Proteomics: Principles and Techniques
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Lecture No. # 21
Matrix assisted laser desorption/ionization-
Time of Flight (MALDI-TOF)

Welcome to the proteomics course, today we will talk about matrix assisted laser desorption ionization time of flight MALDI TOF. In previous lecture, we talked about basics of mass spectrum try the various combinations of mass analysers and ionization sources. Now it is time for us to combine that 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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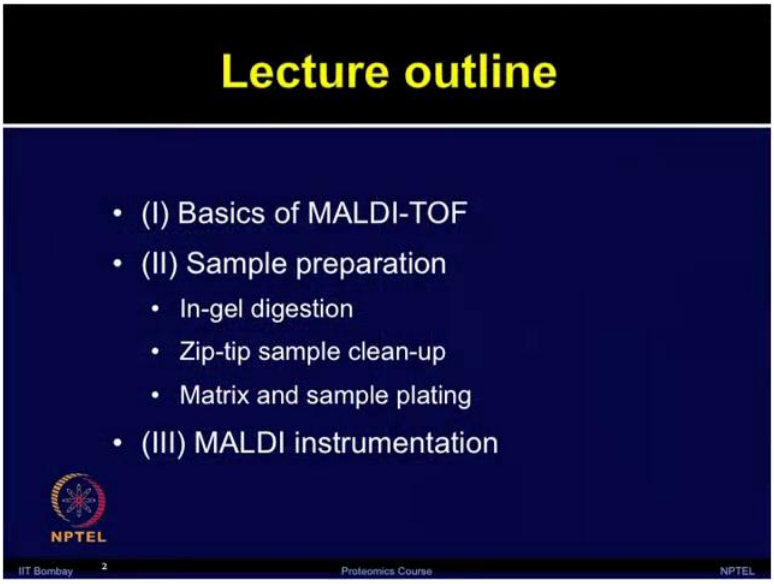

So, in today's lecture, we will talk about basics of MALDI-TOF how to prepare the sample for MALDI TOF or TOF t of analysis which will include in gel digestion, you want to do the photolytic digestion of the samples, followed by cleaning up strip which requires zip tip, then how to prepare the matrix and place those on the sample plating then, we will talk about MALDI instrumentation.

Let us first start about basics of MALDI. 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 Keechi Naka and Helen camp. Then, Naka 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 expect that in two parts one is MALDI which is ionization source another is TOF which is a mass analyser.

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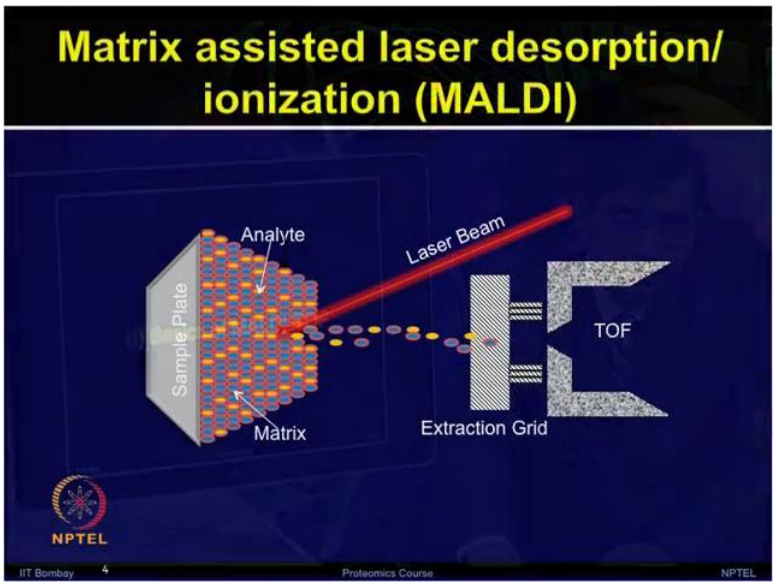

Let us first talk about matrix assisted laser desorption ionization or MALDI. So, analyse or the proteins of interest are mixed with the matrix, which is usually an aromatic compound. There are various type of matrixes available which we will talk in more detail when we come to the sample preparation and matrix selection, but just for your reference we can use 2 5 dihydroxybenzoic acid, we can use sinapinic acid and there are several other choice. Once you selected a matrix for the experiment, then analyse and matrix can be dissolved in a organic solvent. After which then it can be placed on the metallic target. As you can see in the slide the first left section shows you, how to place the analyse and matrix together on the sample plate.

Now, once you have placed the matrix and the analyse on the target plate, you can put that in the vacuum chamber and apply high voltage. Now these crystals are targeted with the short laser beams, as you can see in the slide, then rapid sublimation can convert analyse into the gas phase ions. Now these ions once generated they can accelerate away from the

target plate through the mass analyser, which is time of flight TOFU and they can reach towards the detector. Now this process is shown in the right hand side of the slide.

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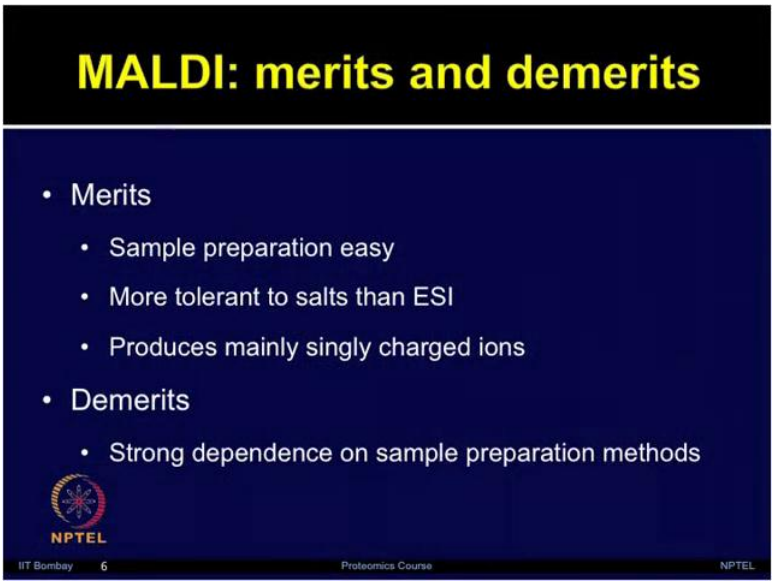

The various advantages, and disadvantages of using MALDI as an ionization source. The sample preparation is very easy, the MALDI provides high tolerance to salt as compared to the electro spray ionization methods. The MALDI produces single charged species most analyses can accept the single photon. The single charge characters can result in some molecules having large mass to charge values. So, therefore, the MALDI is typically integrated with the TOF mass analysers, which can provide the m by z range for the large ions as well. Now these are various methods of using MALDI; obviously, it has to be connected with the TOF. Now, the various demerits of using the system, there is a strong dependence on how to prepare good sample for the this analysis. So, sample preparation methods here will influence the spectrum generated from these experiments.

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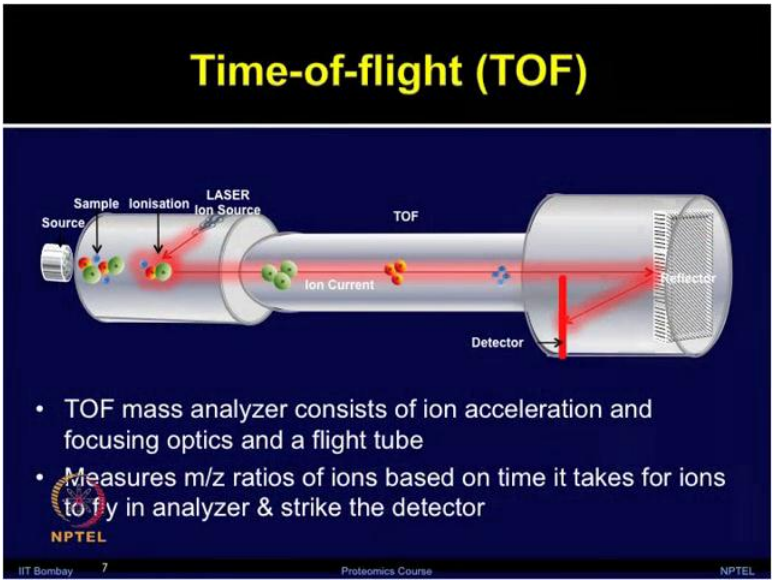

So, the TOF mass analysers they consist of ion acceleration and focussing optics and a flight tube. As shown in the slide you have a source where sample ionization is occurring due to the laser beam bombardment. Then ions are moving in the time of flight tube, and reaching towards the detector. Now often we can also add the reflector and ion mirror which can increase the path length. So, this time of flight tube it measures the mass to charge ratio of ions, based on time it takes for ions to fly in the analyser and strike to the detector. Now, the mass is exponentially proportional to the flight time. How much time it takes to travel in the time of flight tube. So, ions of the lower masses are accelerated to the higher velocities. Now time of flight tubes now time of flight tubes often outperforms these scanning mass analysers in its sensitivity and scan speed.

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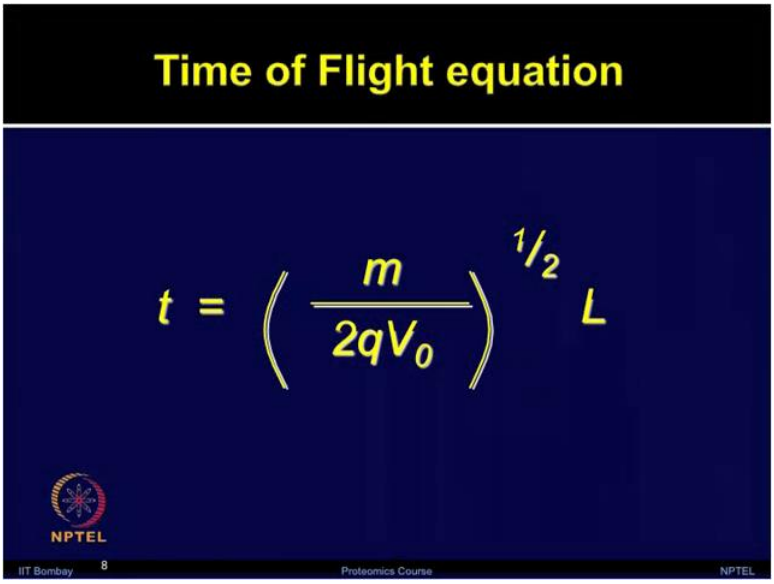

The time of flight of a charged ion can be calculated by using the equation shown in the slide. The flight time is directly proportional to the square root of mass of the ion. Now in this equation t represents time of flight, m is mass of the ion, q charge on ion, V 0 is accelerating potential and L is the length of flight tube.

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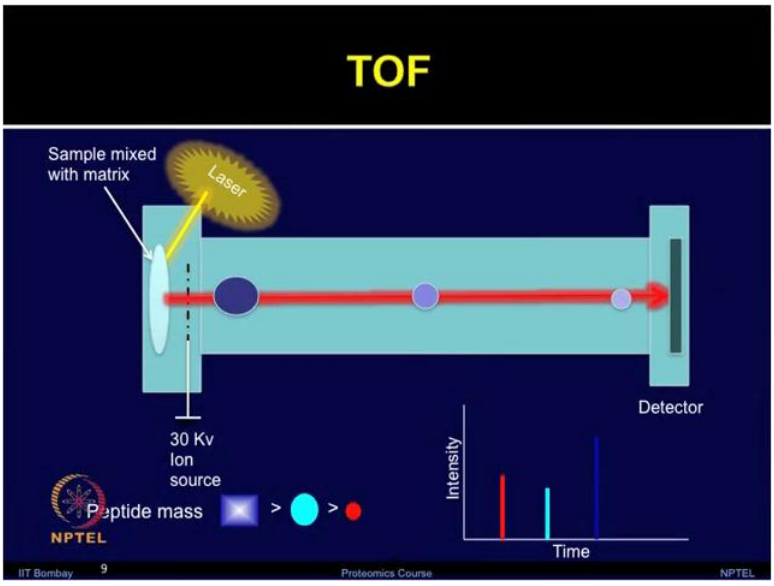

In time of flight tube the ions are accelerated to high kinetic energy, and due to the different velocities they are separated in a flight tube. As I mentioned earlier by adding the reflection on a reflector, the ions can turn around in the reflector that can compensate for minor

differences in the kinetic energy. Now, if you take an example where you have three ions as shown in the dark blue, light blue, and the red colour in the slide. Now you will expect that the small ion which is the red one will show the first peak, followed by the blue ion, and then the dark blue one after discussing some of the basic concepts of using MALDI and TOF. Now let me give you an overview of entire MALDI TOF experiment by showing you the following animation.

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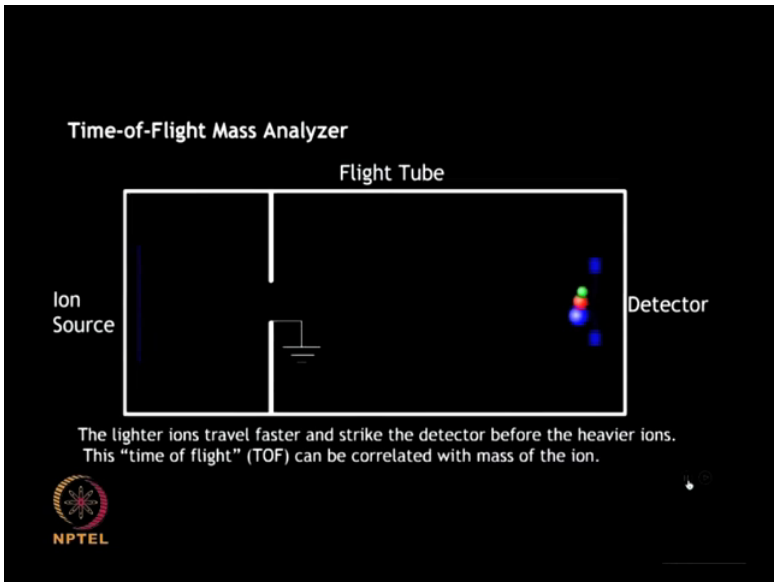

Fundamentals of MALDI-TOF ms. The time of flight analyser resolves ions, produced by the ionization source, on the basis of their mass to charge ratio.

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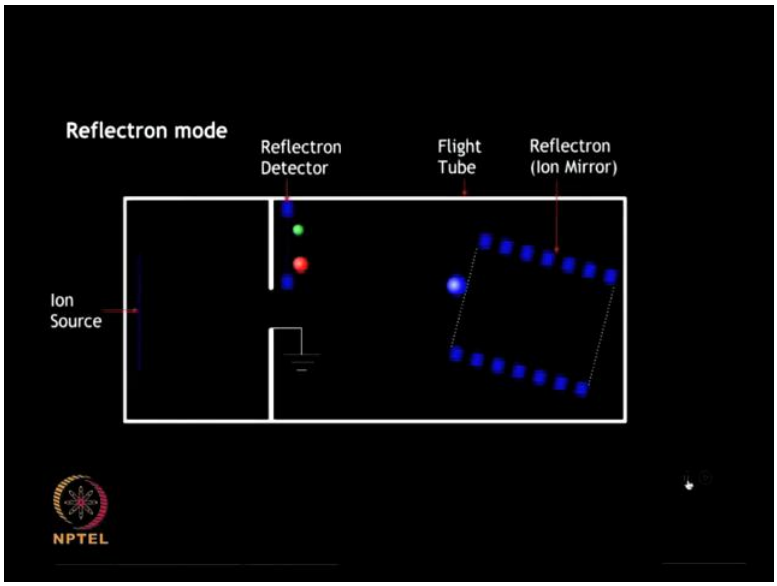

The time of flight tube can be operated in the linear mode or the reflection mode which depends on the sample to be analysed. In case of small molecules this mode usually provides sufficient resolution. The generated ions are accelerated towards the detector, with the lighter ion travelling through the TOF tube faster than the heavier ions. So, the lighter ion travels faster and it strikes the detector before the heavier ion reaches to the detector. The time of flight or the TOF tube can be correlated with the mass of the ion. So, the flight of time of the ions can be correlated with the mass to charge ratio. As we talked earlier the TOF analyser can also be operated in the reflection mode. So, this is more commonly used for the proteomic studies.

An electron which acts as a ion mirror is incorporated at one end of the time of flight tube. This helps in extending the path length and in turn the flight time of the ion without having to increase the actual size of the instrument. So, rather than using very long time of light tubes by including the reflectron ion mirrors, we can increase the path length. This helps to even out any kinetic energy differences between ions having the same mass and thereby improving the resolution.

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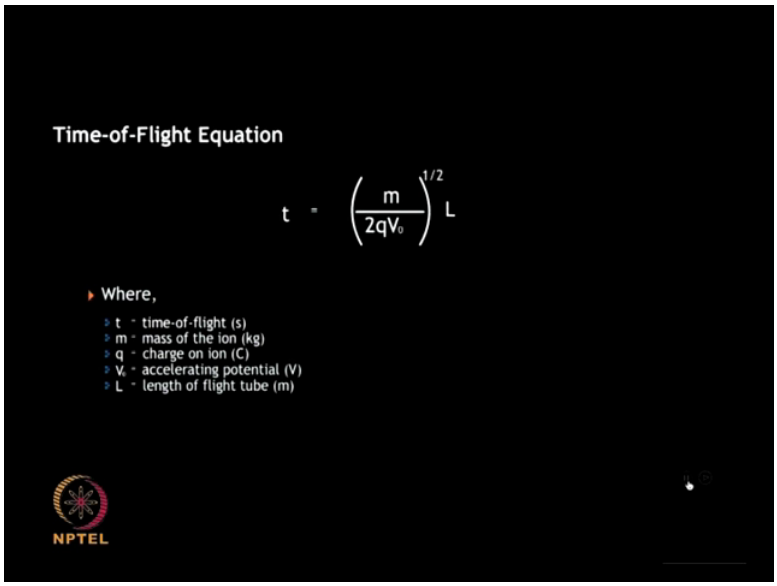

The time of flight of a charged ion can be calculated by a means, of the equation shown here. The flight time is directly proportional to the square root of mass of the ion.

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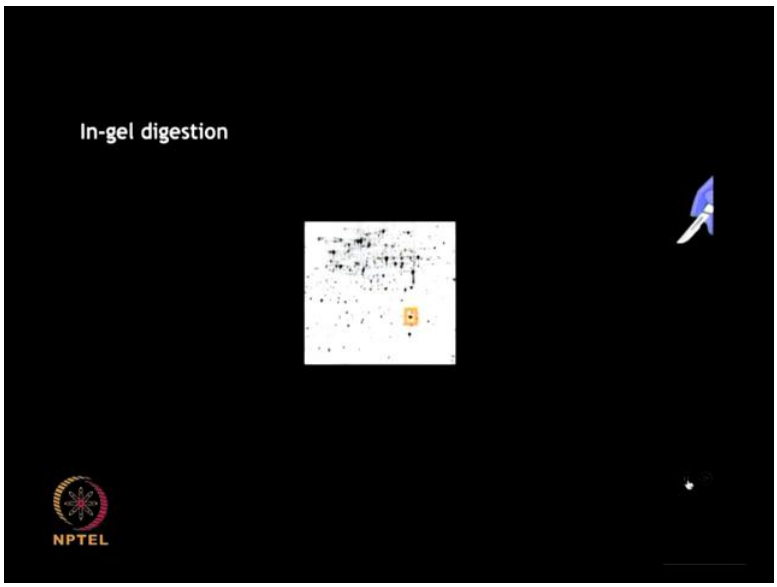

Sample preparation and spotting, the protein sample must be prepared suitably before it can be analysed by the mass spectrometer. If you have run a two d gel. So, first of all the purified protein of interest need to be excised from the gel on which it has been electrophoreses and dissolved in the suitable buffer. So, depending upon the application if

you have purified a protein you can separate that on the gel, and the cut that band. Or if you have a mixture of proteins in two d gel, you can just excise that particular spot a spot.

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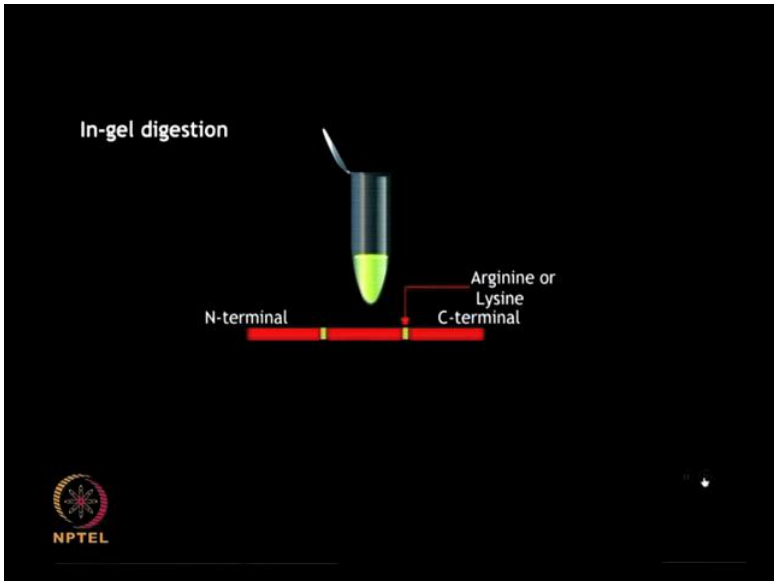

Spot can be dissolved in a suitable buffer, trysin is then added to this mixture in order to carry out digestion of the protein.

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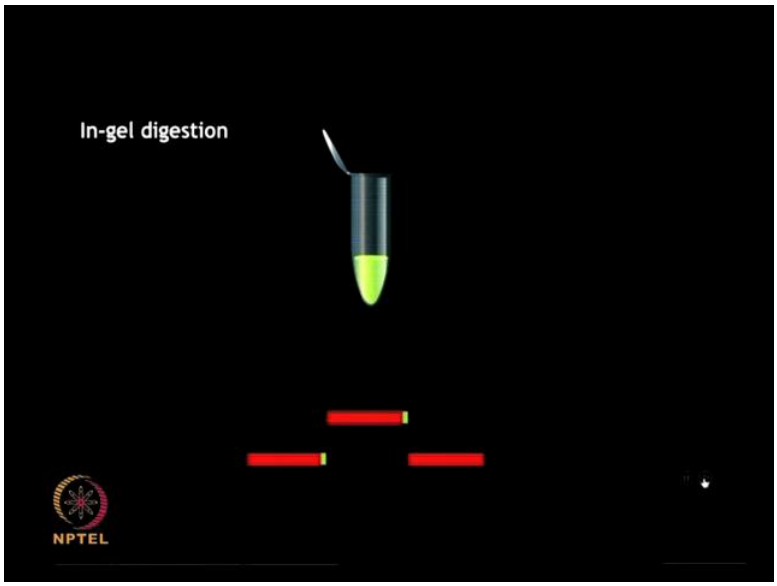

Try sin cleaves the protein at the c terminal of the argentine and lysine residues, but that is not always irreversible if you have a praline present immediately after, then it will hinder that, but overall the protein is digested into smaller fragments of manageable size.

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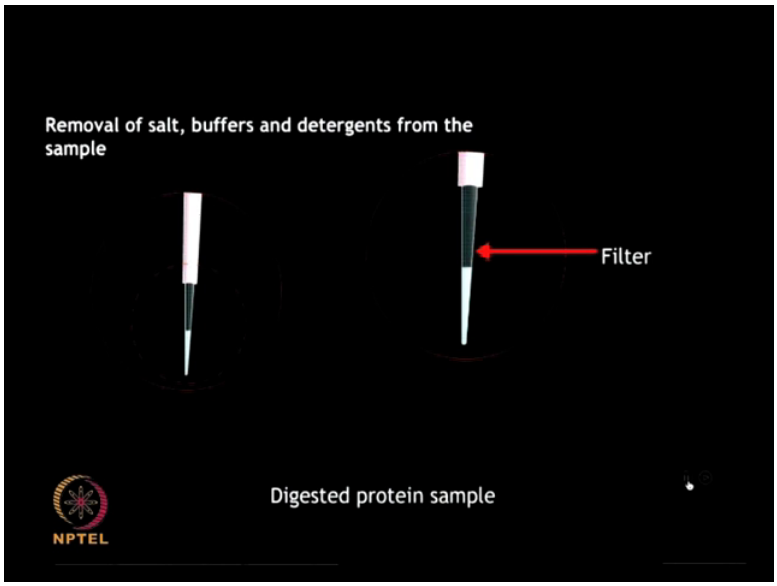

Once the protein sample has been digested all the salt buffer and any detergent must be removed from the sample. So, after doing in gel digestion and before proceeding further mass spectrum try analysis, in between an efficient step is to use some filters or zip tip which can eliminate some of these contaminants and salts. It offers several advantages such as quick verification sample embellishment and ensures that there is no contamination.

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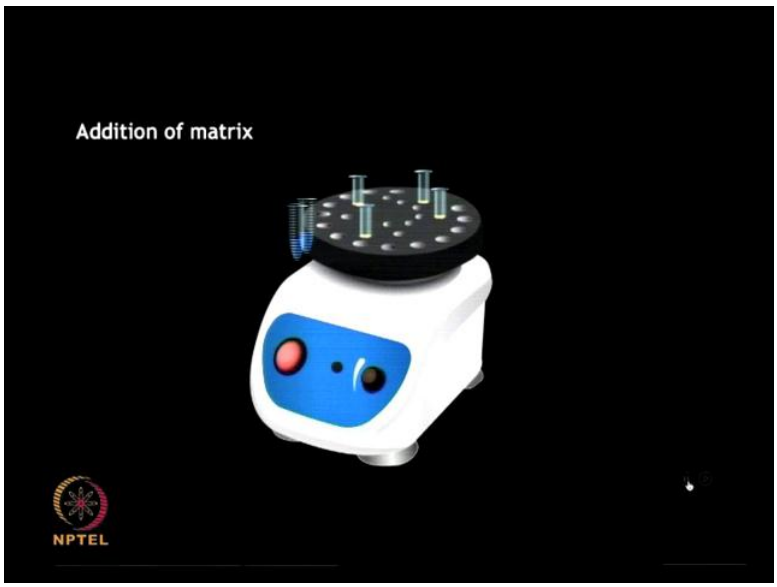

So, there are multiple advantages of using zip tips; however, it can purify only limited volume of the sample, and also it adsorbs some amount of the protein sample thereby

leading to losses. The purified protein sample can be mixed with an aromatic matrix compound such as alpha Sino, four hydroxyl, cinnamon acid or sinapinic acid in the presence of an organic solvent.

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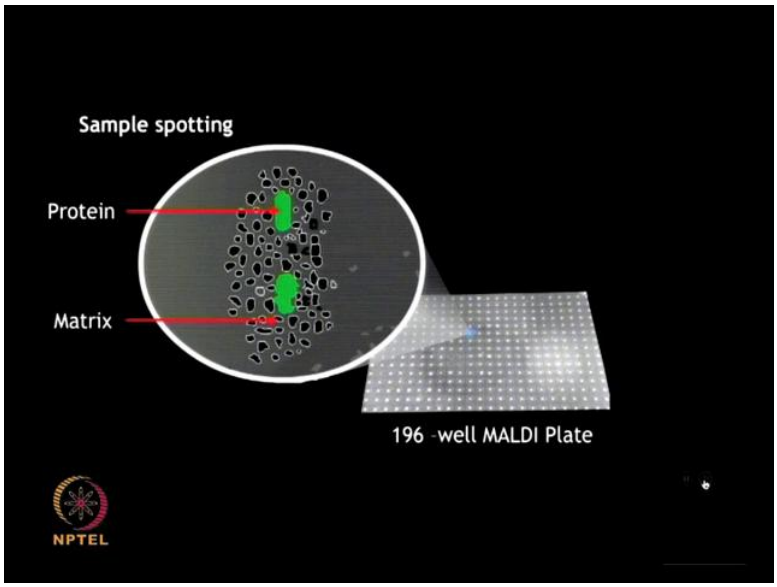

The components are mixed thoroughly and then the solution containing the organic matrix with embedded analyse of interest can be spotted onto a metallic MALDI sample plate. MALDI gives you an opportunity to analyse large number of samples in high throughput fashion.

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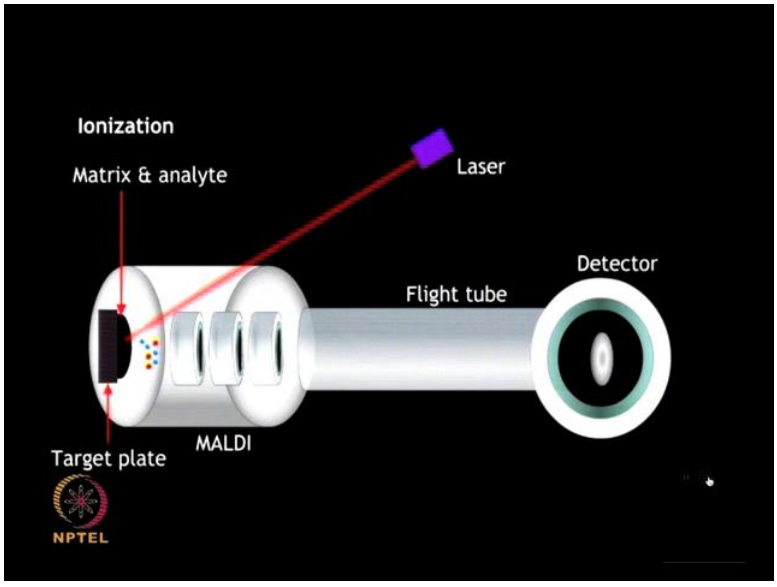

The target plate containing the spotted matrix and analyse can be further placed in a vacuum chamber, with high voltage and short laser pulse are applied. The laser energy gets absorbed by the matrix and is transferred to the analyse molecules which undergo rapid sublimation resulting in gas phase ions.

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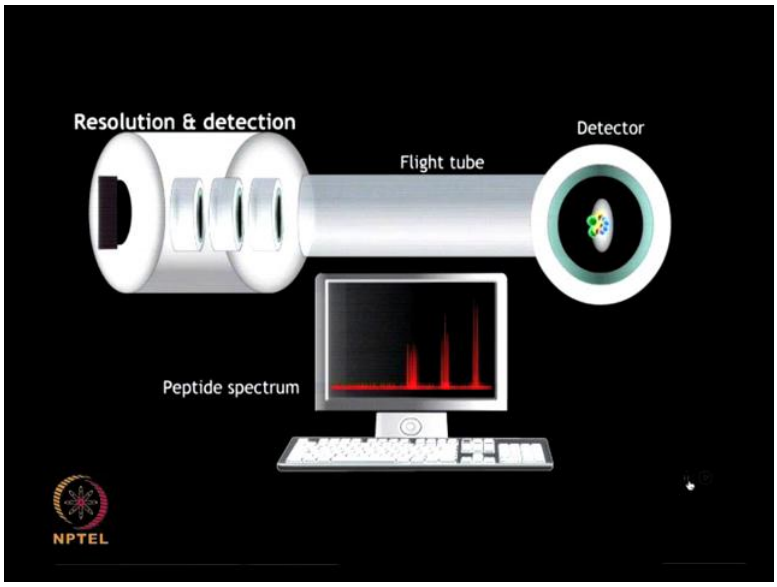

The gas phase ions generated are accelerated and travel through the side tube at different rates. The lighter ion move rapidly and reaches the detector first, while the heavier ions migrate slowly. These ions are resolved and detected on the basis of their mass to charge ratio and a mass spectrum is generated. Parameters such as geometry design, power supply quality, calibration method, sample morphology, ion beam velocity etcetera all of these factors affect the accuracy of mass detection. After looking at the animation now let us talk about how to prepare the sample let discuss these steps in more detail. The first part will be in gel digestion of the protein samples. So, the mass spectrometric identification of target protein greatly depends on the efficacy of in gel digestion process that generates a mixture of peptides from the target protein through proteolysis digestion.

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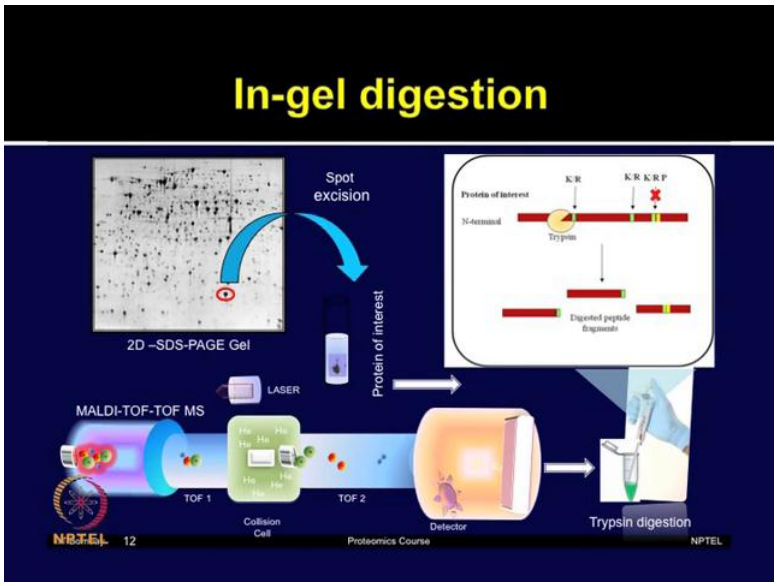

This slide gives you an overview since in the last module we discussed about, two dimensional gel electrophoresis. It shows that if you have a spot of interest, you can excise that spot from the two d gel and then subject that to in gel digestion followed by the mass spectrometric analysis. In gel digestion is a multistep procedure which includes spot selection, spot excision, removal of stain, reduction, alkylation, and proteolysis cleavage as well as peptides extraction. So, multiple steps are involved and how good your in gel digestion is is going to ensure the success of the spectrum generated from the mass spectrometry. Now although this overview shows you the process to start with the two d gel, but same can be also applied for even the gel free proteomic techniques. If you want to analyze a sample even from the gel free proteomic based approaches, it is often good idea to separate those protein complex mixture on the s d s page gel, exile the bands and then extract the proteins on that perform the in gel digestion. So, that you can simplify the proteome and then you can increase the overall proteome coverage.

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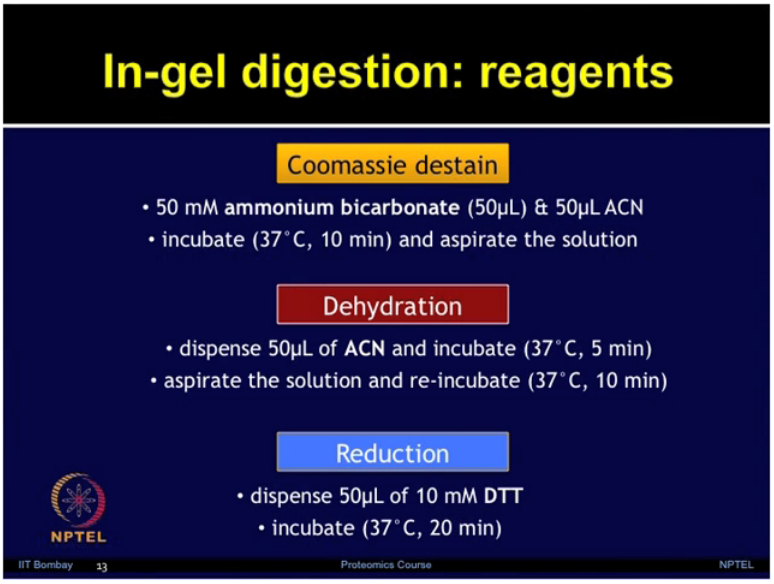

So, similar protocol can be modified and used for various types of applications in the proteomics. So, this slide gives you various recipes for performing in gel digestion. The first step is the distaining of the spots or the band, because you have stained the gels with the coomassie brilliant blue or some other or some other stains. And first of all you would like to remove these stains. So, stain removal is essential prior to the mass spectrometric analysis, the exile gel pieces should be vast with the bicarbonate buffer and acetonitrile for removal of the staining agent.

You can see the recipe in the slide; we will talk more about how to do these experiments when we come to the animation of these steps. Acetonitrile reduces the hydrophobic interaction between protein and the stain while, the ionic solution decreases the ionic interaction between negatively charged coomassie brilliant blue dye and the positively charged protein. Once the coomassie is detaining or the destining step is performed then we need to dehydrate the gel pieces, which can be done by using addition of acetonitrile. After this incubation is done then you are ready for performing reduction step. Now, why reduction step is required? So, after this stain removal the next steps are including reduction as well as alkylation of protein residues. So, that you can denature the protein into it is primary structure, continuing on to the same theme of in gel digestion and various steps required performing such experiment. lets now look at the next step which is alkylation.

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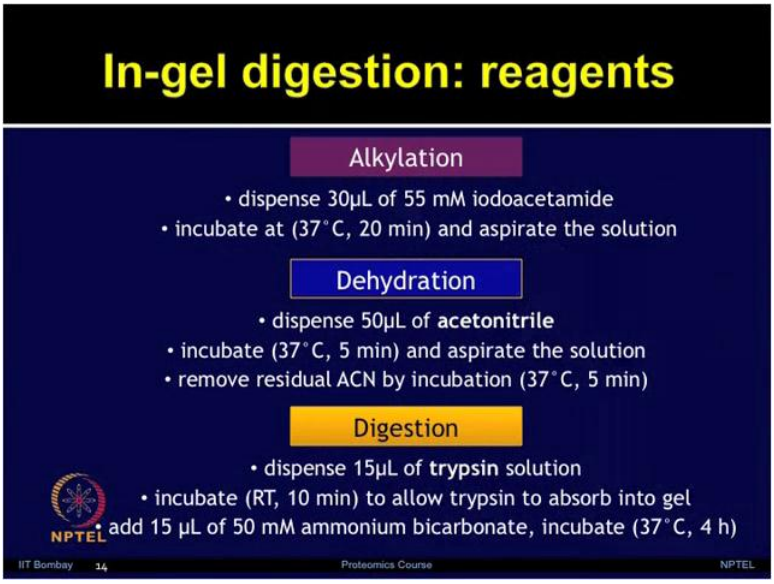

So, in the alkylation you need to add the iodoacetamide. The reformation of disulphide bonds may occur. So, to prevent that iodoacetamide which is an alkylation agent is used here. Again you need to do the dehydration step, as recipe is mentioned in the slide, you can add the acetonitrile and then we are ready perform the digestion which usually done by using trip sin. So, prior to the m s identification proteins are digested to generate peptides, there are various enzymes which one can use for performing this step, but trip sin is most widely used proteolysis enzyme, used for the protein digestion. It breaks a peptide bonds at the carboxyl terminals of a basic amino acids, such argentine and lysine. While digestion is done this is usually the overnight step then one need to do the peptides extraction of the digested proteins. So, peptides generated through the proteolysis cleavage can be extracted by using recipe including formic acid or trifluoroacetic acid t f a in the 50 percent acetonitrile solution. Now, coming back to the importance of reduction and alkylation of the proteins. We mentioned that we need to add DTT and I a a in various steps during the in gel digestion process.

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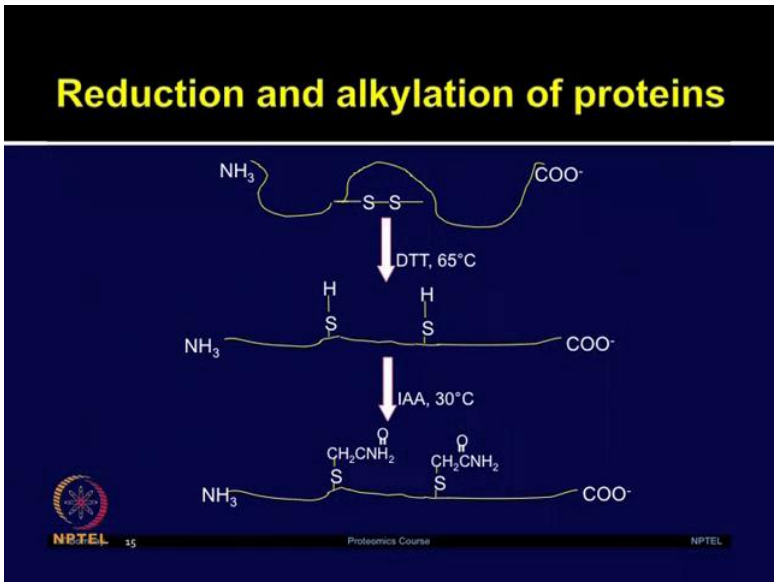

So, DTT is important for treatment of proteins to break the disulphide bonds, which we have also talked in the sample preparation when we discussed earlier. Now iodoacetamide it adds the iodoacetamide group to the sulphide group and prevents disulphide bond formation. So, these steps are quiet important in gel digestion process. Now, coming to the specificity of trip sin first of all let discuss why we need to do the proteolysis digestion. So, you want to generate the peptides with the molecular weight within the mass range of mass spectrometer he always want to simplify the process for even very superior analytical instruments.

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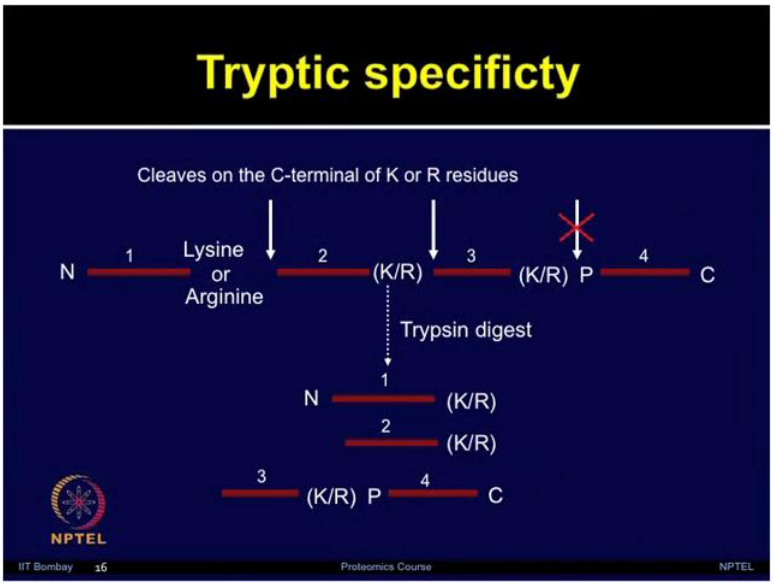

So, that you can increase the efficiency of the process, the enzymatic digestion are performed with the various enzymes, but typically with the trip sin which cleaves at the c terminal of lysine and argentine residues, but exceptions can occur with the praline, if praline is present then that breakage will not happen. So, one can use the modified trip sin which is a serine end peptidase; however, it cleaves at the praline lysine and the praline argentine bonds at the much lower rate. You can see the cleavage process and the specificity in the slide, where it shows if you have the lysine or argentine residue it can break the bonds, but when there is a praline residue present their then it cannot cleave.

So, the in gel digestion of proteins isolated by the gel electrophoresis remains core area in the mass spectrometry or in the any of the proteomics applications. So, the following video which we are going to watch, is going to provide the broad guideline for the in gel digestion; however, the recipe is very flexible and it varies from lab to lab to meet the specific requirement of particular proteomic experiment. The in gel digestion procedure is compatible with the downstream mass spectrometry analysis, whether you want to continue with the MALDI TOF or you want to do the l c m s based mass spectrometry analysis.

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The in gel digested protein samples are further processed by using zip tip pipettes which contains c 18 or c 4 media for enrichment of peptides prior to m s analysis. Zip tip pipette tip within 10 microfiber pipette tip with a bed of chromatography media fixed at

it end. It is used for concentrating and purifying peptides as well as removing salts detergents and interfering agents.

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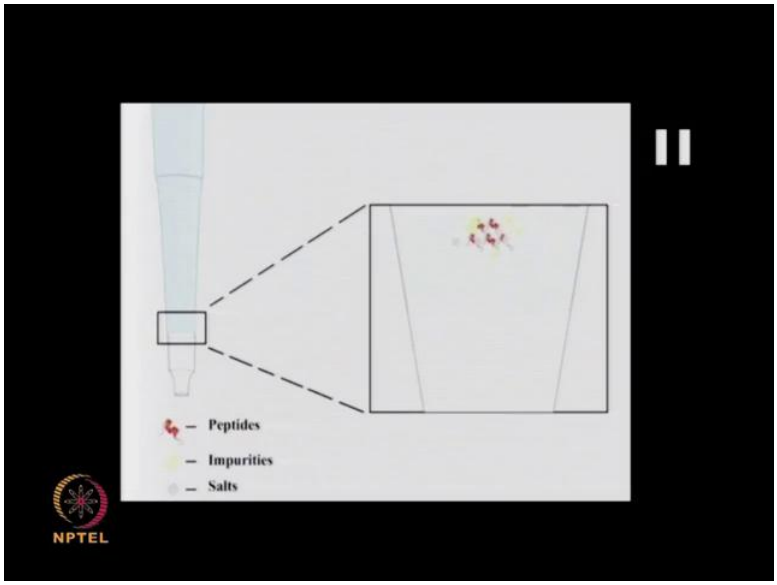

So, first we need to attach the zip tip pipette tip on top of a suitable micro pipette, condition the zip tip with 10 microfiber of acetonitrile, perform the washing steps three times and wash it with point one percent trifluoroacetic acid or t f a. So, after watching this video now you are very clear about the in gel digestion process how various steps are important to perform these experiments. Now once you have done the in gel digestion you can directly use these cryptic digest for further mass spectrometry analysis, but it is often recommended that in between you add one more step which is sample clean up, you do not want your columns or your MALDI instruments to get clocked due to the salt or some other interfering residue present in the mixture. So, it is recommended that one should use a cleanup step in between.

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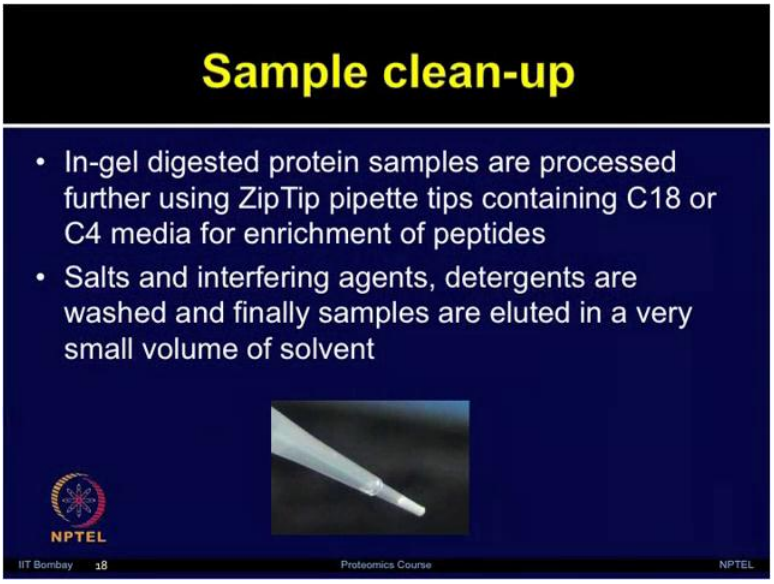

So, the in gel digested protein they can be cleaned up by processing further using zip tip pipette tip which contain c 18 or c 4 media for enrichment of the peptides. Salts and interfering agents the detergents are washed and finally, the samples can be eluted in a very small volume of the solvent. So, this zip tip is very small tip like device for removal of salts, as well as other interfering components from the protein sample, and it is performed before injecting the sample for the mass spectrometry analysis. The zip tip's can be incorporated into high throughput robotic devices or multichannel pipettes for the high throughput applications. Let me show you this video for the sample clean up by using zip tips. In gel digested protein samples can be further processed by using zip tip. This animation shows the washing solution of 0.1 percent t f a passing through the zip tip column. Now load the sample of an interest onto the zip tip by pupating 5 to 10 microfiber of samples and this step has to be repeated 10 to 15 times. They can do the binding of peptides to this activated zip tip by aspirating and dispensing 10 cycles.

So, samples are passed through the activated zip tip where they are captured in particular bed of chromatography media. Now wash this c 18 tip thrice with 10 microfiber of 0.1 percent t f a to remove the salt another interfering components. So, these steps ensure the salts and detergents are washed and finally, samples can be eluted in a very small volume of solvent. Now elute the sample from the zip tip with 10 microfiber of 50 to 70 percent acetonitrile in 0.1 percent of trifluoroacetic acid. After the elution is done then you can keep this process samples in cry boxes and store in minus 20 degree centigrade freezers.

The in gel digested samples which have been processed by using zip tip's can be further analyzed by using mass spectrometry. So, now you know how to performing the cleaning step by using zip tips. Now you have the sample ready and you have selected the matrix.

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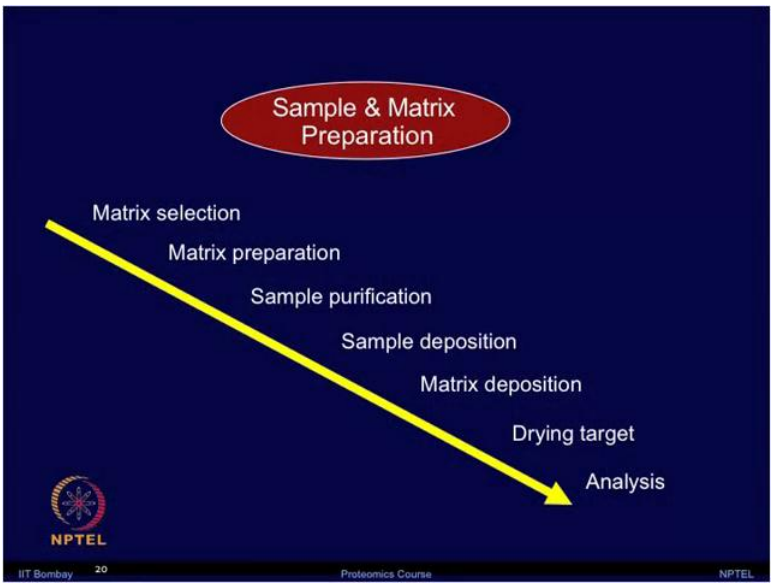

So, now, let me show you this various steps involved before you can actually start the MALDI experiment. So, you need to select the matrix you need to prepare matrix, you have already done the sample purification. Now sample need to be deposited on the MALDI plate, either, you can mix with the matrix or you can this to separately there are various combinations one can try, and then once both sample and matrix is deposited on the MALDI target plate, then you are ready to do the drying and then plates can used for MALDI TOF instrument for further analysis. Let us first talk about matrix selection.

So, the important step in MALDI TOF analysis is 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 compound with low vapour pressure and volatile natures, most of the matrices are acidic in nature. So, it can easily excide the photon and ionize analyse for the analysis; however, there are few matrix basic matrices are also available. In the slides I am giving you an overview of few matrices and some of their properties.

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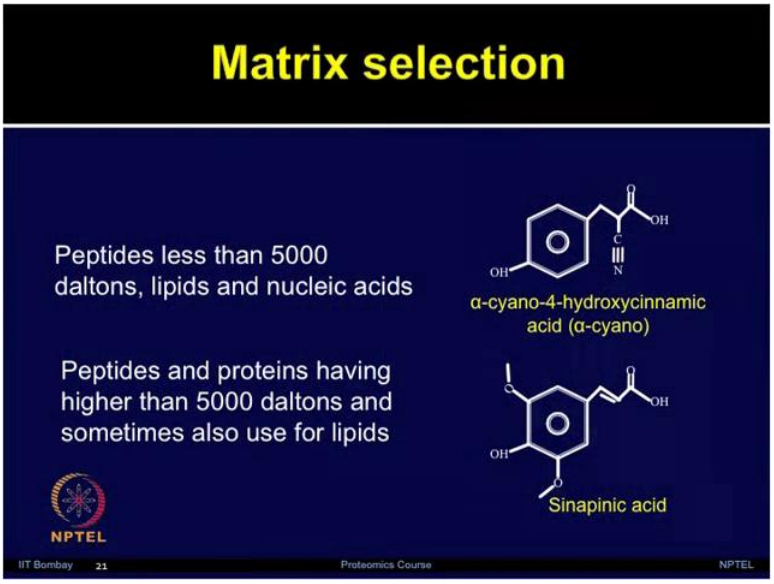

But, there are many more properties which is not mentioned here, but just to give you certain major features of these matrices commonly used for the various applications. So, one is alpha cyan 4 hydroxycinnamic acid. When you have peptides less than 5000 Daltons or lipids and nucleic acid, one can use this matrix, one can also use sinapinic acid if peptides and proteins are having more than 5000 Daltons and it can also be used for the lipids. Then you have options such as 2 5 dihydroxybenzoic acid also known as d h b, small molecules and peptides which are not ionized by the other molecules can be analyzed by using this matrix. Trihydroxyacetophenone t h a p it is used for small nucleotides and also used for phosphorylation and specialized application. 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 option available for selecting the matrix depending on the molecular weight and the type of applications, but regardless of these these matrices absorb energy from the laser source and converge both matrix and analyse into the gaseous phase. Matrix can also ionize analyse molecule by providing energy which comes from the laser bombardment.

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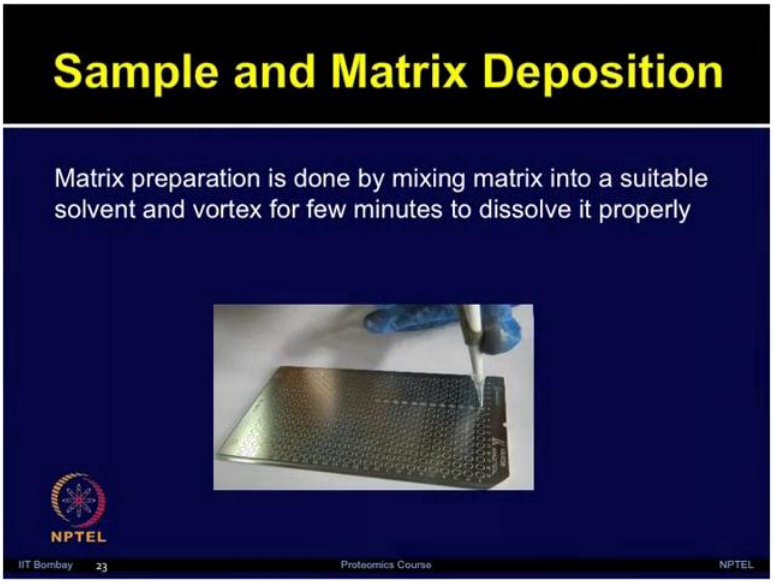

Now, once we have selected a matrix, matrix can be prepared by mixing it into a suitable solvent and vertex it for few minutes. So, that it can dissolve properly now you are ready with both your analyse and the protein which you want analyze as well as the matrix which you have selected for your application. Now one needs to think how to deposit that 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 a eppendorf tube and then the mixture is directly deposited by using a micro pipette on to 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 matrix is deposited above it and then it is properly mixed before drying process can happen. Other way of doing is to apply that with the sandwich based method, in which first a small amount of matrix is deposited on the plate, then you add the protein sample and again matrix is spotted on top of it.

So, that you have enough matrix in the below and the above of the analyse. So, one can try different combination of placing the matrix and the analyse. And then once you have placed all of these sample of interest on the MALDI plate, then you are ready to dry the target plate. So, after spotting is done and MALDI plate is dried almost 30 minutes then the instrument can be turned on and m s analysis can be performed. Now there are various type of configuration of these instruments available, as well as there are various type of commercial software which help to operate the hardware. It is 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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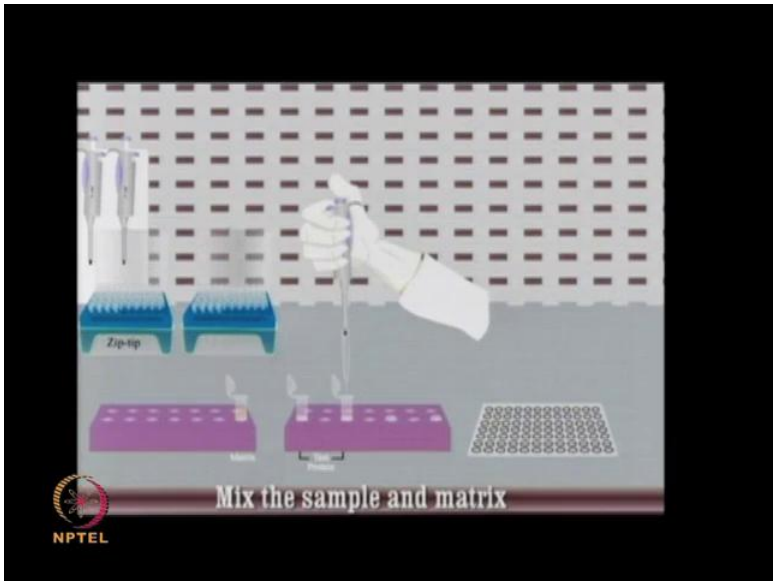

MALDI's perform in two steps. In first step the compound for the analysis should be dissolved in solvent containing small organic molecules known as matrix. This mixture is dried

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Before analysis and liquid solvent used in the preparation of solution is removed.

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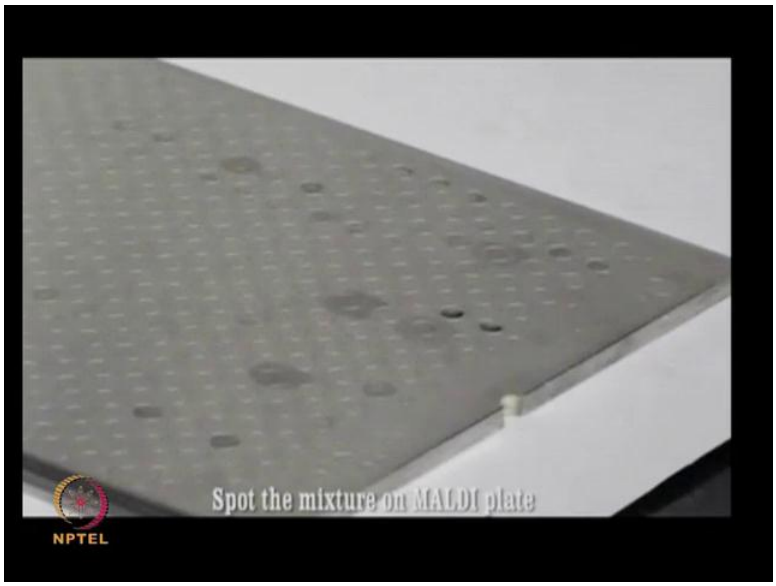

So, in this video by depicting the matrix preparation, as well as instrumentation will try to give you overview of the MALDI TOF instrumentation.

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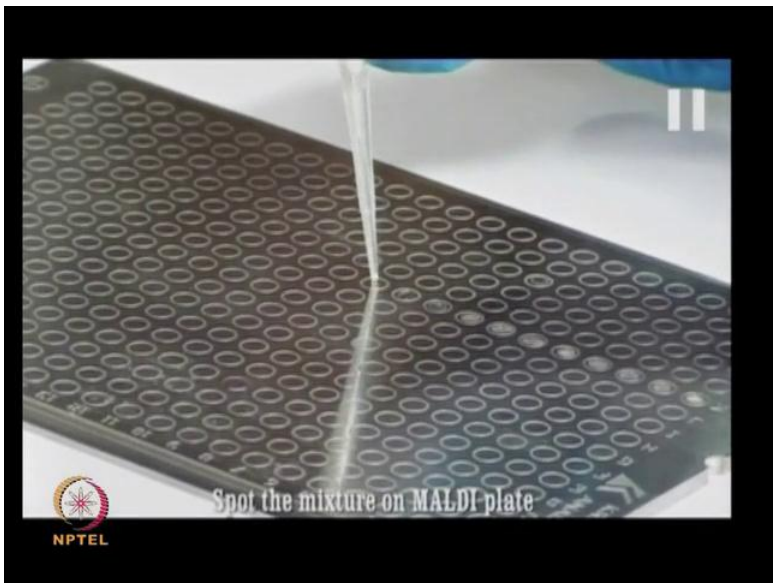

So, spot the mixture on the MALDI plate. How uniformly you can plate this mixtures on the MALDI plate ensure your good spectra and data quality later on.

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Completed on the MALDI plates the samples are allowed to dry for 30 minutes after which the instrument is switched on and m s analysis.

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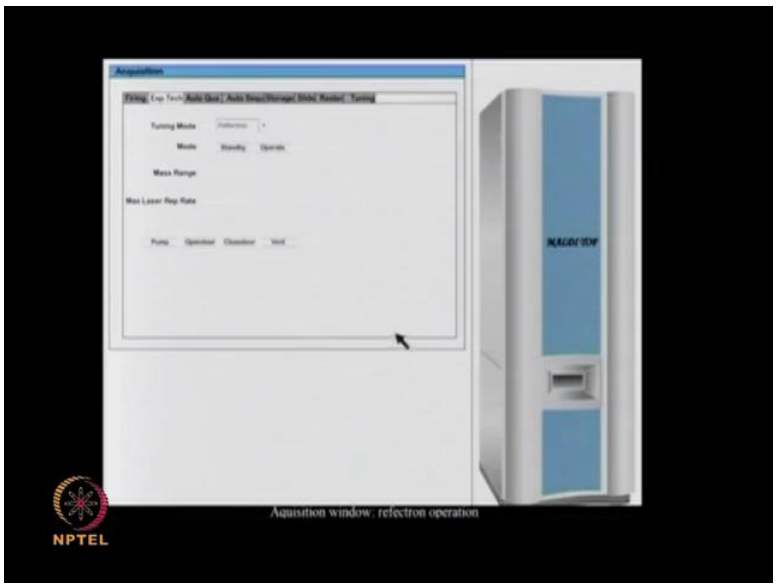

Can be performed while these steps are happening you need to ensure that instrument is on.

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So, click on the software and open the acquisition window and then click on the open door, insert MALDI target plate face up with the cut-off corner to the front and

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Now by using software close door. The door of insertion chamber is now closed you can select the plate; you can view the overall plate on the screen and then select a spot which you want to analyze.

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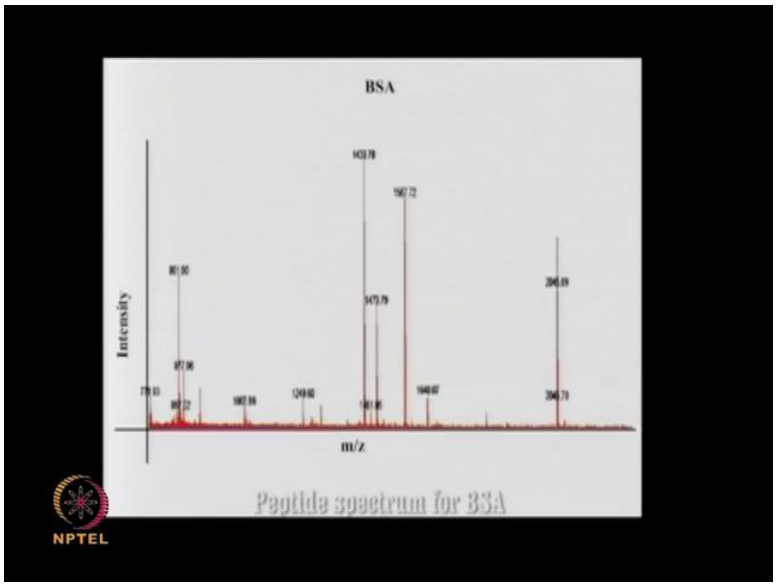

So, click on the yellow target in the acquisition window and select go to the location. You can now do the laser bombarding and peptide spectrum is generated we have shown here one standard protein bovine serum albumin. So, you have to look at various locations where you can get best spectra from that spot and then you can freeze it.

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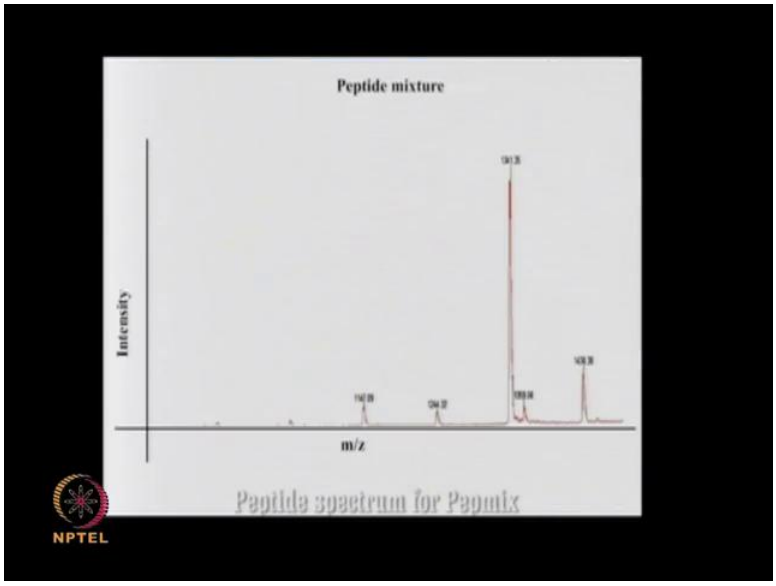

Same process can be performed for different spots and different regions. Now we have shown here a spectra for the pep mix.

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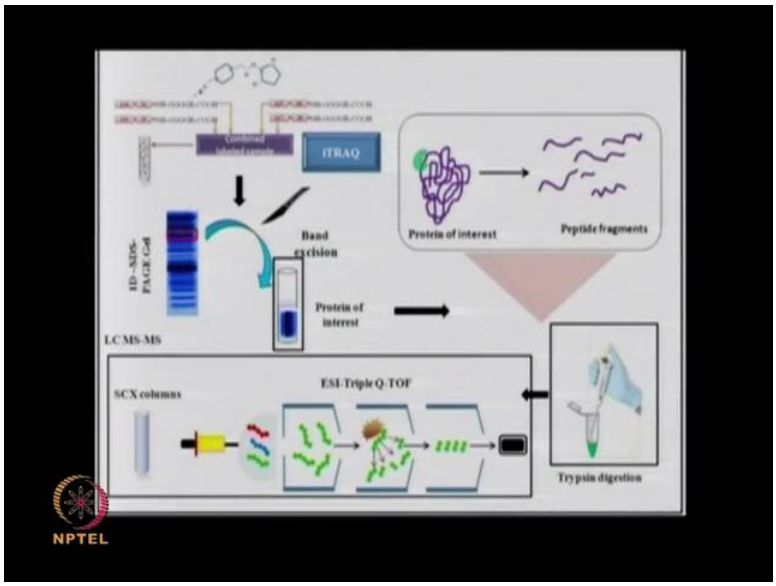

In gel digestion of proteins for m s analysis video. Often one or two dimensional gel electrophoresis is applied for separation of complex mixtures of proteins prior to mass spectrometric analysis. In gel protonated digestion of separated proteins is performed to cleave the proteins of interest present within the polyacrylamide matrix. You can see the overview of various steps involved in this process. One can use one dimensional electrophoresis or take the protein samples directly from the mass spectrometry based experiments. Separate those on s d s page or two d gel and then excise the band of interest or spot of interest. The gel based technique increases the dynamic range of analysis, since they involve sequential separation of proteins based on the molecular weight lower to higher molecular weight. The mass spectrometric identification of the target protein greatly depends on efficacy of the in gel digestion process.

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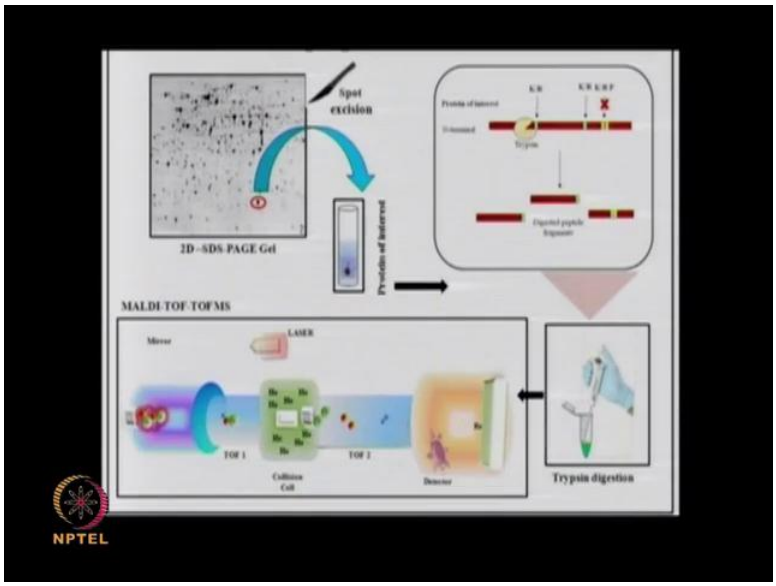

That generates mixture of peptides on the target protein through proteolysis digestion.

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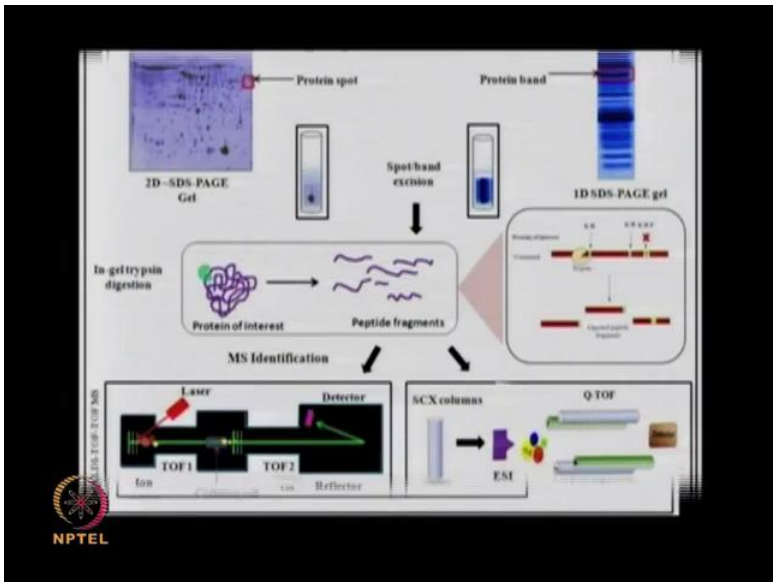

This slide gives you the overview of various steps involved in the in gel digestion process, and also how various type of samples can be analyzed by using this method, whether you have one d s d s page gel, two d s d s page gel, in either way you have to do the in gel tryptic digestion. Once the peptide fragments are generated, then those can be analyzed using l c m s m s or madli TOF or different type of hybrid mass spectrometry

techniques. So, in gel digestion it is a multistep procedure, but remains central to the proteomic applications.

In gel digestion includes spot selection, spot excision, stain removal, reduction, alkylation, proteolytic cleavage and finally, extraction of the peptides. So, let us say you have run a gel first of all you need rinse the entire gel with water for few hours with intermittent changing of the water. Now you would like to exile the band of interest or the spot of interest. So, keep a glass plate inside a lamina hood and clean the surface carefully excise protein spot with a sterile scalpel and place gel slice into eppendorf tube. It is possible you have exile the large spot or large band, then cut the slice into cubes while avoiding too small pieces as they can clog the pipe at tips.

So, this is the lager spot you cannot directly take this one for doing the in gel digestion. So, you need to exile that into small cubes. Now keep this small gel pieces into sterile micro centrifuge tube. Now you can add 50 to 100 micro litter of stain removal solution for the large gel pieces take enough liquid to cover it completely. So, you can adjust the volume depending on the size of your spots or the band. After adding off then stain removal solution rotate it on a shaker for 30 minutes at room temperature for complete removal of the stain from the gel pieces. It is recommended that you change the solution after every 10 minutes.

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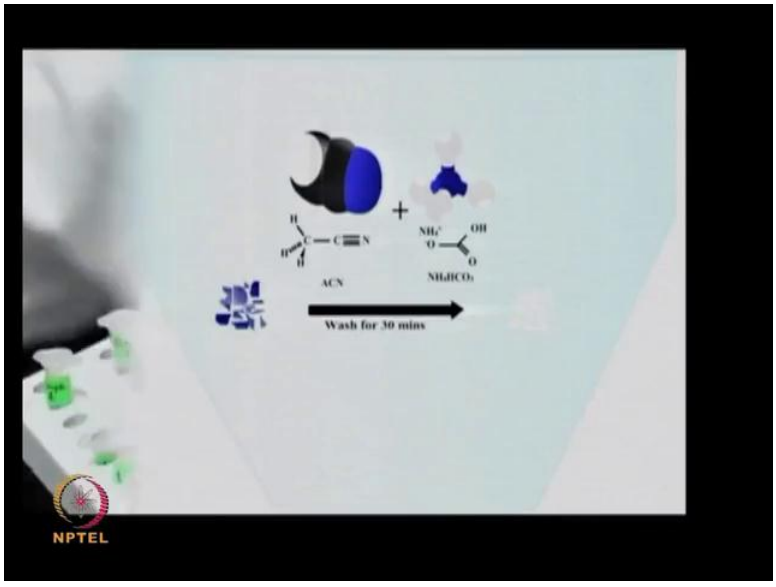

By changing the solutions and removing it after every 10 minutes. The coomassie brilliant blue stain gel piece becomes colourless. We have seen the animation. Now once the staining is removed, then you can add dehydration solution. So, add 50 to 100 micro litter of dehydration solution and rotate that on the room temperature. You need ensure that you change the solutions after every 10 minutes. So, that gel pieces becomes wide and stick together.

Now, spin the gel pieces down at 1000 g for 30 seconds. Once centrifugation is complete then remove all the liquid. After removing the solution completely then add reduction solution. So, add 30 to 50 micro litre of reduction solution to completely cover the gel pieces, incubate it 30 minutes at 56 degree centigrade, for treatment of protein residues with dithiothreitol breaks the disulfide bonds. Now chill down the tubes to room temperature, add 50 micro litre of dehydration solution, mix it properly and incubate for 10 minutes to remove all the liquids.

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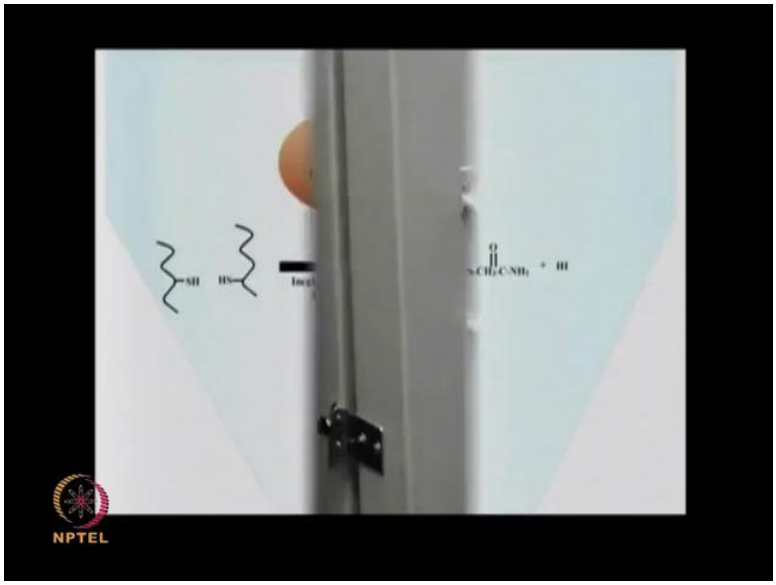

Then add alkylation solution. So, add 30 to 50 micro litres of alkylation solution and incubate it for 20 minutes at the room temperature in dark condition. The iodoacetamide prevents the deformation disulfide bonds it is an alkylation agent, it adds iodoacetamide group to the sulfhydryl group and prevents disulfide bond formation. Now your incubation is over. So, you can remove the tubes, and now remove the solution. Now add 50 micro litre of dehydration solution mix it mix it properly by overtaxing and incubate it

for 10 minutes. Again you need to remove all the solution, air dry the gel pieces and then add 25 micro litre of trip sin solution around 500 nana gram.

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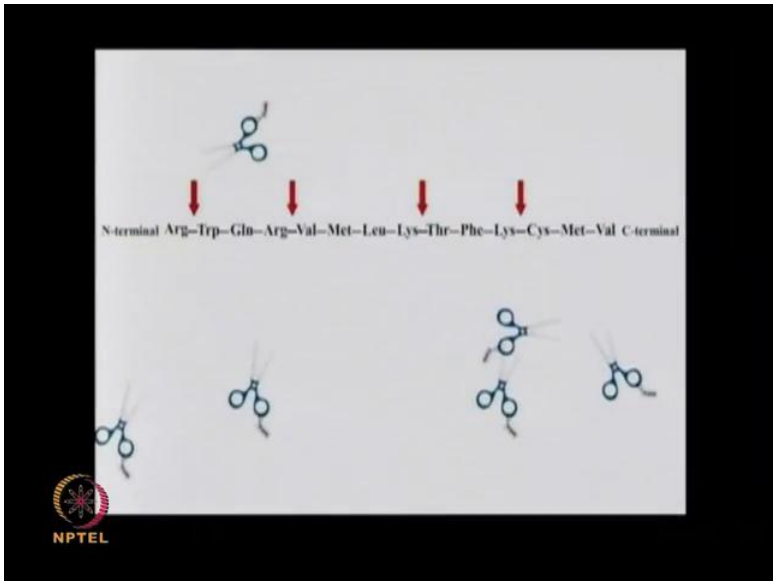

So, add trip sin to the dry gel pieces and keep it ice for 30 minutes, for absorption of enzyme by the gel pieces. Add 25 micro litre of ammonium bicarbonate buffer, it is same buffer in which trip sin is prepared and incubate at 37 degrees for overnight 12 to 16 hours for proper proteolysis cleavage. So, prior to m s identification proteins are digested to generate peptides, there are several proteolysis enzymes are available chymo trip sin, trip sin, pepsin are some of the enzymes commonly used for proteolysis. After the overnight step you have to stop the reaction by keeping the reaction mixture in ice.

Now, after the overnight incubation the peptides generated through the proteolysis digestion can be extracted by using extraction buffer, containing 0.1 percent formic acid or trifluoroacetic acid in 50 percent of acetonitrile solution. Now collect the supernatant in the small aliquots and extracted samples can be stored in these small aliquots. The efficient extraction process is essential to ensure the release of peptides from the gel matrix to the solution. Now all the solution volumes described in the protocols are recommended volumes, but depending upon the experimental requirement, you can optimize and change these volume and the incubation timing according to your experimental requirements.

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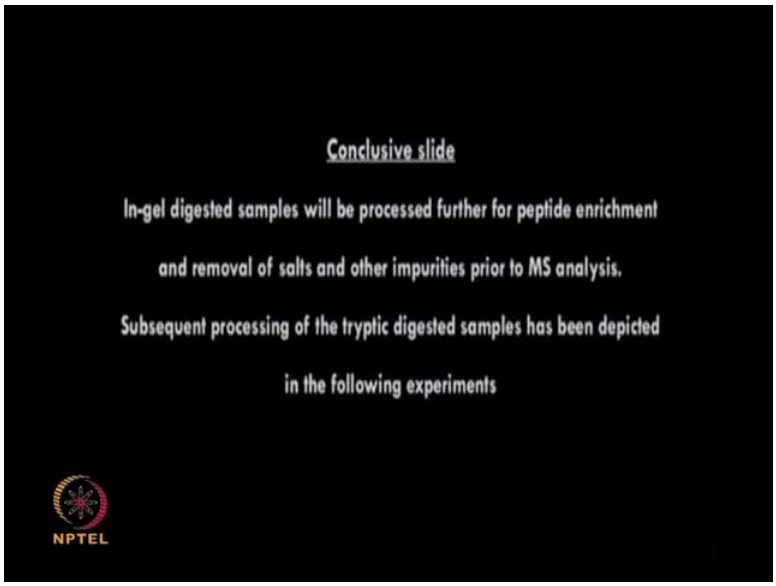

Now, these samples can be stored and used for the further mass spectrometry analysis. So, now you are clear with how to perform the amide TOF experiment. Now let us add one more mass analyzer. So, now we have configuration of MALDI TOF-TOF.

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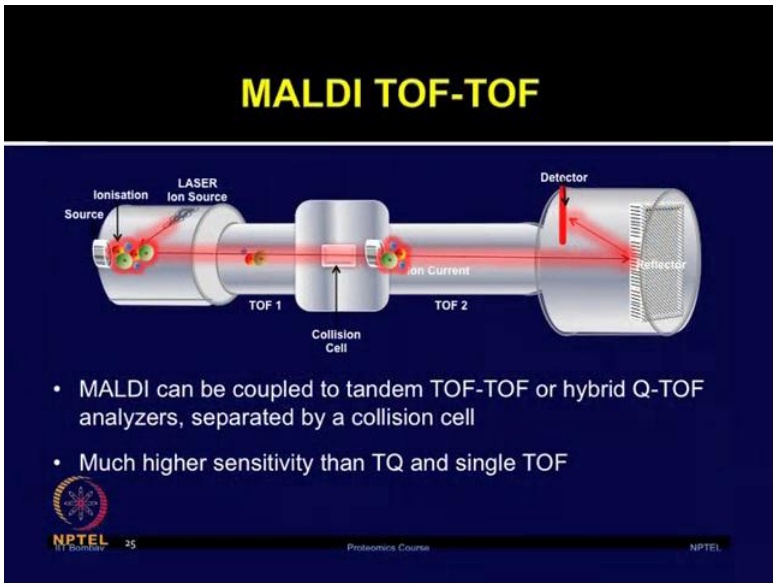

So, MALDI can be coupled to the tandem time of light in combination with another time of light. So, TOF-TOF or with hybrid quadruple time of light analyzers which are separated by the collision cells. Now for proteomic application it is recommended to use the TOF-TOF or q TOF the peptide ions are accelerated through the first time of light

tube, as you can in the slide. And then they are dissociated by introducing an inert gas into the collision cell. This process allows the collision induced dissociation spectra from the madly 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 finger printing and high throughput analysis of the protein or proteome is possible with the hybrid TOF analyzers.

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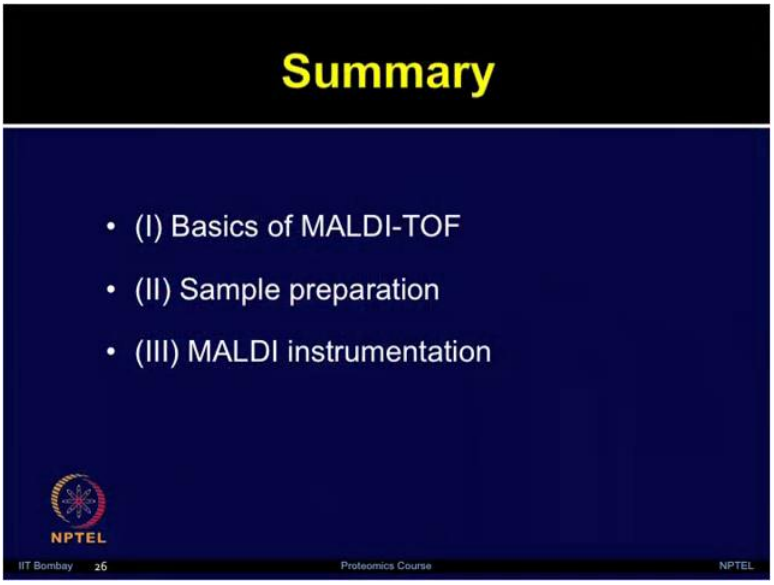

So, in summary today we talk about basics of MALDI TOF we have also discussed the sample preparation various steps involved including in gel digestion, matrix selection, zip tipping and after that very briefly we discussed about MALDI TOF instrumentation. And then we talked about how various type o hybrid configurations can be used to increase the overall sensitivity and various applications for the proteomics. We will continue our discussion on the mass spectrometry in the next lecture; we will talk on the liquid chromatography based methods, thank you.