Introduction to Proteomics
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Lecture - 15
Lab session - Protein/Peptide Pre-Fractionation using OFFGEL FRACTIONATOR & Data Analysis

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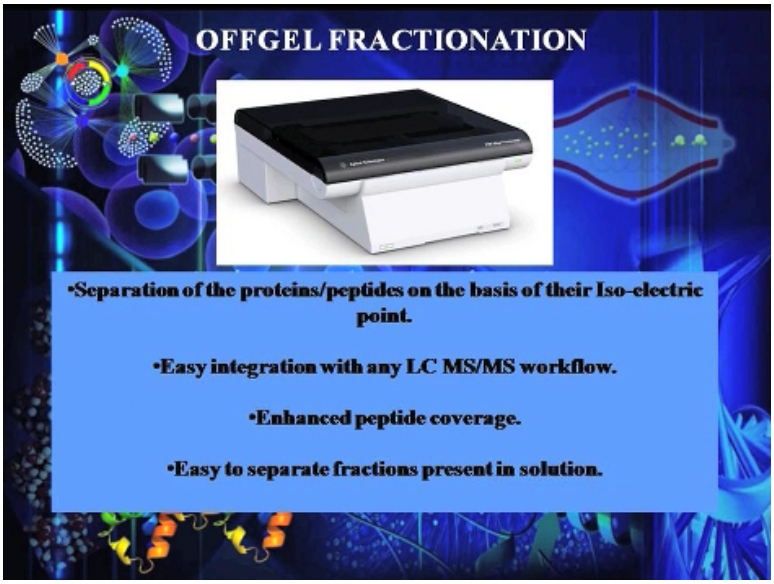

Today we will do a lab demonstration of the OFFGEL fractionation and we will see how the OFFGEL fractionator works. This device helps in fractionation of proteins and peptides the principle is very simple like that of the two-dimensional gel electrophoresis.

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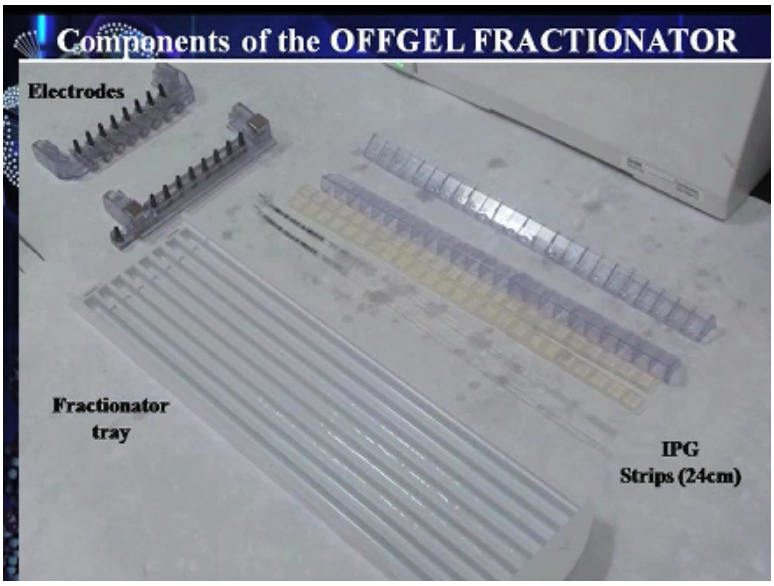

Here we also use a IPG strips to separate the protein or peptides based on its isoelectric focusing point why we do OFFGEL.

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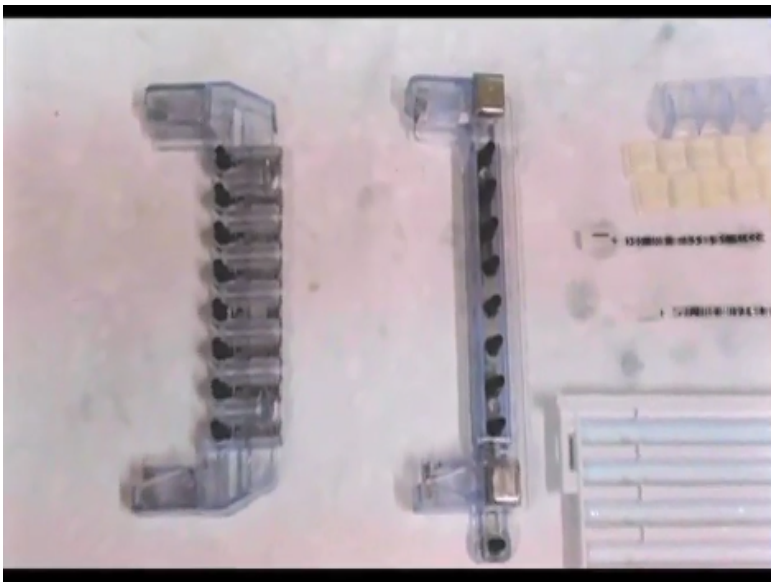

We do it not only to reduce its complexity of the samples also to get enhanced coverage of the proteome.

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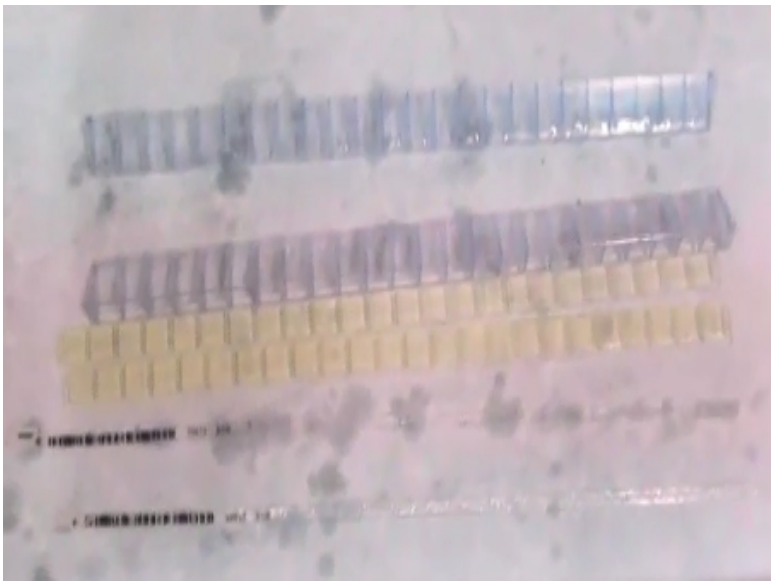

So, as you can see these other different components that are used for fractionation. So, we have electrodes we have the IPG strips of different strip lengths and we have the collector cup and the over layering cover. So, the IPG strip is a very important component as it is on this that the peptides are separated. We use the IPG strip on the tray.

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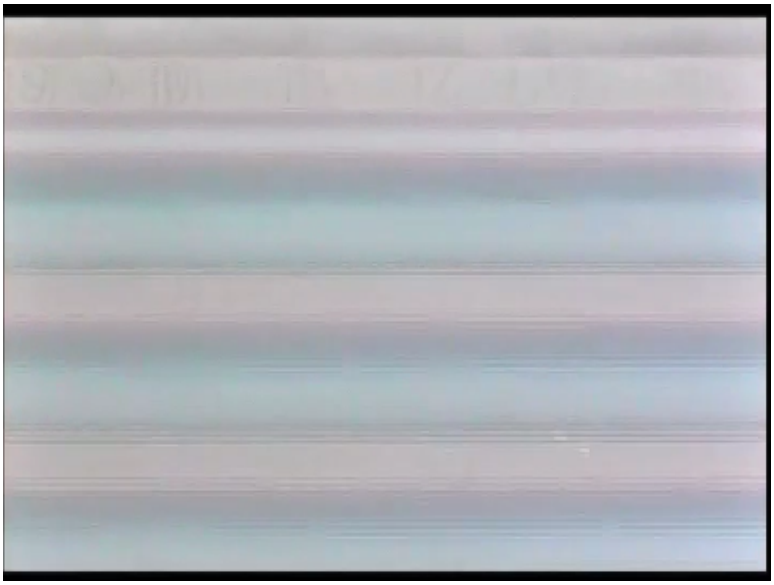

And we overlay it with our sample before we do the fractionation. We have multiple number of lanes in the tray so as to do parallel object fractionation of several different samples.

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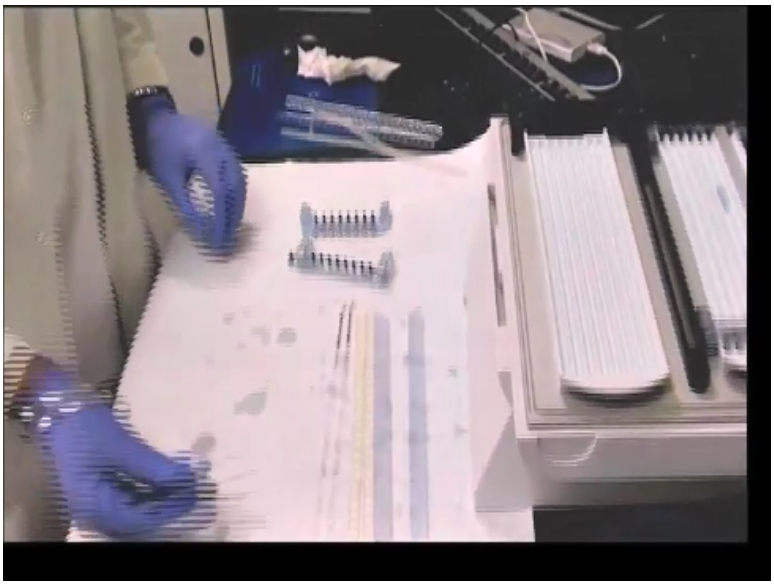

So, in this step we will see that how we will place the strip on the tray. We can place parallelly multiple strips for the object fractionation this step we will remove the cover of the strip gently so as not to disturb the sense of the surface.

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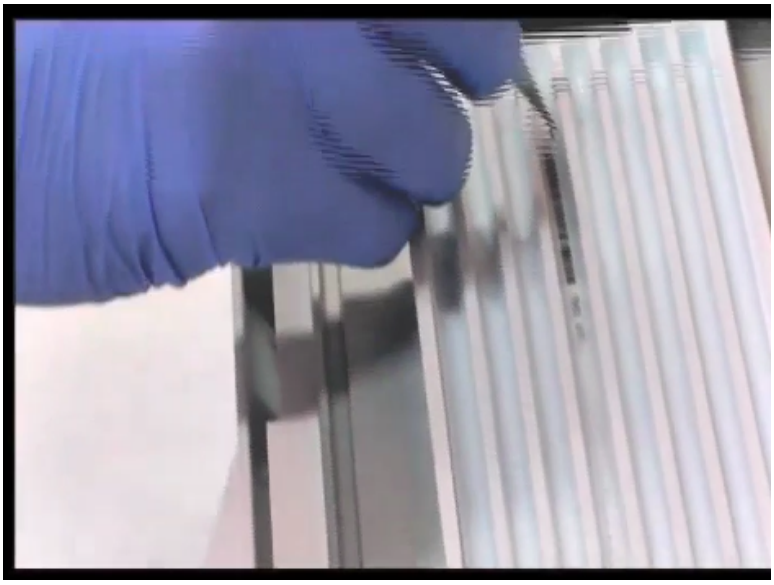

It has to be done very carefully using forceps we will place the gel on the tray. The gel side has to be up otherwise the peptide separation of the protein separation will not be able to occur properly. Now, we will place the cups on which the factions will be separated and place it tightly on the gel. Using your fingers press it gently so as to make it tight on the cup.

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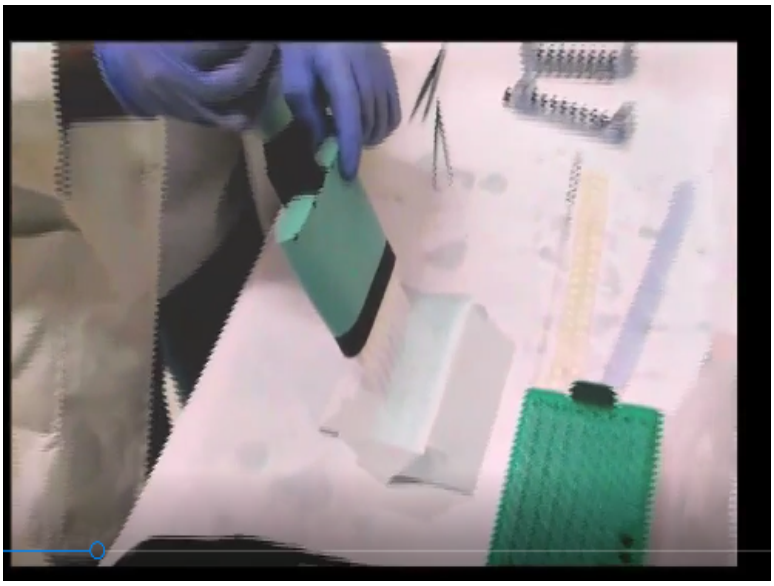

This is the step where we will rehydrate the gel. We will use 50 Micro liters of Milli cube water and place it on the cup.

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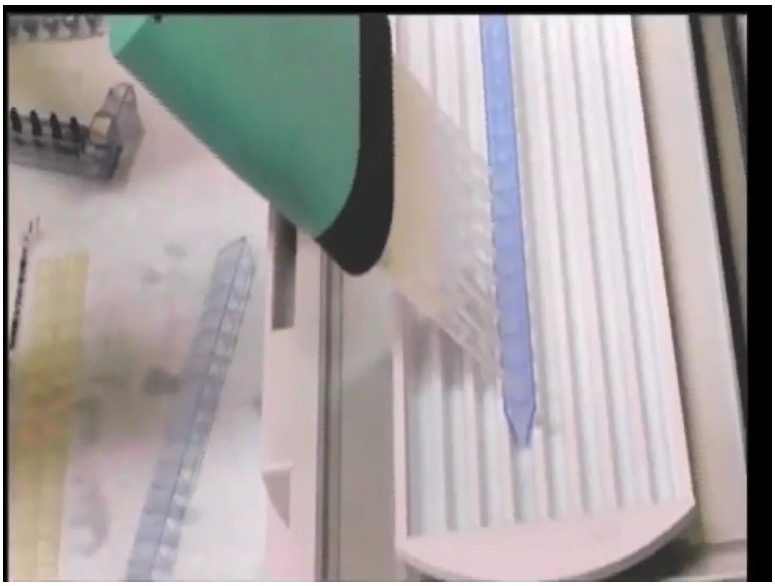

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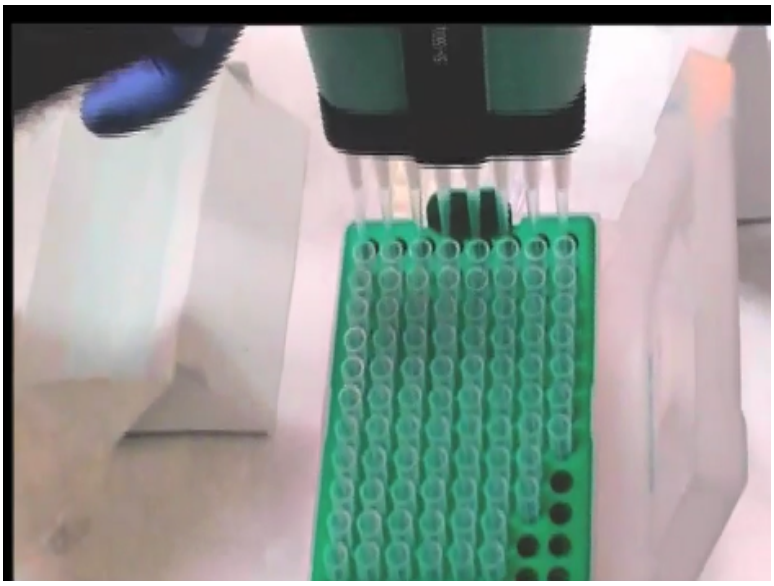

We will do it using the multi-channel pipette so as to maintain the uniformity in the volume.

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Paper mix are used in the sides of the well so as to maintain the conductivity of the current because during the fractionization high voltage of current is passed through the IPG strip and sometimes the conductivity is lost. These paper mix will allow the current to flow properly the fractionation to happen in the proper manner.

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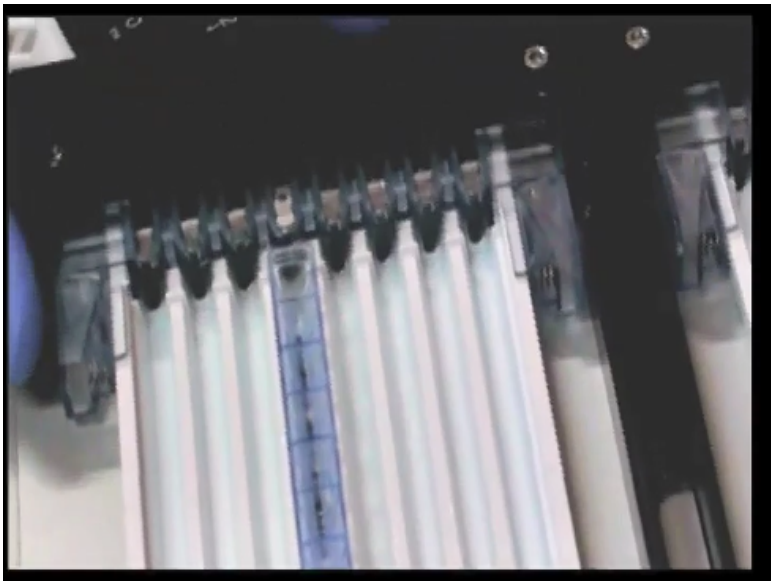

And the electrodes are displaced on top of the tray in both the sides.

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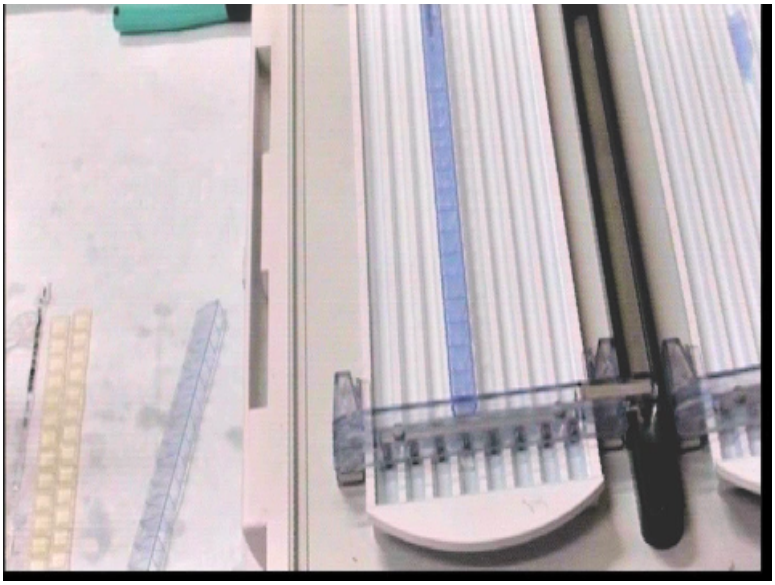

And finally, will seal the cup so as to stop evaporation. After 15 minutes of incubation we can put the samples on the IPG strip in the similar manner.

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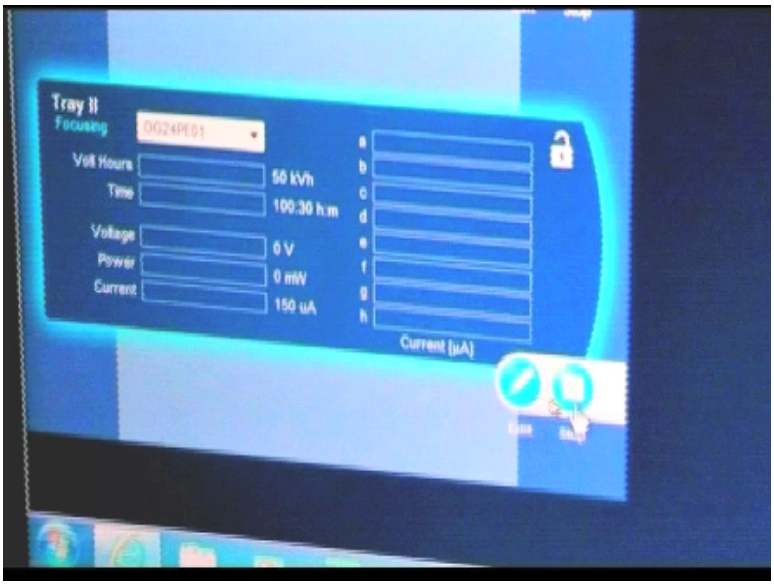

In this step we will see the various parameters with which we can monitor the fractionation process like the voltage and current passing through. When we click on the start button the fractionization process starts and as you can see the lane on which the IPG strip is placed will display the amount of current passing through. So, if you use multiple strip there will be different display in those lanes as well.

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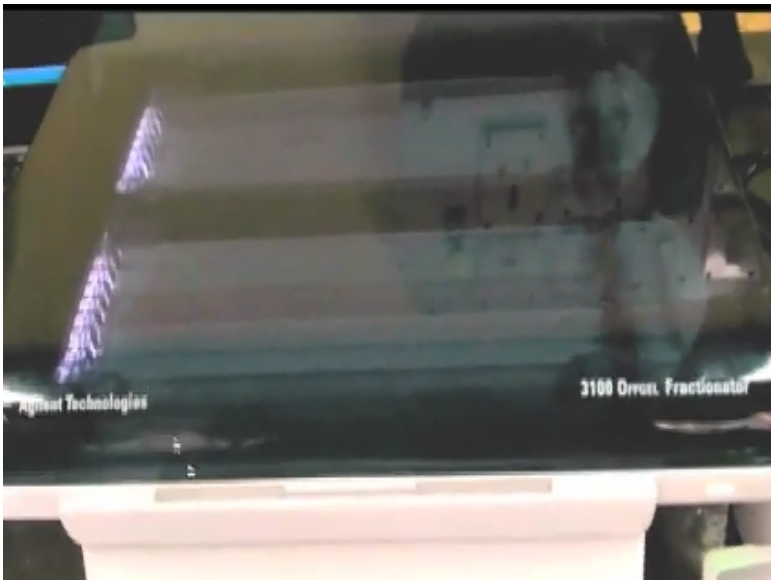

The machine will show a light indicating the conductivity that is happening once the process is done the machine starts blinking and now is the time where we can collect the object fractionated samples.

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Once the object fractionation process is over.

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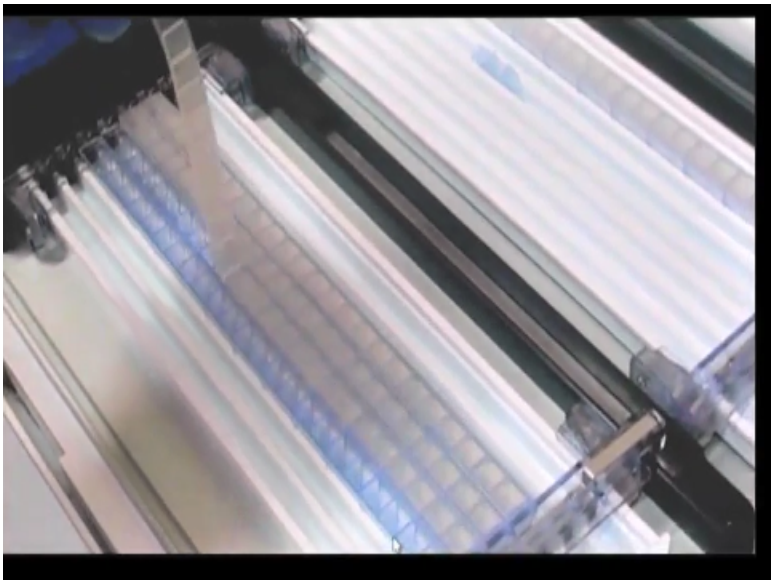

We have to remove the overlaying cap gently. After this process we will collect the individual fractions in different Eppendorf.

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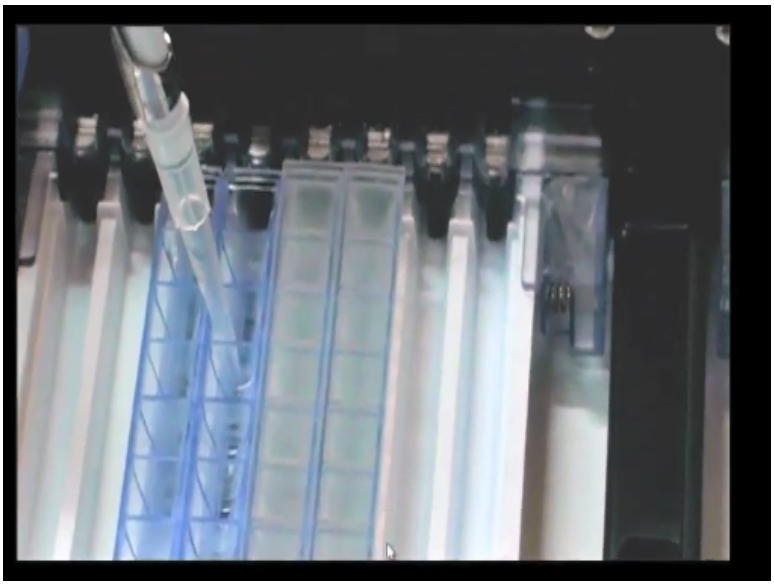

We can use a micro pipette to take out the different fractions and make sure that all the sample is taken out avoiding the gel pieces. Each fraction is collected in different Eppendorf tubes and often vacuumed right before it is subjective to mass spectrometry. Thus, in this way a simple procedure you can help us in getting a greater coverage of the proteome before mass spectrometry method and also this helps in reducing the complexity of the sample.

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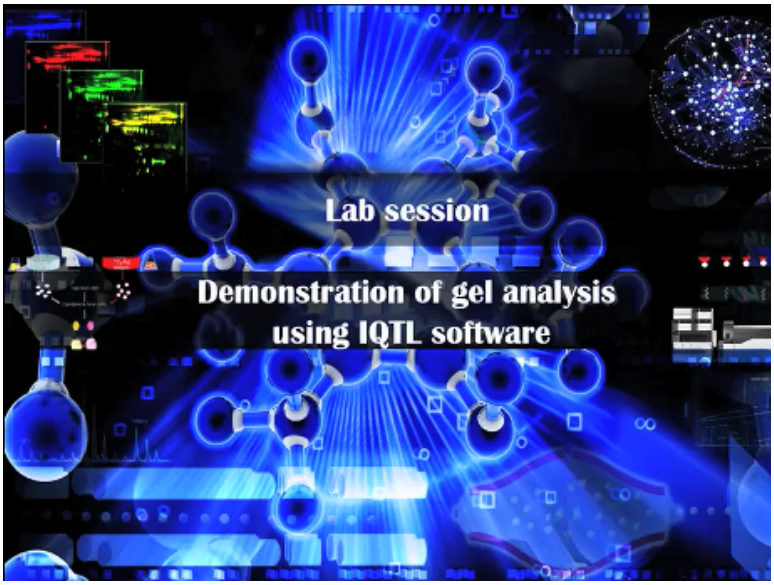

Hello everyone.

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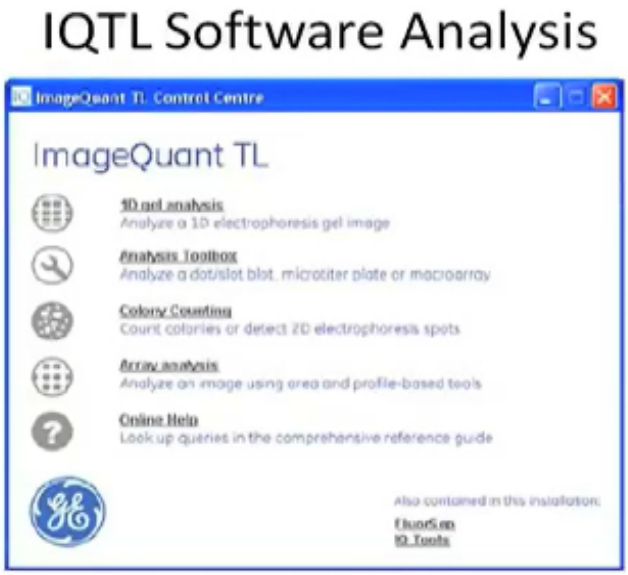

Today we will have a lab demonstration of a gel analysis using IQTL software. Gel analysis is imperative to study differentially expressed proteins IQTL is high performance easy to use software package for the quantitative analysis of images from a wide range of biological samples a wide array of gel analysis software are used in different quantitative proteomics studies such as 1DL electrophoresis gel array, array analysis colony counting/2d spot analysis, general image analysis using toolbox.

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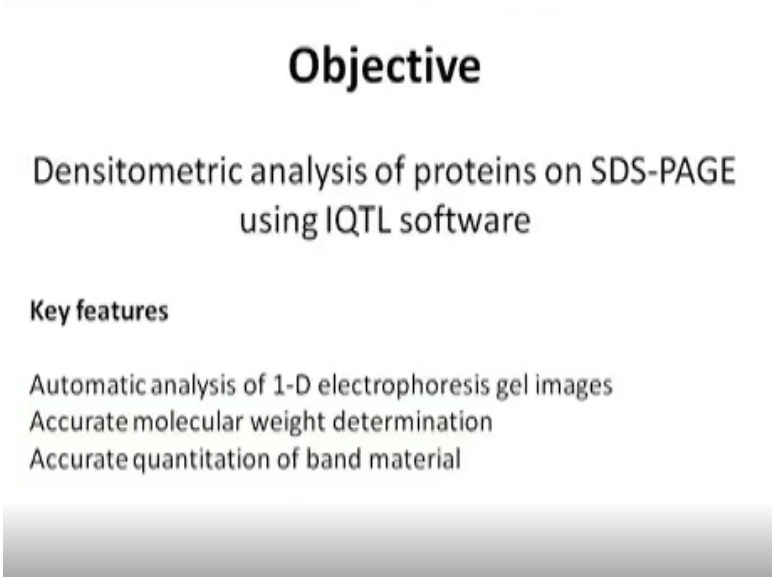

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We will open the image which has been saved on the local desktop.

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We can analyze gels either automatically or stepwise. First, we will analyze the gel automatically once we click on automatic method it will detect the band and give analysis result. But there is one drawback it skips some bands which is undetected. So, we prefer to go stepwise method where we can manually select the bands.

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In case of step wise there are different steps first we will create the lanes by clicking on manual.

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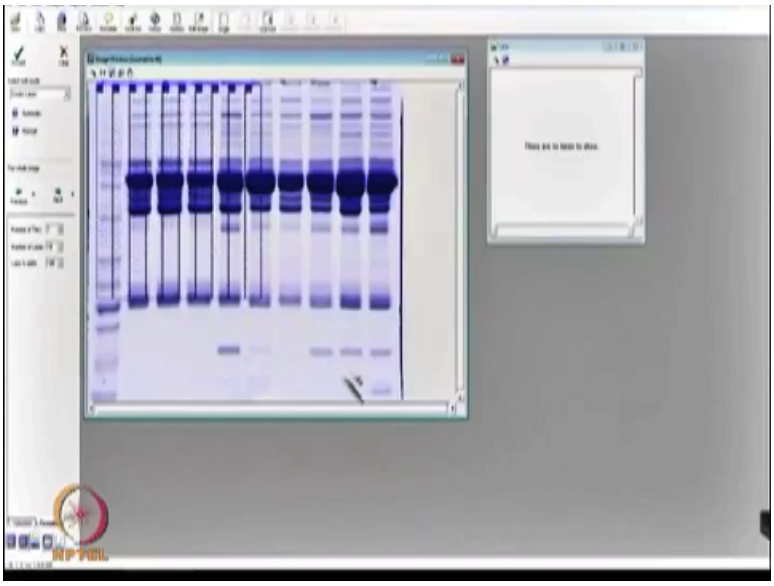

Now we click on the top left of the gel and scroll down till bottom right after that if bands are not coming under the lane. We can edit the lane by clicking on edit single lane and we can move the lane at their right place.

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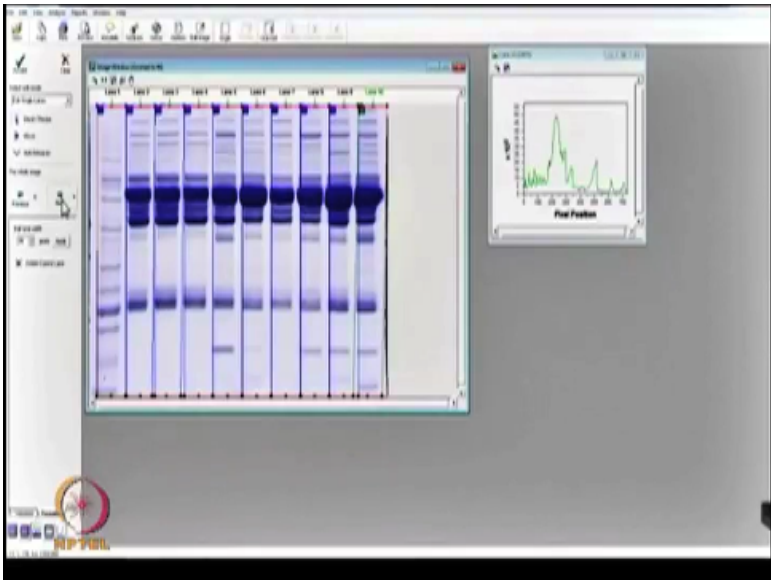

Background subtraction action will minus the background the values from the real intensity of band and will give accurate measurement. It can be performed by clicking on the subtract band detection. Band detection can be carried out automatically using parameters such as minimum slope noise reduction etc with band edges determined automatically or set to a fixed width.

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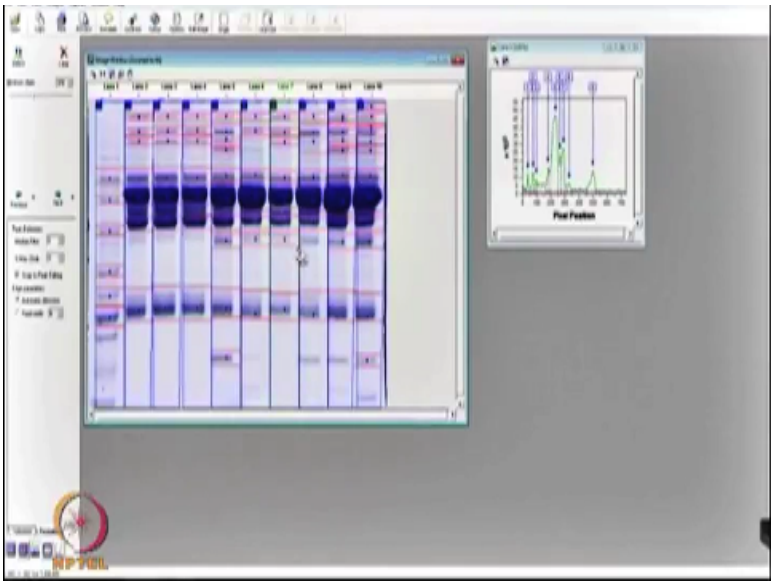

Here we will detect the bandwidth automatically it skips some low intensity bands that we can select manually.

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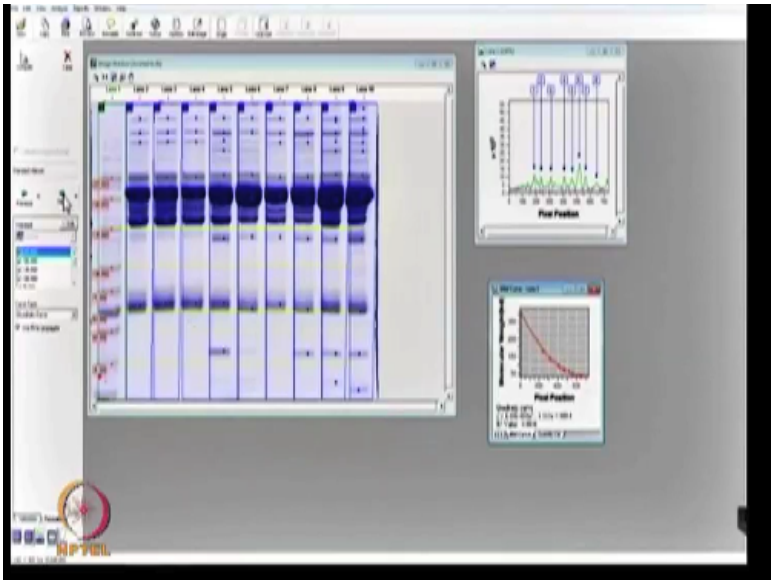

Molecular size molecular size allows users to select from a library of 13 standards. Or to create their own new standard here we have selected Abcam standards. Since we have run molecular size standards for Abcam and assign the molecular weight size to each band in first lane. Molecular weight curve can be seen on the right side of the open tab the molecular weight of bands automatically displayed in measurements table.

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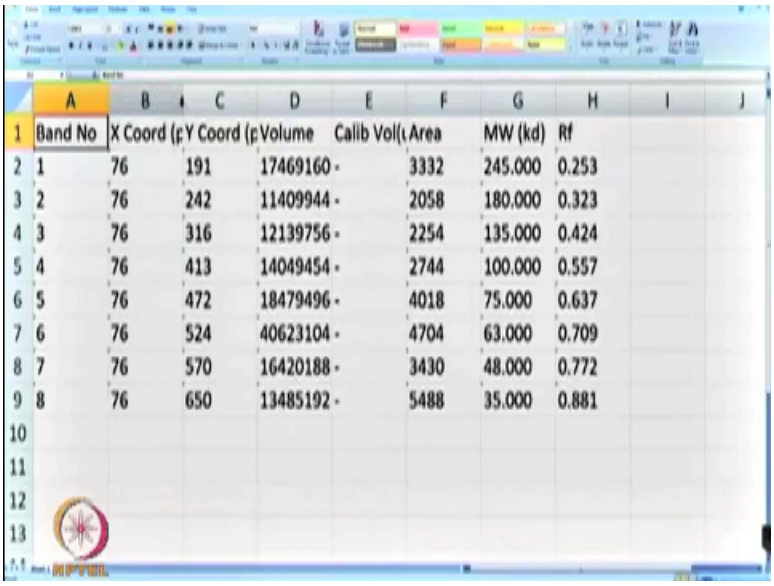

	A	B	C	D	E	F	G	H	I	J
1	Band No	X Coord (p	Y Coord (p	Volume	Calib Vol(u	Area	MW (kd)	Rf		
2	1	76	191	17469160	-	3332	245.000	0.253		
3	2	76	242	11409944	-	2058	180.000	0.323		
4	3	76	316	12139756	-	2254	135.000	0.424		
5	4	76	413	14049454	-	2744	100.000	0.557		
6	5	76	472	18479496	-	4018	75.000	0.637		
7	6	76	524	40623104	-	4704	63.000	0.709		
8	7	76	570	16420188	-	3430	48.000	0.772		
9	8	76	650	13485192	-	5488	35.000	0.881		
10										
11										
12										
13										

Datasheet can be exported as excel file go to edit and click on export as an excel file this excel file have the data for selected lane here. We can see band known coordinate of bands molecular weight and RF values. In the similar way we can export the data for all lanes and can compare the band intensities

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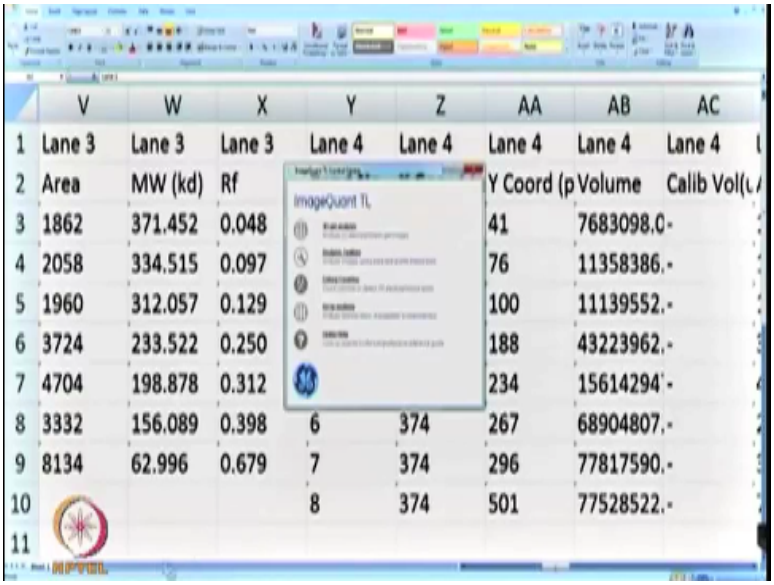

	V	W	X	Y	Z	AA	AB	AC
1	Lane 3	Lane 3	Lane 3	Lane 4	Lane 4	Lane 4	Lane 4	Lane 4
2	Area	MW (kd)	Rf			Y Coord (p	Volume	Calib Vol(u
3	1862	371.452	0.048			41	7683098.0	-
4	2058	334.515	0.097			76	11358386.	-
5	1960	312.057	0.129			100	11139552.	-
6	3724	233.522	0.250			188	43223962.	-
7	4704	198.878	0.312			234	15614294	-
8	3332	156.089	0.398	6	374	267	68904807.	-
9	8134	62.996	0.679	7	374	296	77817590.	-
10				8	374	501	77528522.	-
11								

So, in this way we can do 1D Gel analysis IQTL software. Thank you.

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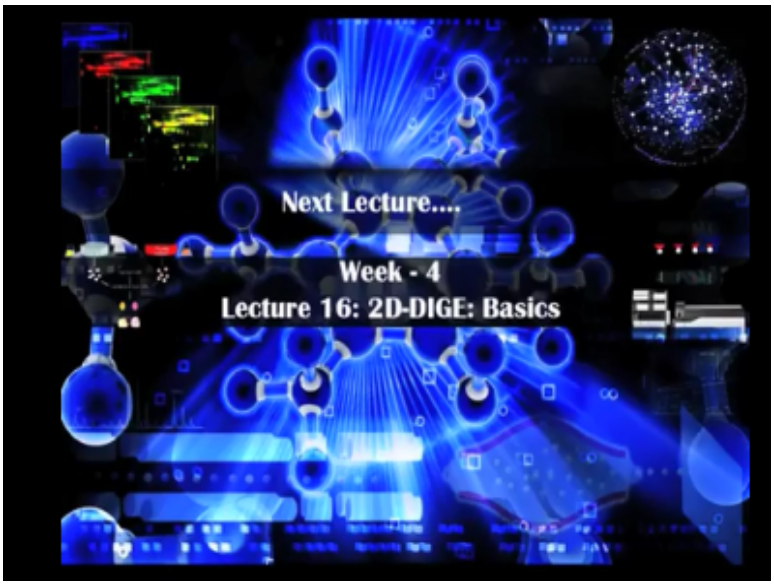
Next Lecture....
Week - 4
Lecture 16: 2D-DIGE: Basics