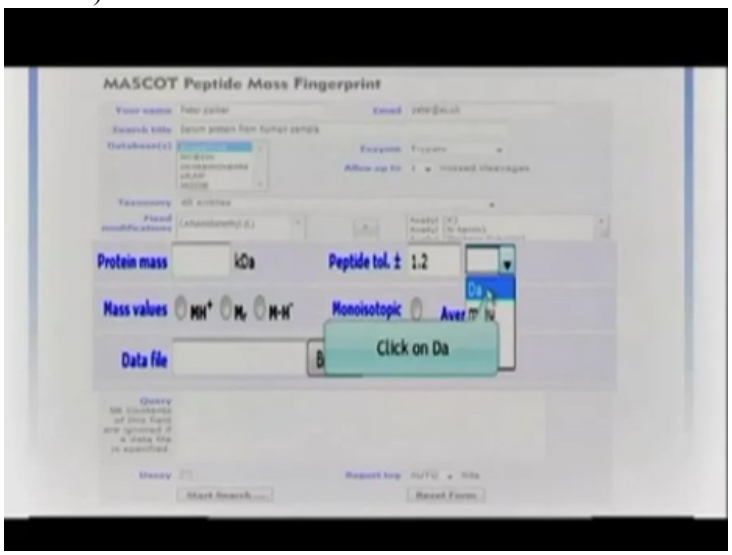
Depending upon user needs the parameters can be changed. Protein mass is the mass in that protein and is optional.

(Refer Slide Time 23:26)



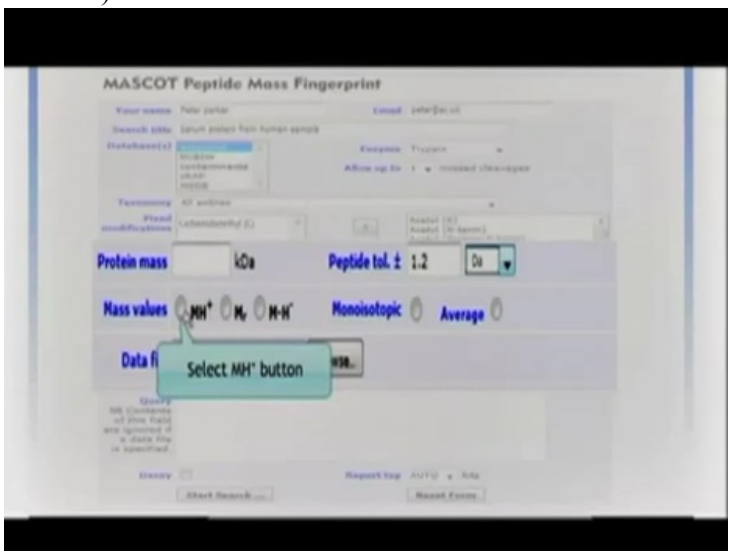
Set peptide tolerance as…

(Refer Slide Time 23:29)



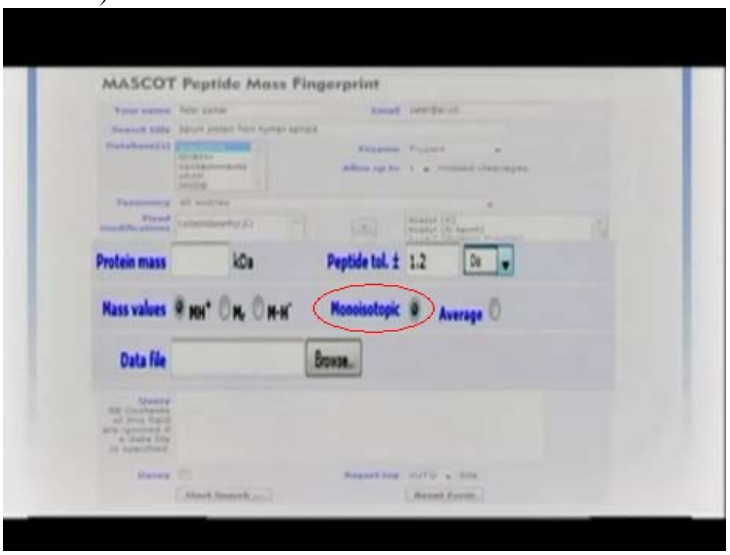
… +/- 1.2 Daltons. Mass values it specifies the type of charge to be examined.

(Refer Slide Time 23:38)



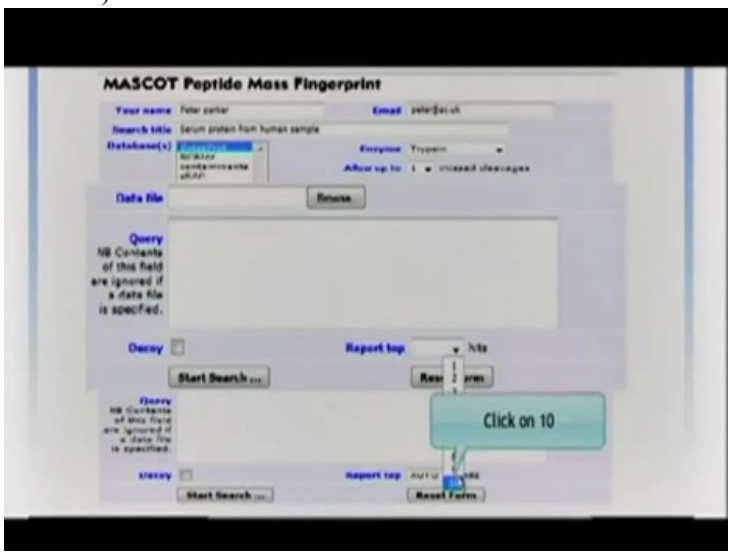
Select MH positive.

(Refer Slide Time 23:42)



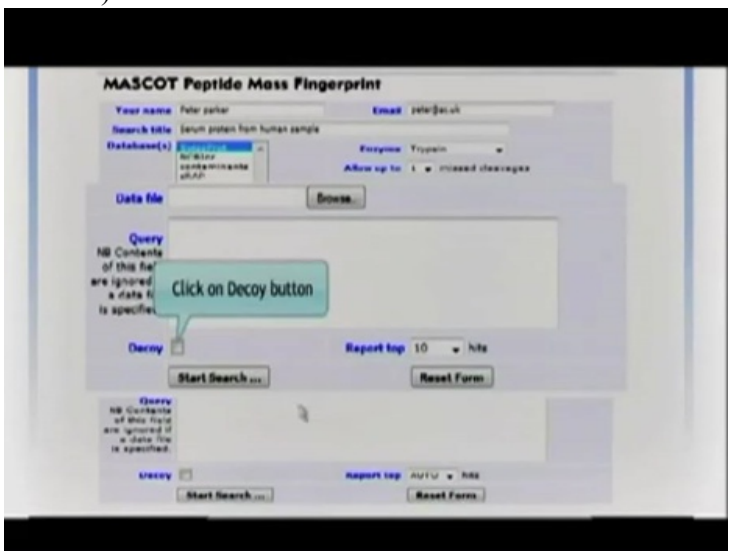
Select mono-isotopic.

(Refer Slide Time 23:45)



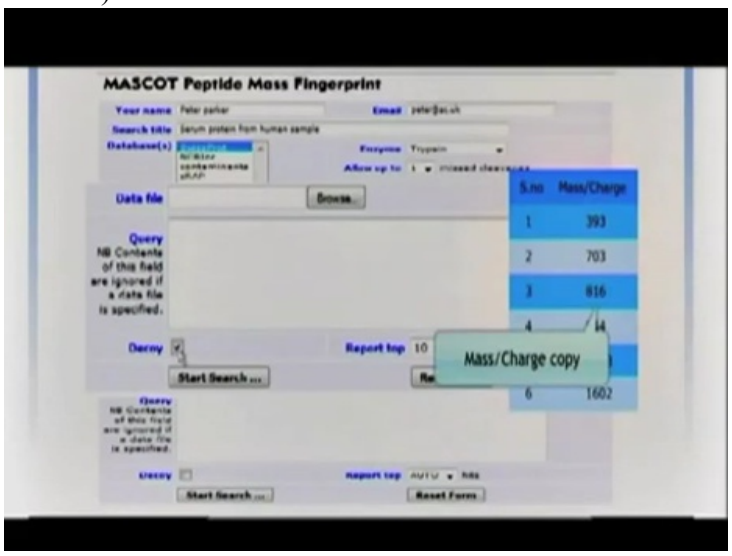
Report top 10 hits.

(Refer Slide Time 23:48)



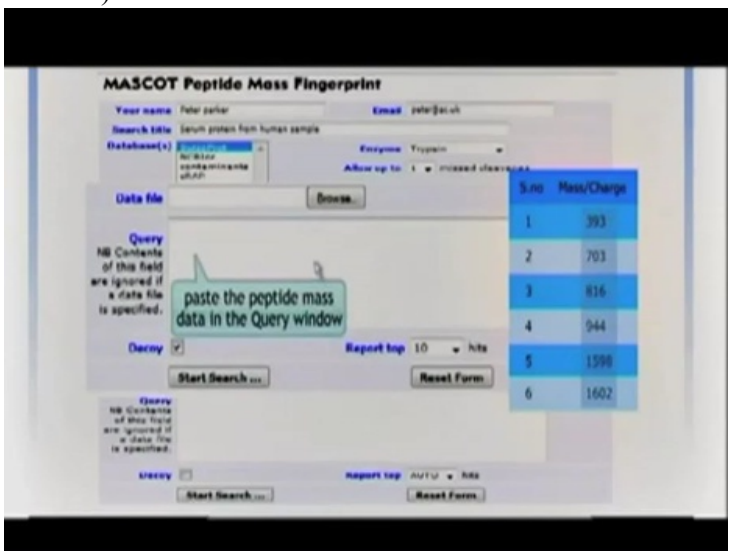
Select Decoy for statistical analysis

(Refer Slide Time 23:51)



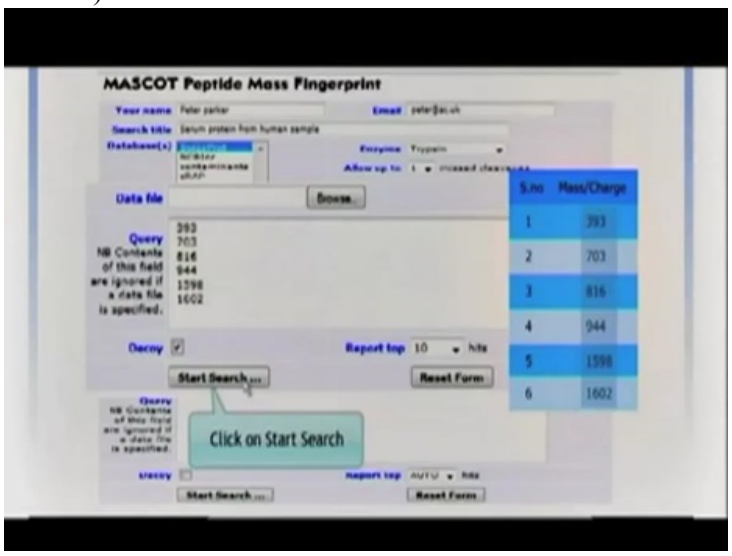
Copy the m by z value …

(Refer Slide Time 23:56)



… and paste in the selected box for Mascot search.

(Refer Slide Time 23:59)

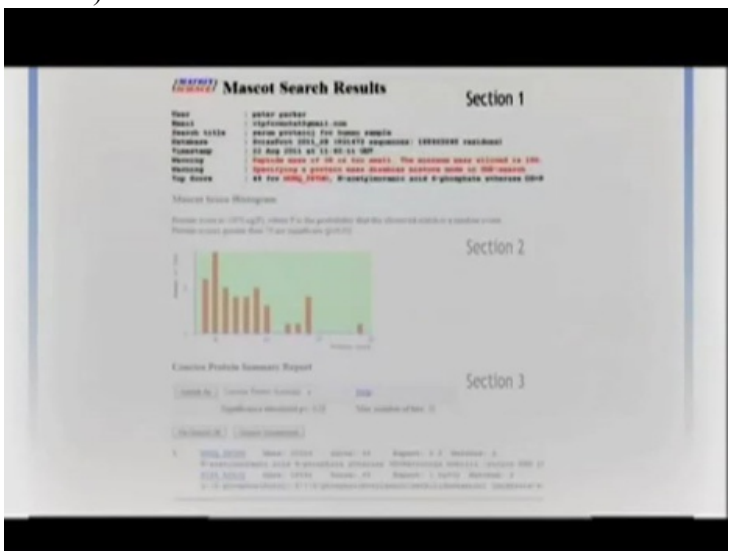


Click on search.

(Refer Slide Time 24:02)

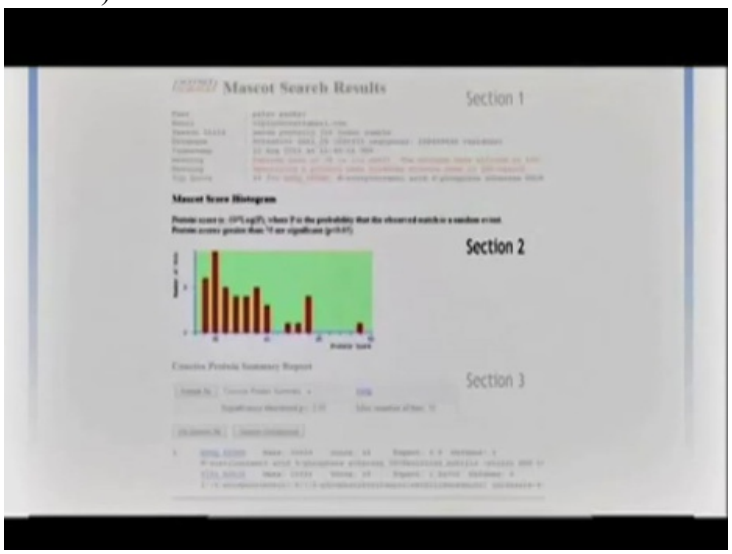
Data output

(Refer Slide Time 24:06)



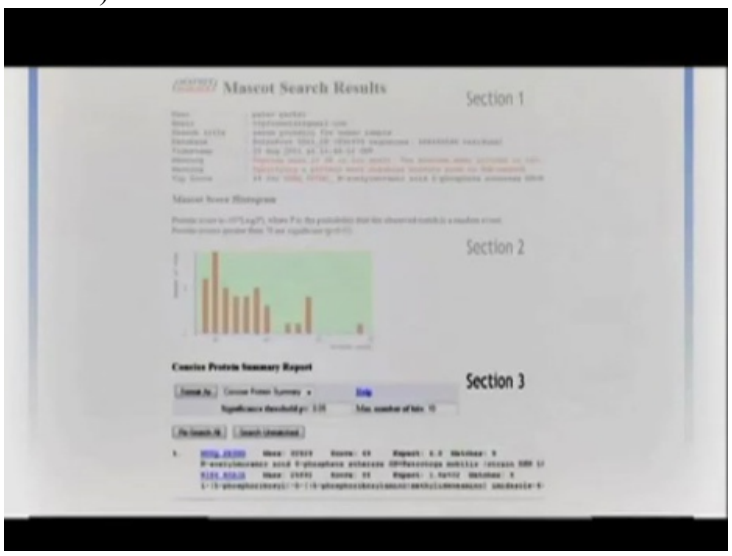
The output can be seen in 3 sections. In section 1 the summary of spec parameters defined by user

(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)

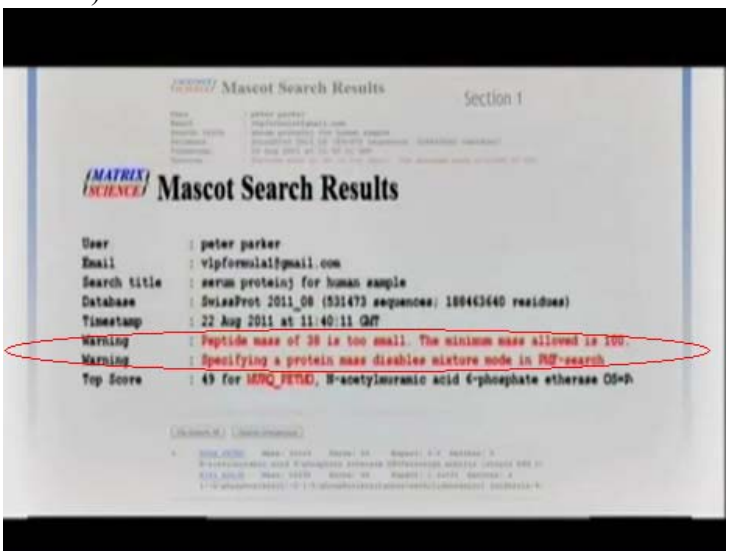


Section 3 summary report in which the matched protein from the database with the details of important parameters are displayed either in concise format protein format and the data can be exported too.

(Refer Slide Time 24:38)

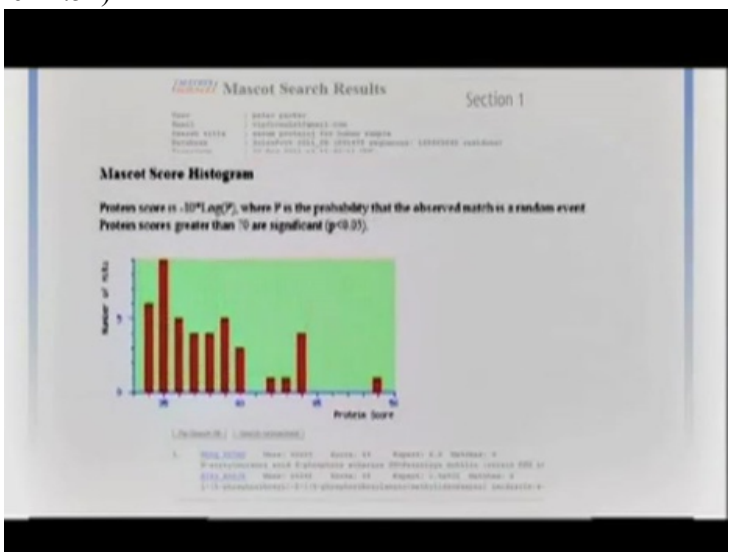
Data Analysis

(Refer Slide Time 24:43)



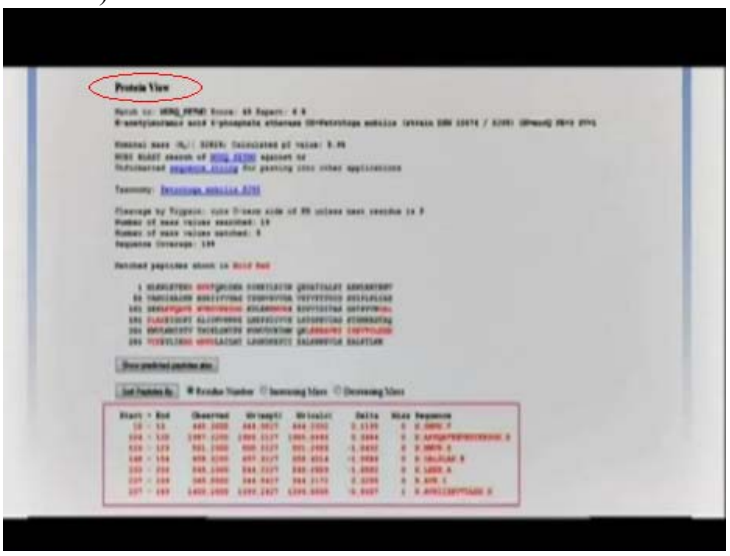
If the search parameters are not the best fit, the software generates the error message. Depending on the error message the user needs to change the parameter setting and do the search again

(Refer Slide Time 24:54)



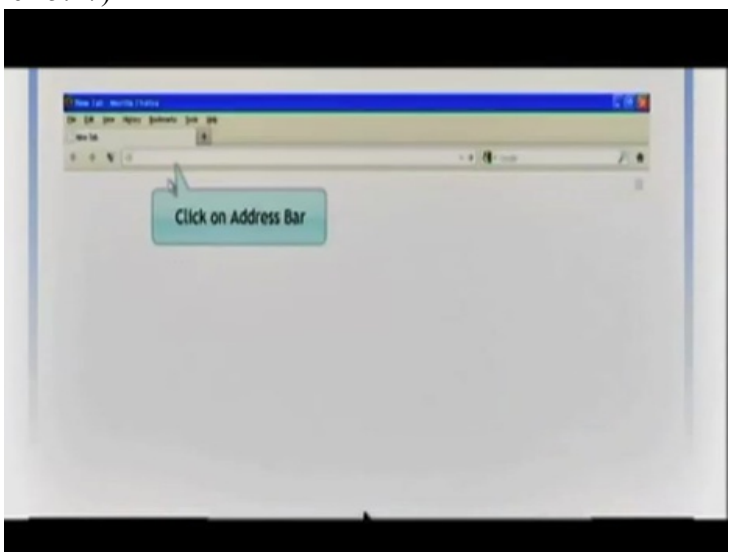
In section 2 the Mascot score histogram the number of protein hits and their score is displayed along the graph

(Refer Slide Time 25:03)



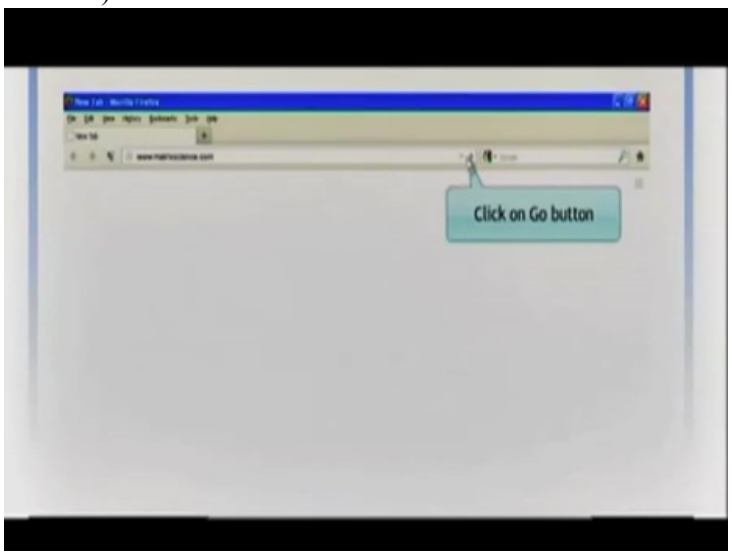
Protein view section displays matching of the query peptide to the protein sequence in the database. The sequence type, the matched region, what is the expected and the calculated value of the query peptide and the sequence details ….

(Refer Slide Time 25:17)



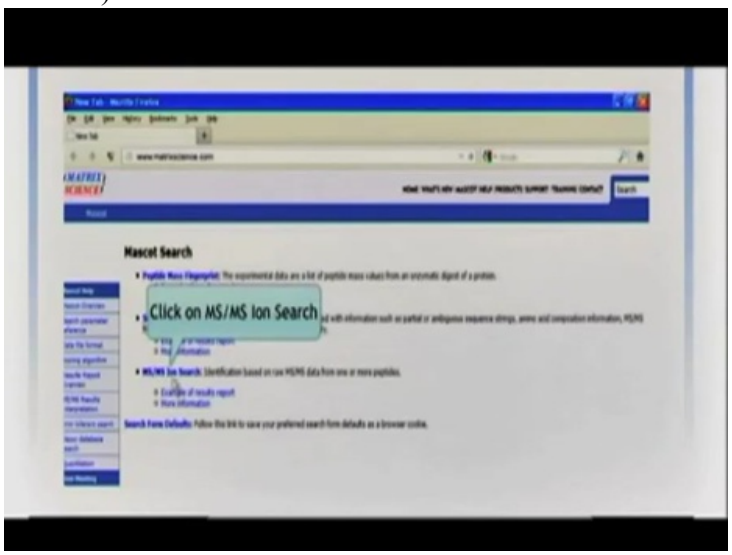
For better protein identification and to increase protein score CID of each peak generated …

(Refer Slide Time 25:23)



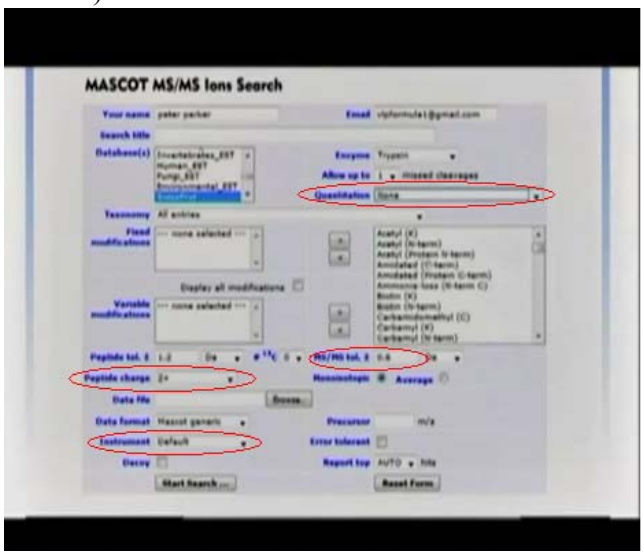
… is carried out to generate MS/MS data

(Refer Slide Time 25:27)



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

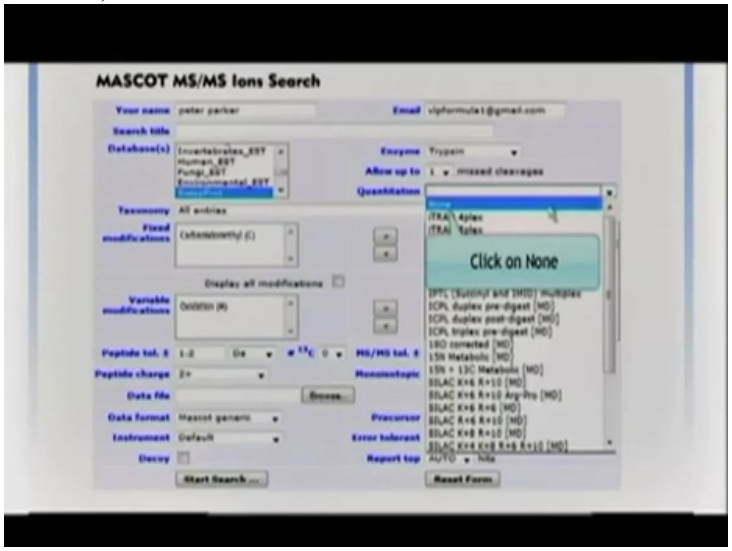
(Refer Slide Time 25:42)



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)

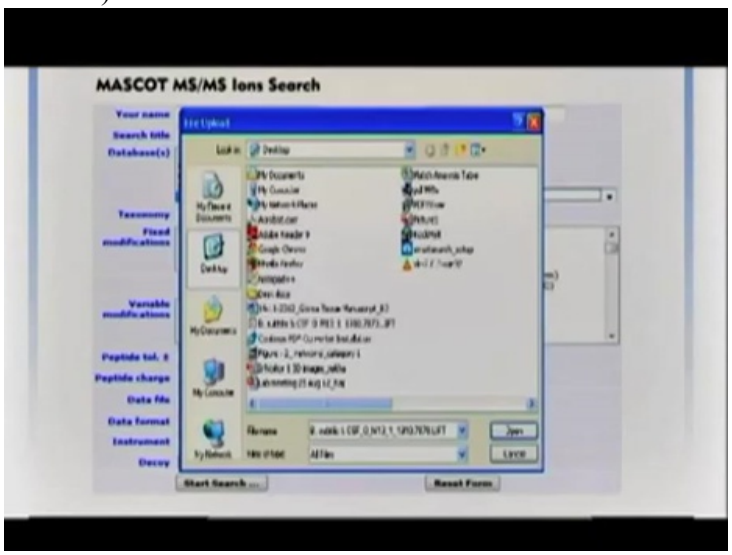


…select none in quantitation tab

(Refer Slide Time 26:02)

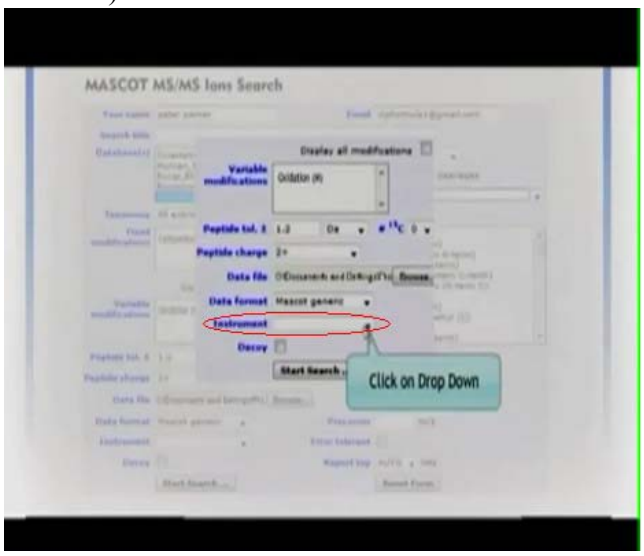
Remaining parameters remain same as PMF. Browse the MS/MS raw data file and …

(Refer Slide Time 26:10)



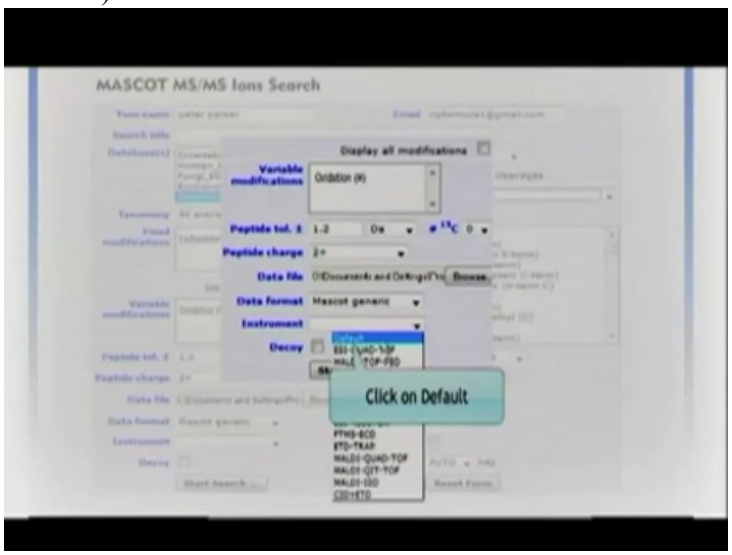
…search the results using Mascot

(Refer Slide Time 26:13)



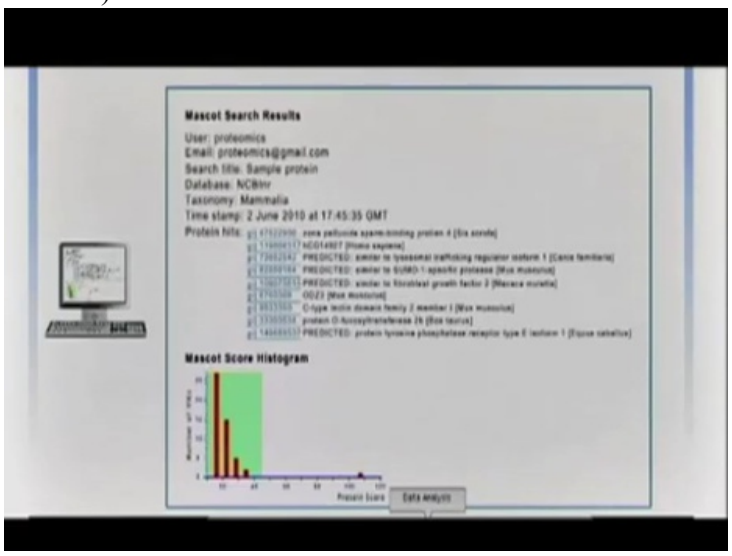
Define the instrument …

(Refer Slide Time 26:15)



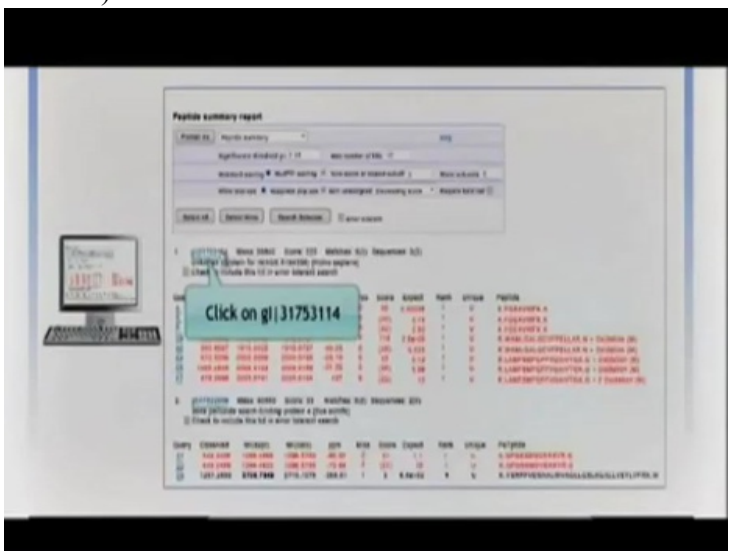
…that has been used to generate the raw data. When we don't know the name of the instrument select default.

(Refer Slide Time 26:27)



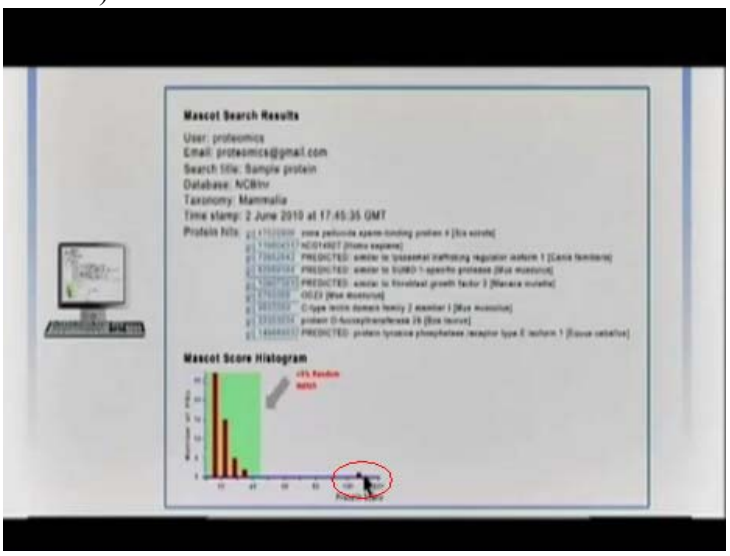
The result output generated is almost similar to PMF output.

(Refer Slide Time 26:29)



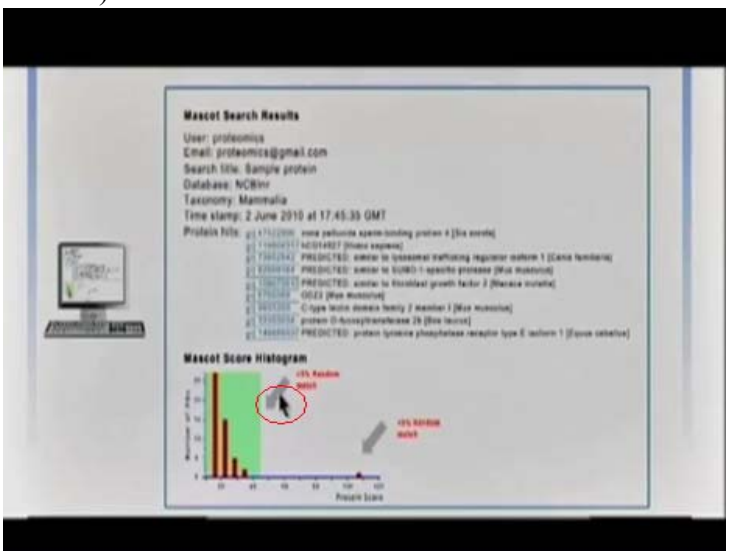
The accession ID indicates the protein information obtained from the database marked as green

(Refer Slide Time 26:37)



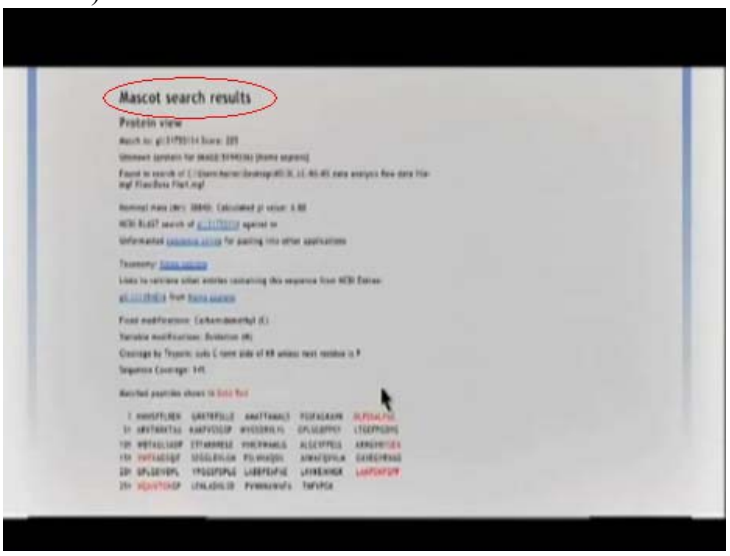
The protein hit outside the green box indicates the p value less than 0.05 which is statistically significant….

(Refer Slide Time 26:45)



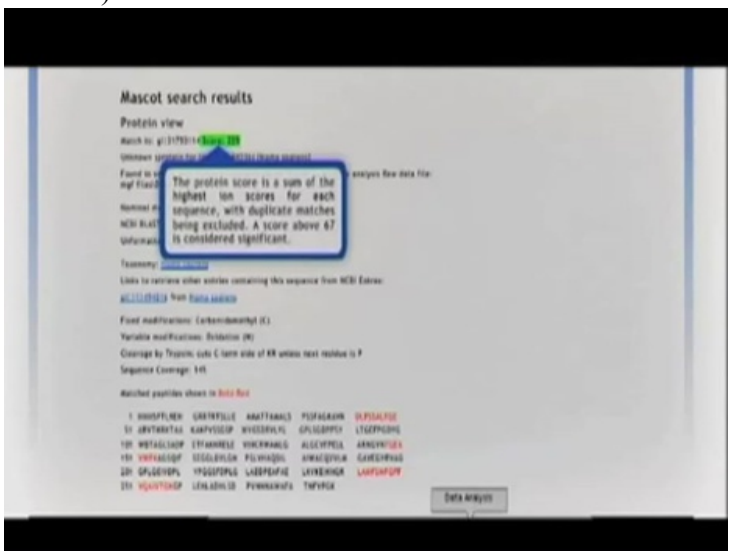
… where as hits inside the green box indicates random matching

(Refer Slide Time 26:50)



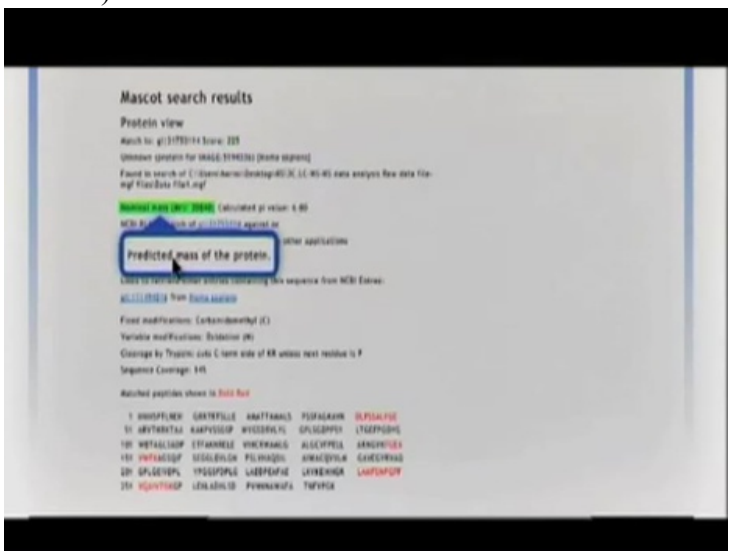
Mascot search Results

(Refer Slide Time 26:56)



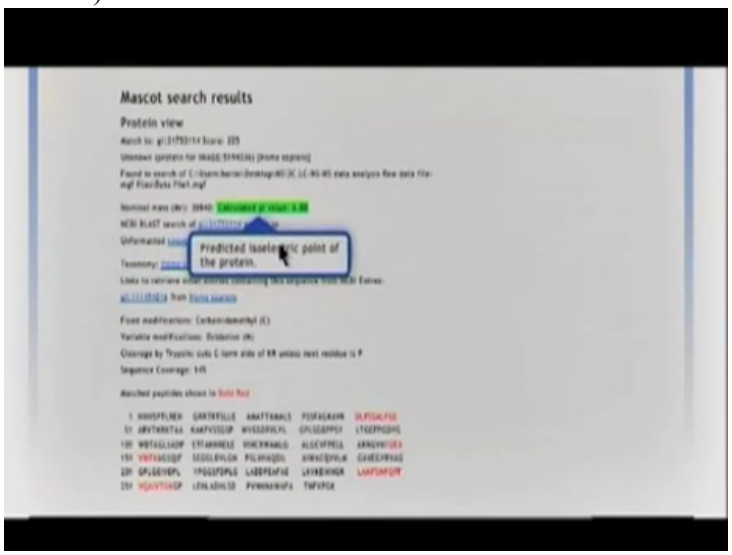
The score the peptide score is the sum of highest ion scores for each sequence with duplicate matches being excluded.

(Refer Slide Time 27:03)



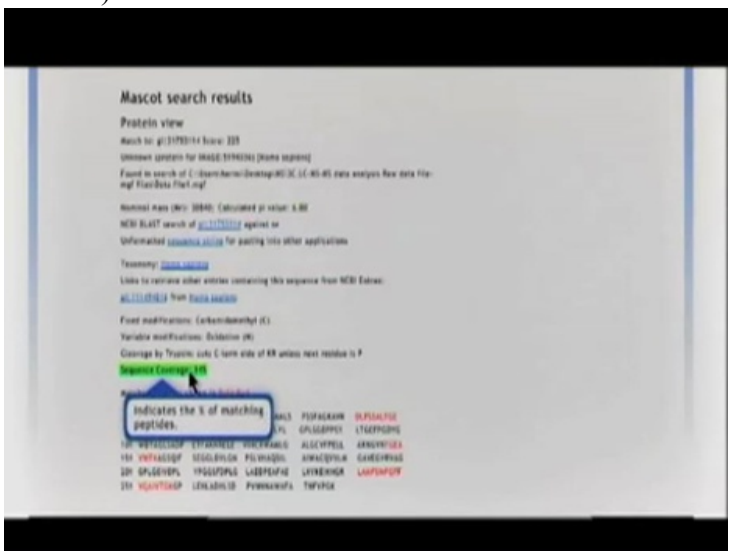
Nominal mass it is predicted mass of the protein

(Refer Slide Time 27:09)



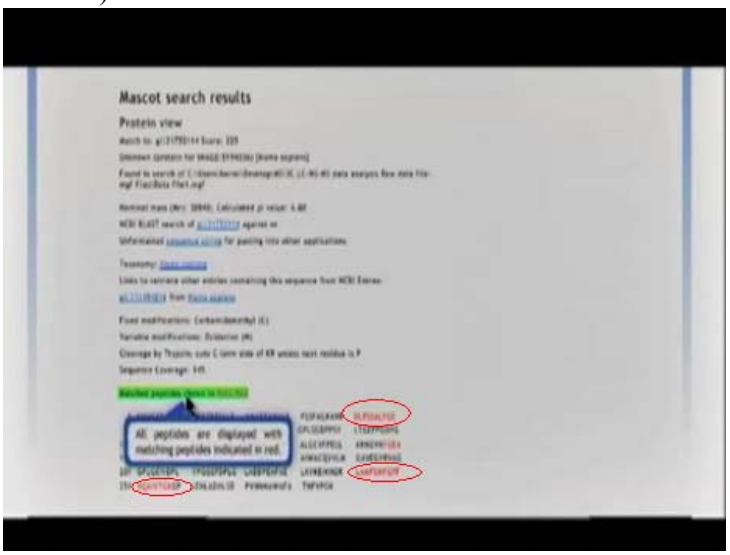
Calculated pI value, predicted isoelectric point of the protein

(Refer Slide Time 27:17)



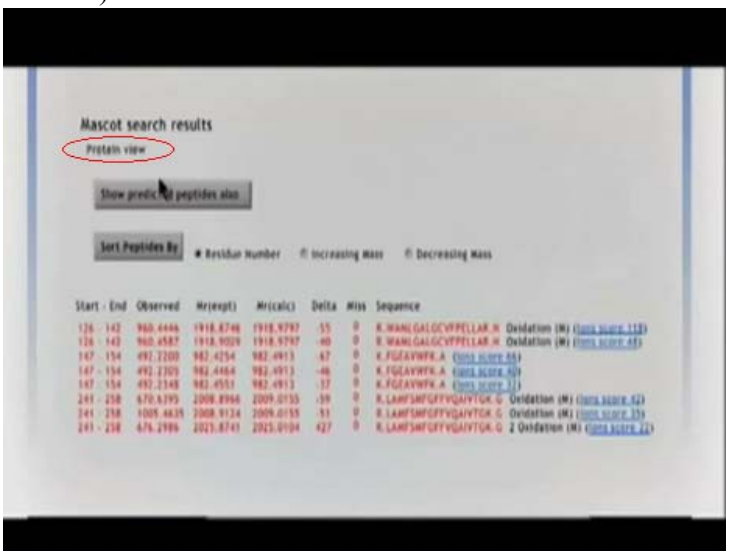
Sequence coverage indicates the percent of matching peptide

(Refer Slide Time 27:24)



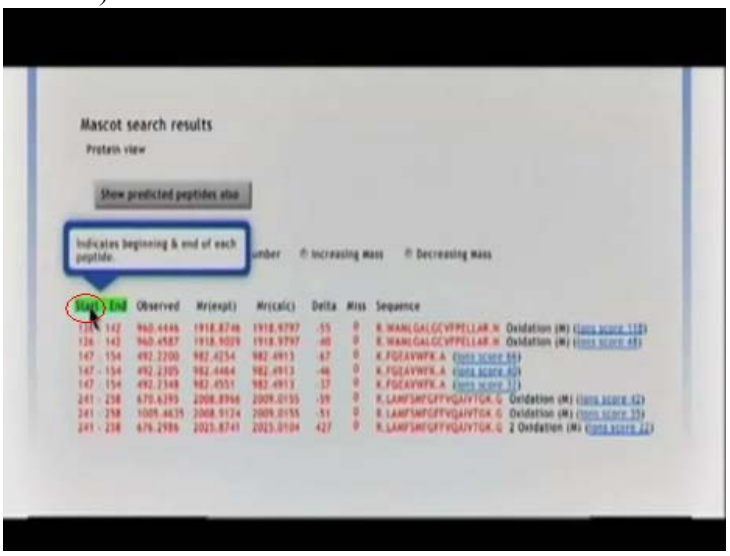
All the peptides are displayed with matching peptides indicated in red

(Refer Slide Time 27:32)



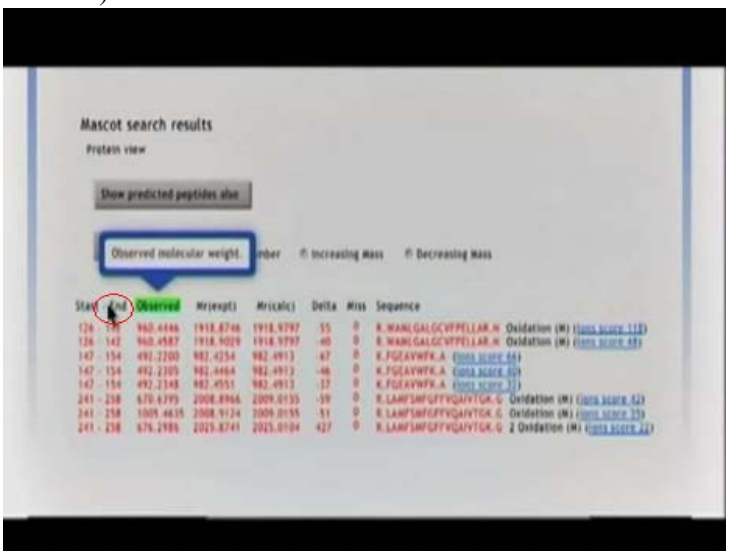
The protein view obtained on selecting the particular protein link is very similar to protein view observed in PMF. It provides detailed information ….

(Refer Slide Time 27:41)



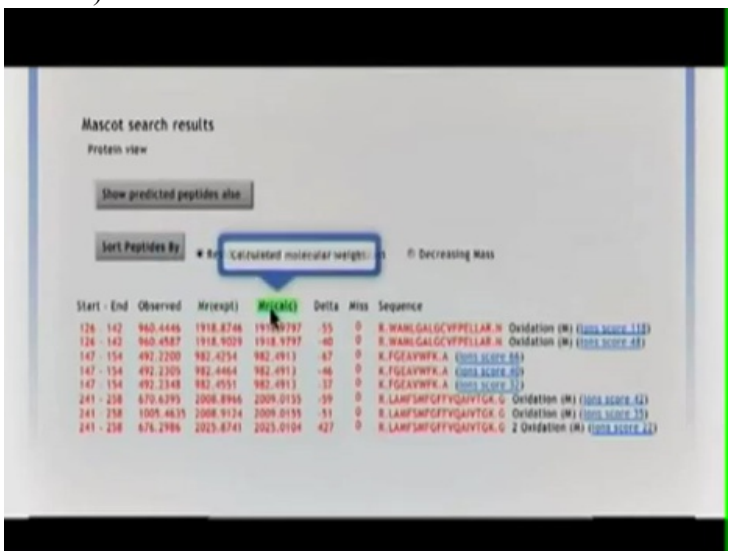
…about each of the matched peptide displayed The start …

(Refer Slide Time 27:46)



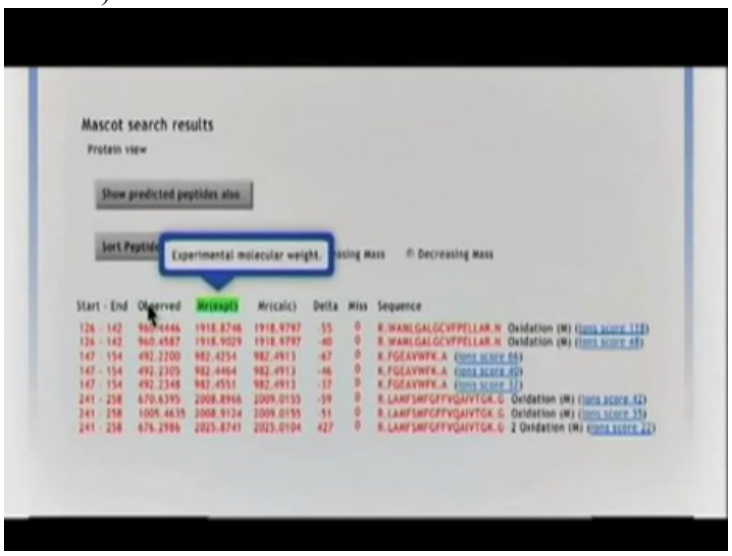
… and end position of amino acids …

(Refer Slide Time 27:50)



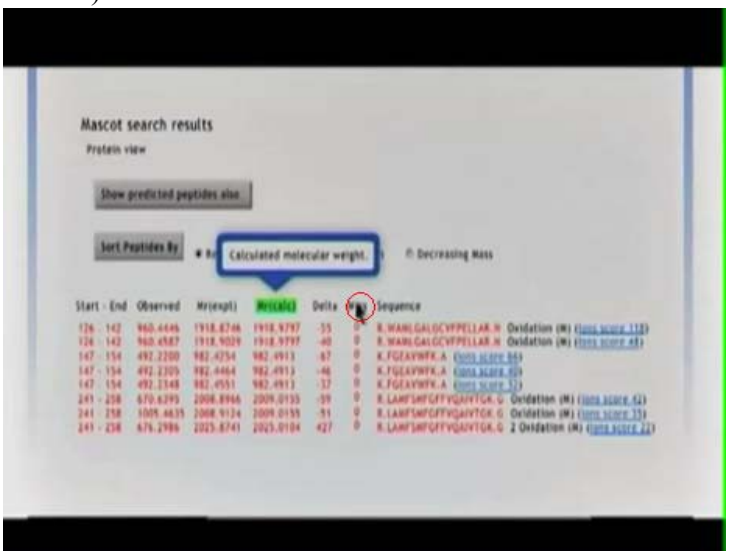
… calculated and …

(Refer Slide Time 27:51)



… experimental molecular weights…

(Refer Slide Time 27:55)



… number of missed tryptic cleavages,

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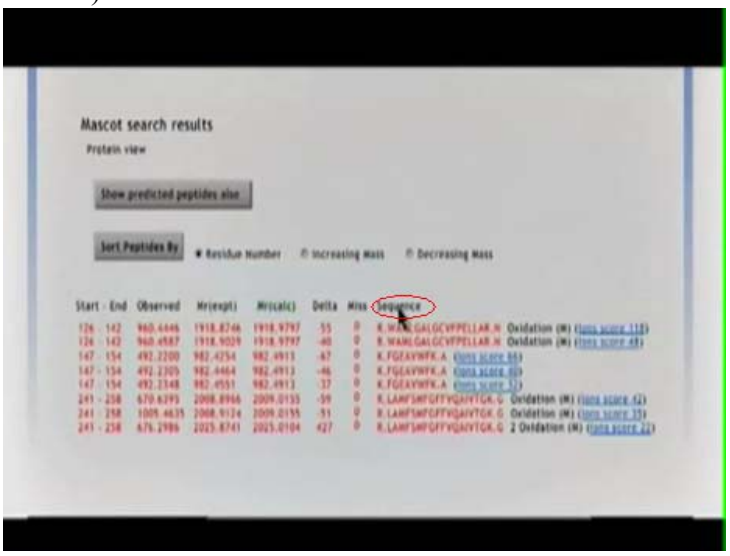

… sequence of each peptide segment and their corresponding ion scores are shown

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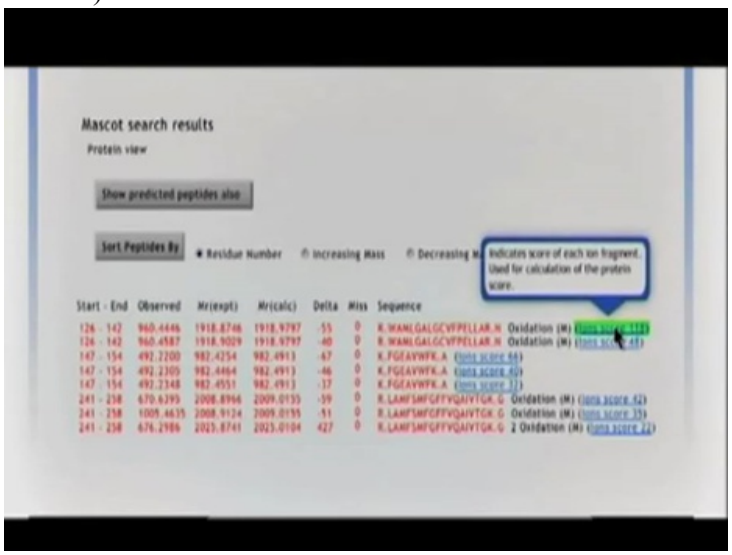

The highest ion scores are used for computing the final protein score

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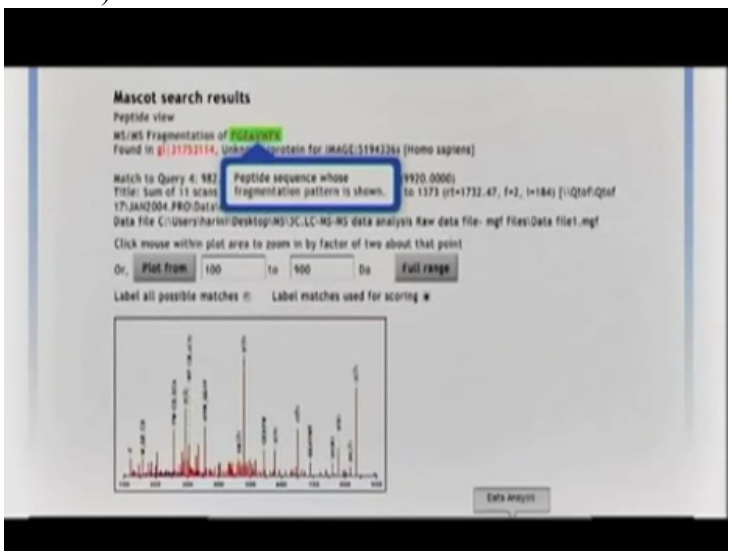

Each peptide in Tandem MS/MS undergoes through second round of fragmentation when it passes through the second mass analyzer before it reaches the detector

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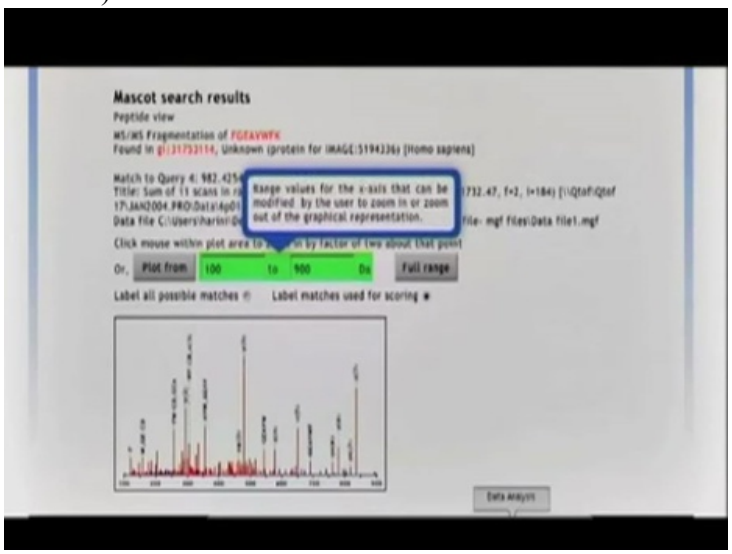

This provides significantly larger amount of information regarding each peptide segment which can be viewed by clicking on the peptide link provided in the summary report. The fragmentation pattern is displayed graphically …

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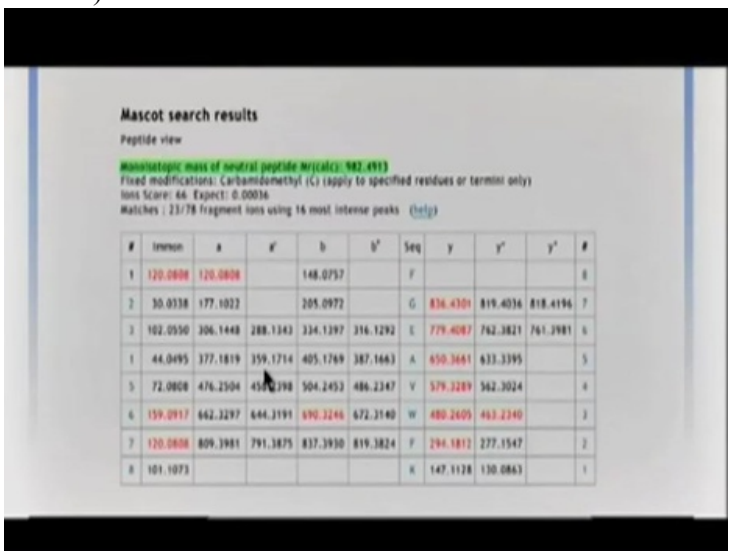

…which can be zoomed into as per the requirement

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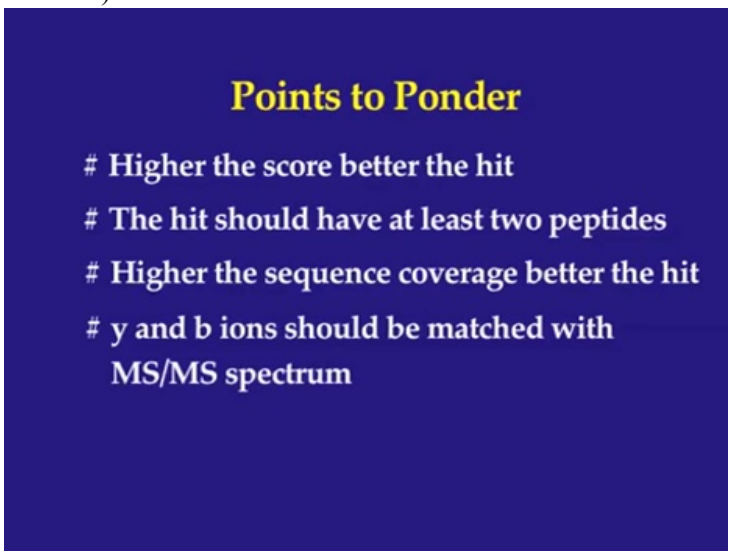

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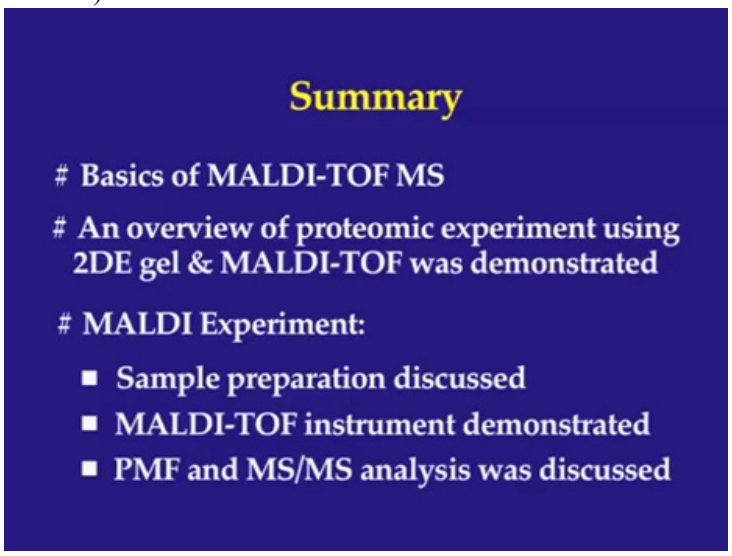

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