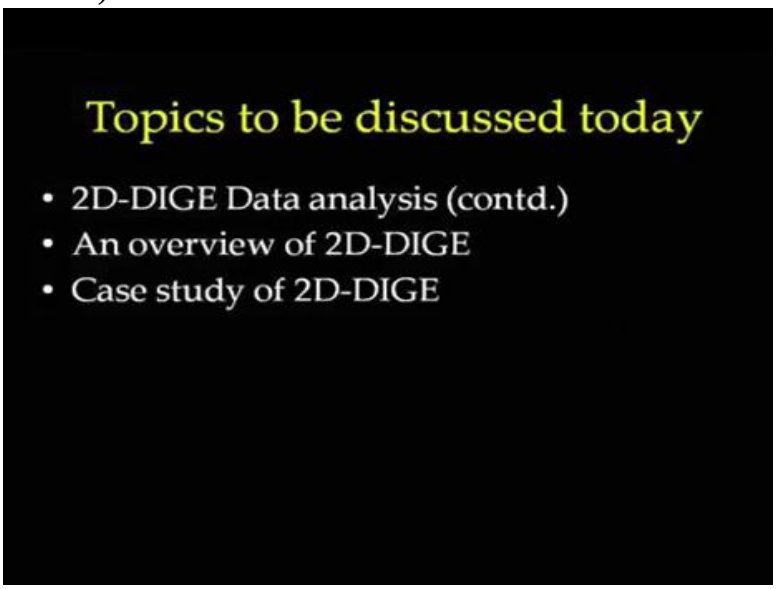
Proteins and Gel-Based Proteomics
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Mod 05 Lecture Number 19

(Refer Slide Time 00:14)



Professor: Doctor Srinivas from GE Healthcare, who is going to talk to us about DIGE technology and give us a demonstration on software to perform DIGE gel analysis.

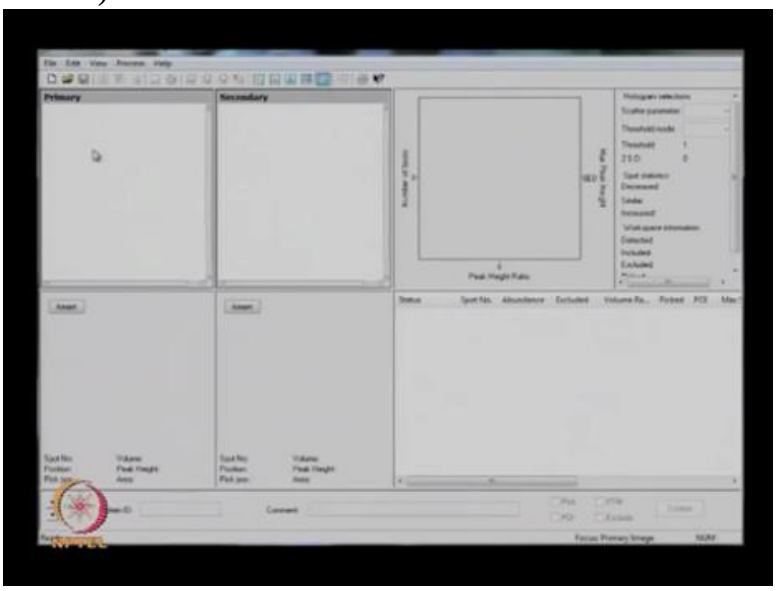
(Refer Slide Time 00:33)

Professor - expert conversation starts

Expert: The next one is Differential in Gel Analysis shortly we call as DIA

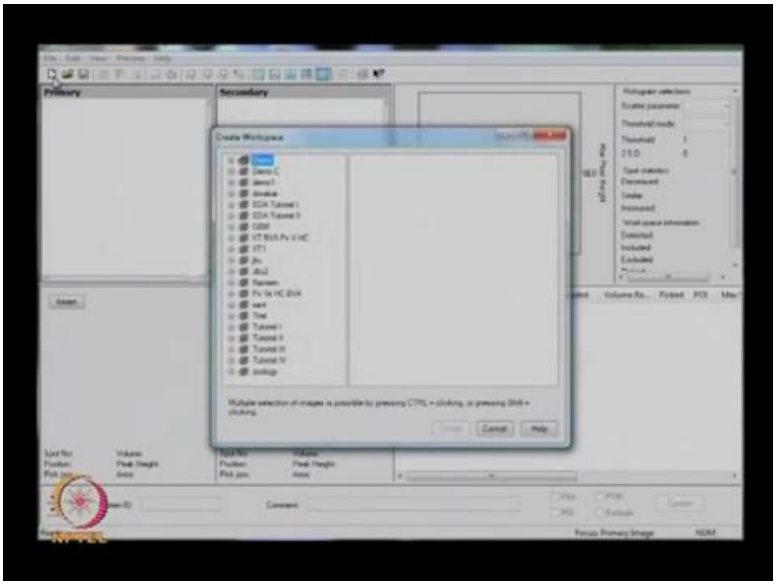
(Refer Slide Time 00:40)

(Refer Slide Time 00:45)



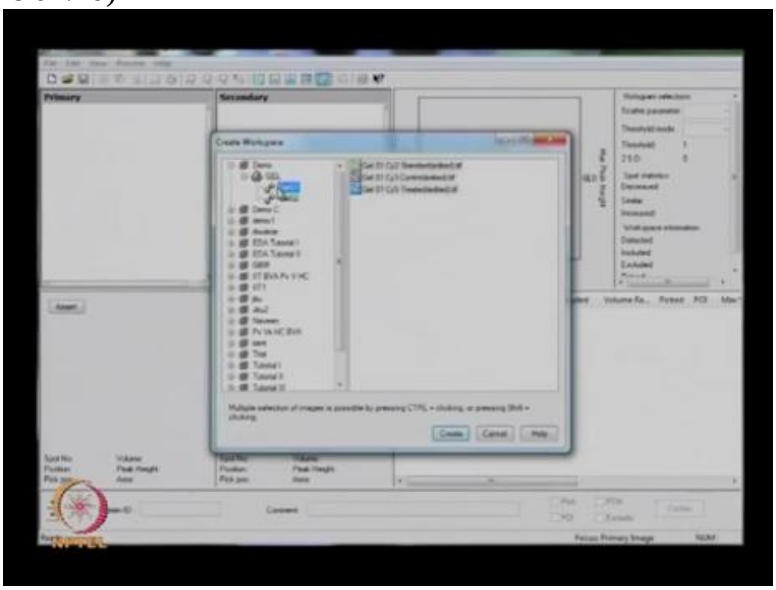
Expert: In this you can see we can create a new project, a new DIA here like there is an option, create workspace.

(Refer Slide Time 00:54)



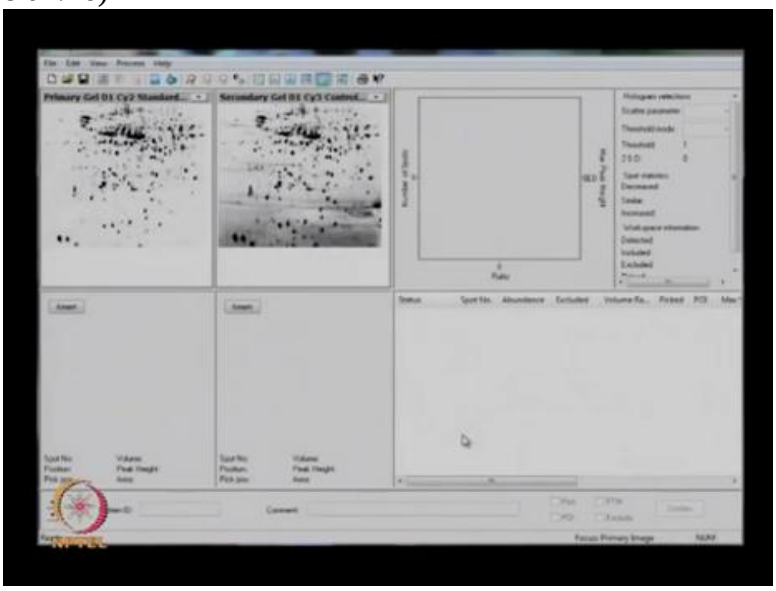
Expert: From here we can .it will take you to where we already saved our gels in our database Now from the database, we can select any particular project and from there.

(Refer Slide Time 01:10)



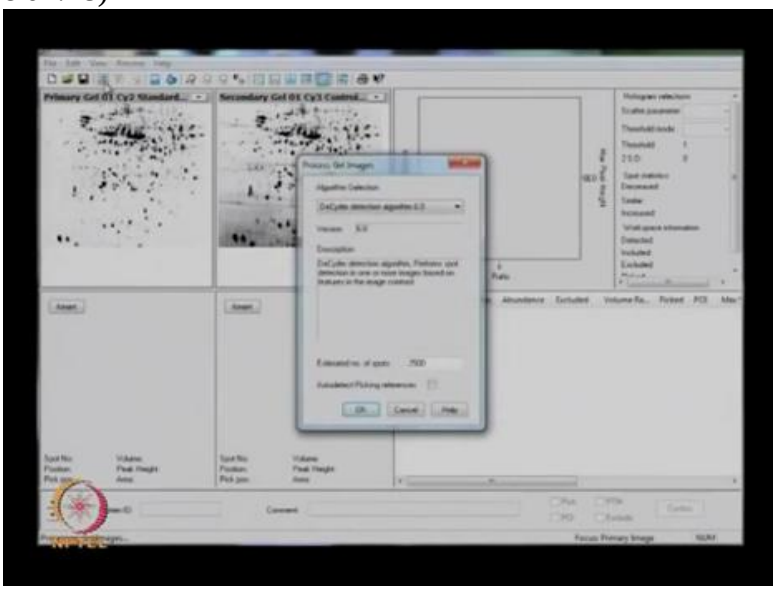
Expert: I am selecting Gel No 1, as we save this one

(Refer Slide Time 01:16)



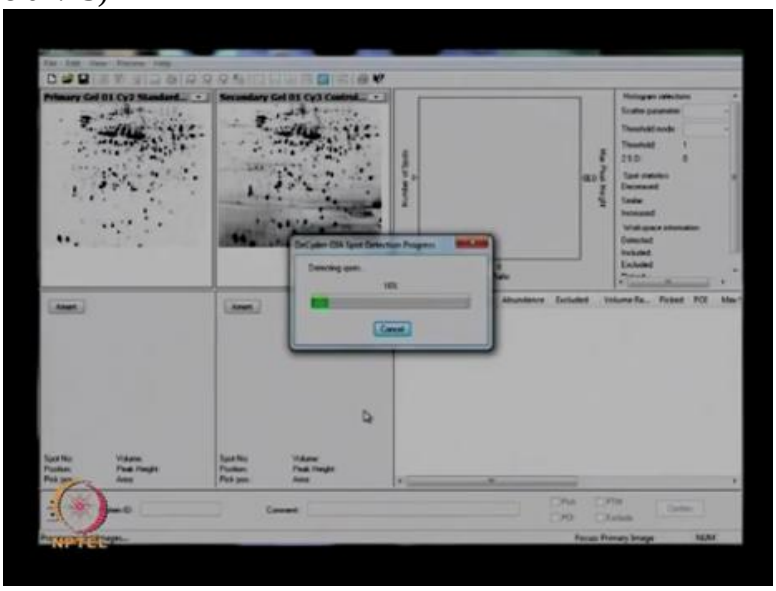
Expert: Now you can see these gels where we have uploaded. So now after uploading here, you can process these gels, then

(Refer Slide Time 01:28)



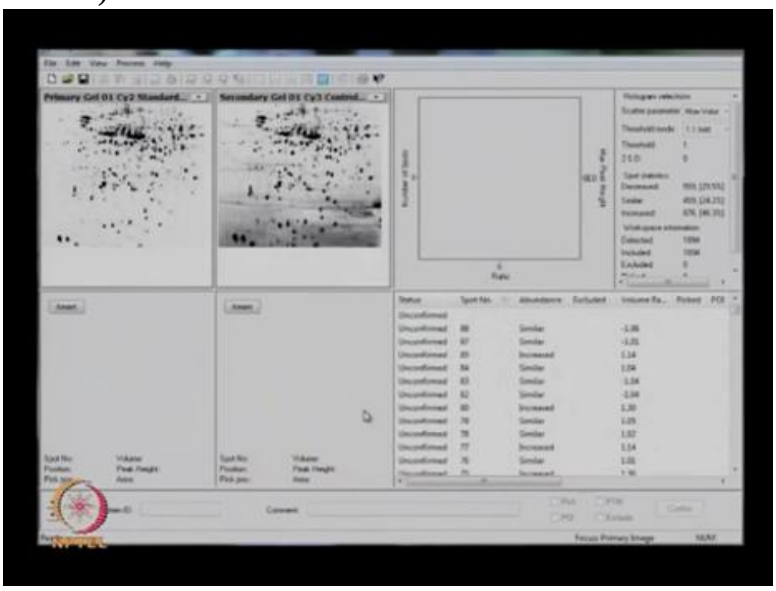
Expert: during process you have to give some number. This is some threshold which you are giving here actually. This will be 2000 so that it will take care of background issues also

(Refer Slide Time 01:45)



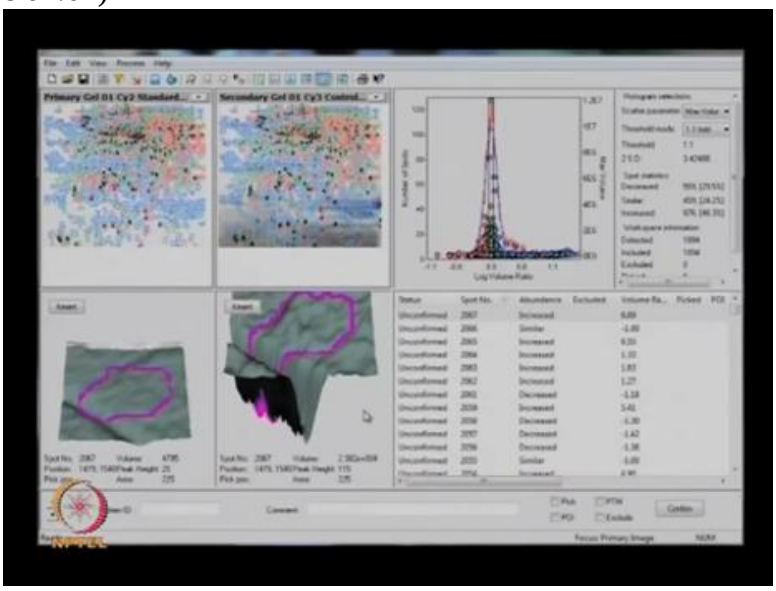
Expert: Then basically in DeCyder, co-detection will happen. But I would like to explain you some more about what is co-detection? This co-detection is, uses the information of all 3 channels and will create

(Refer Slide Time 02:01)



Expert: a geometrically identical

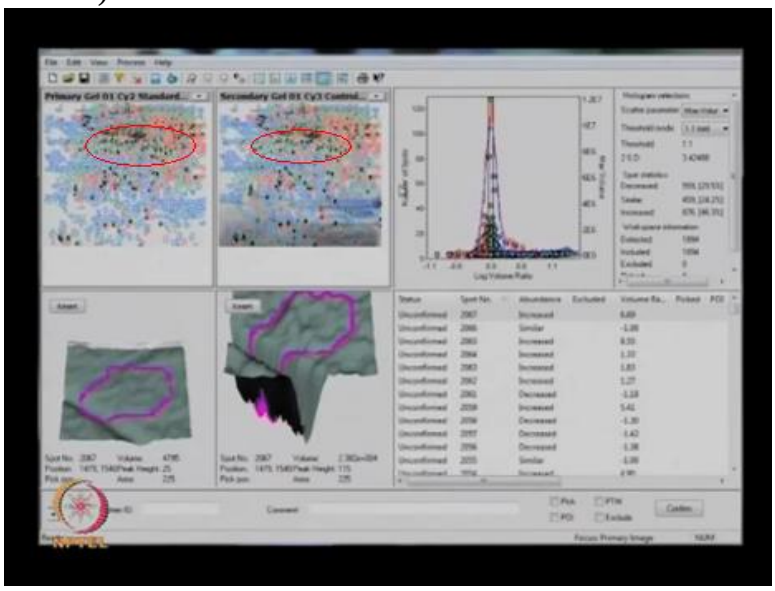
(Refer Slide Time 02:04)



Expert: spot boundary for a spot across all the channels. That means, there are 3 channels; Cy2, Cy3, Cy5. Out of these 3 channels, in Cy2 image, it creates a particular volume and the same area can be applicable for the remaining two gels also This is way it works. In this way quantitative and qualitative results are much more accurate than with a single detection

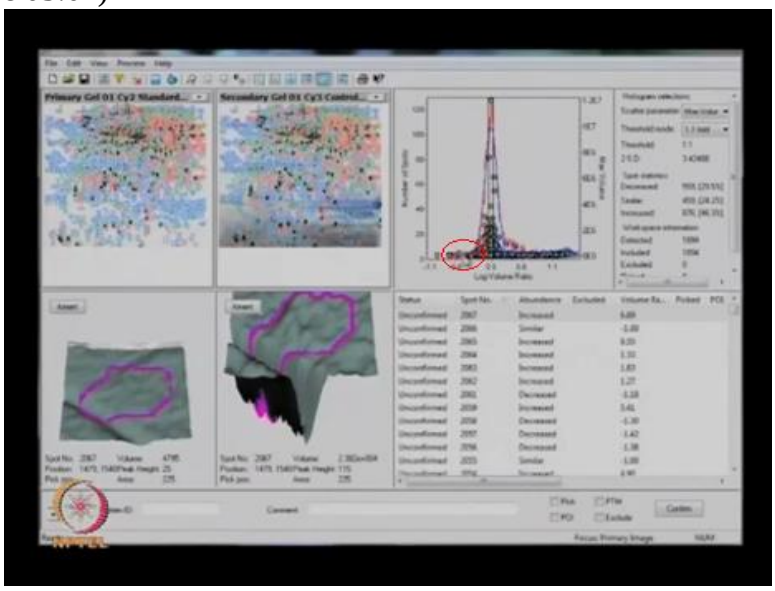
In DIA, each image is co-detected with its internal control producing 2 images pairs. The ratio of standard sample is calculated further Or the ratio of standard sample is calculated for each protein in each image.

(Refer Slide Time 02:57)



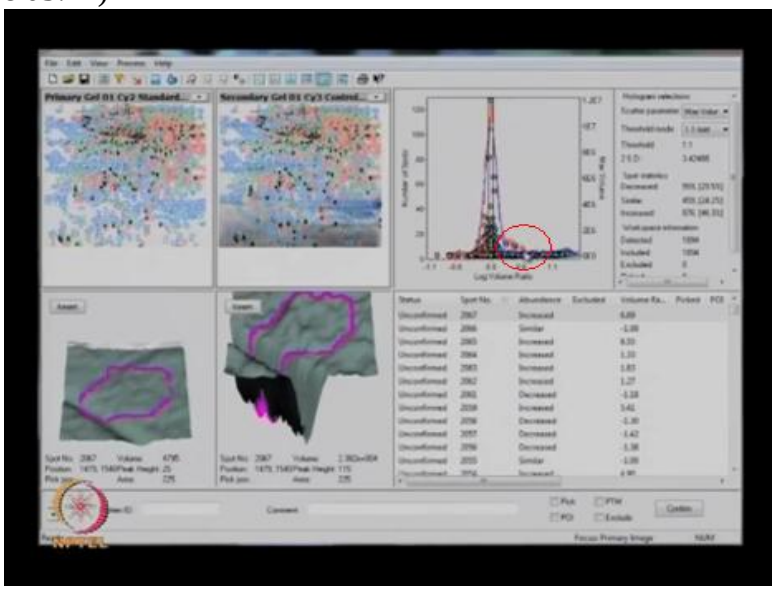
Expert: So as we see here, these are all the number of spots it has been detected and which

(Refer Slide Time 03:04)



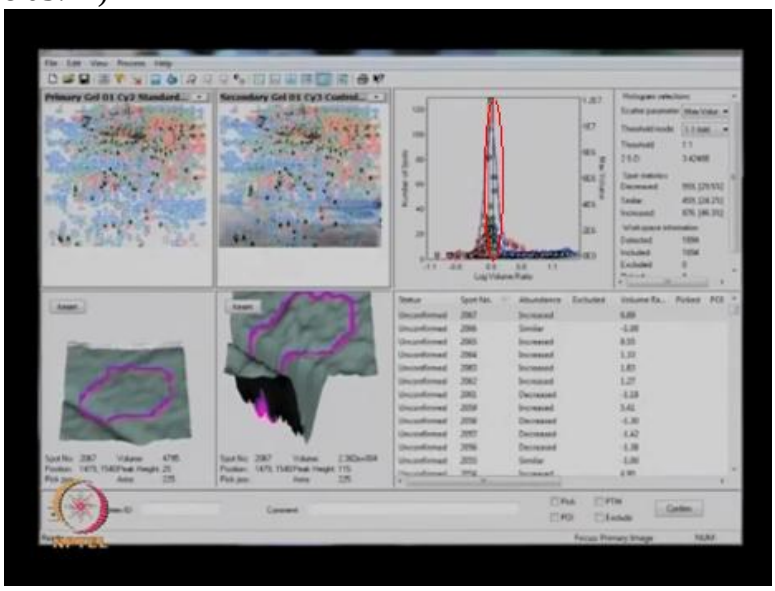
Expert: there are, you can see some red color spots, here these are all down-regulated and compare with control with treated and

(Refer Slide Time 03:11)



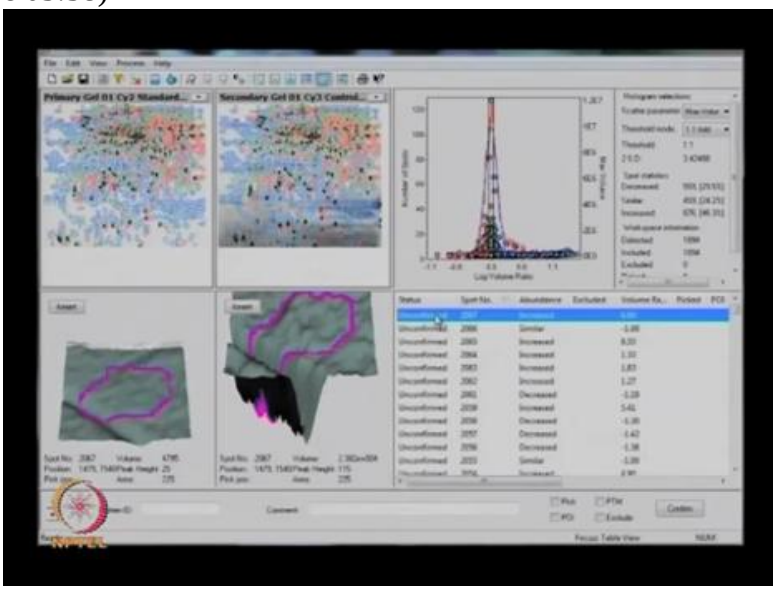
Expert: these blue color spots, there you can see, they are all operated spots when comparing with control and treated. In between these,

(Refer Slide Time 03:24)



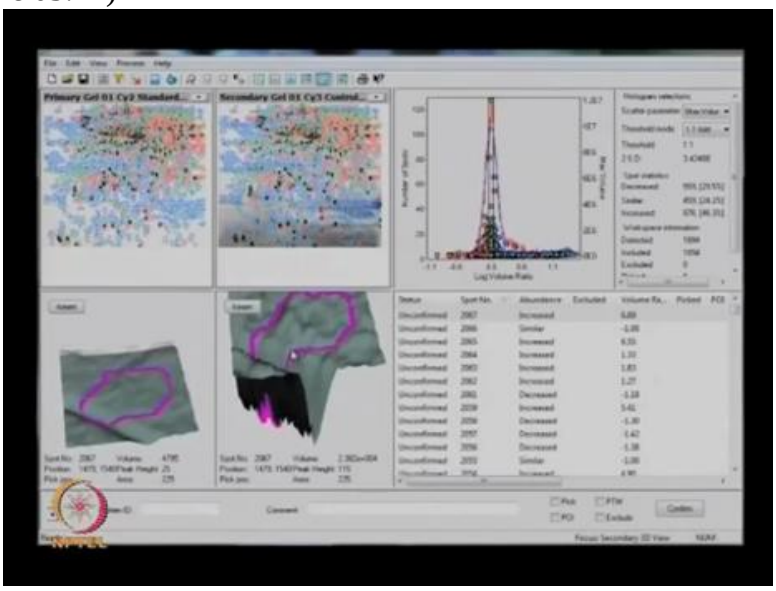
Expert: there is a blue there are some blue color spots. These are all similarly regulated So this is what we can see in DIA. Now you can go through each and individual spot

(Refer Slide Time 03:38)



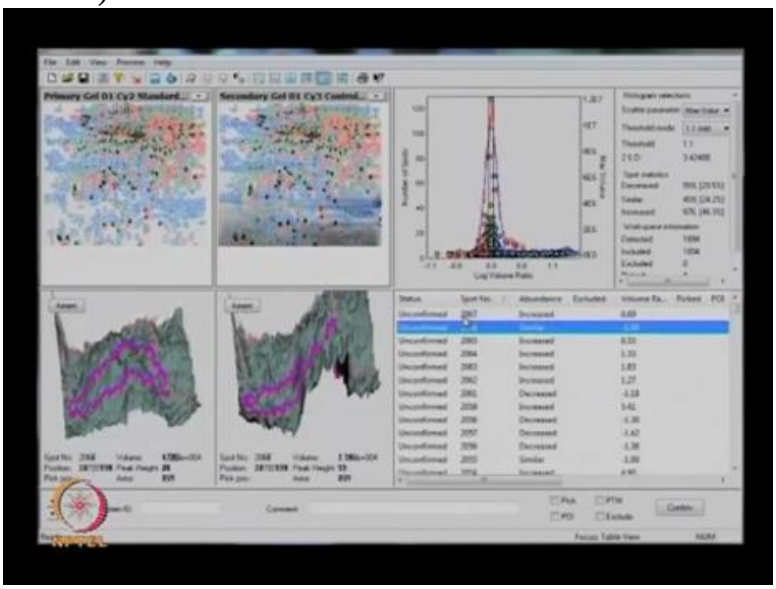
Expert: and you can see the 3D view of that particular spot

(Refer Slide Time 03:42)



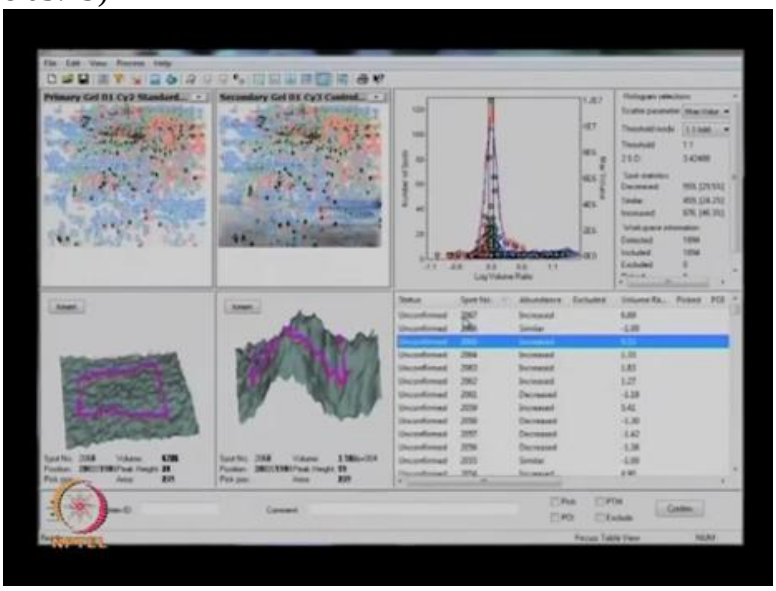
Expert: If suppose, if we can select any...

(Refer Slide Time 03:48)



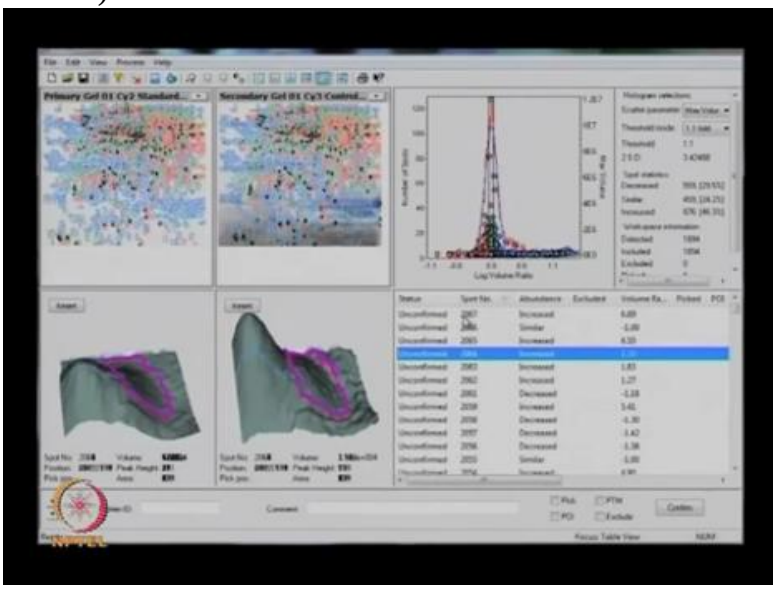
Expert: you can go to one by

(Refer Slide Time 03:49)



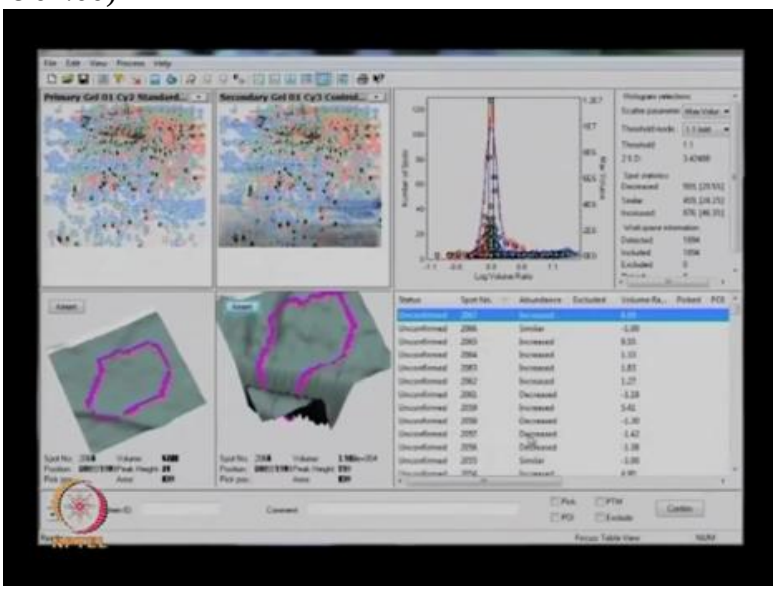
Expert: one and you can see whether it is exactly spot

(Refer Slide Time 03:51)



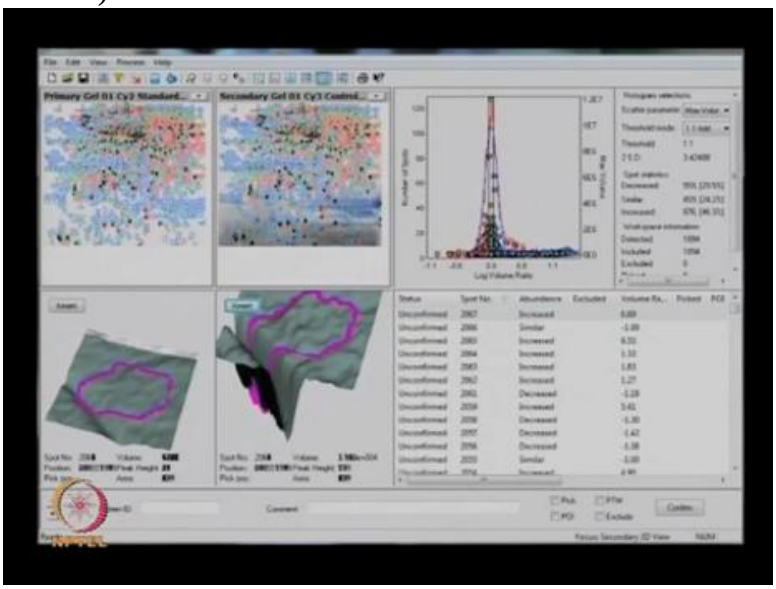
Expert: or some background, or if it is a background, you have to remove that So suppose this is the background,

(Refer Slide Time 04:00)



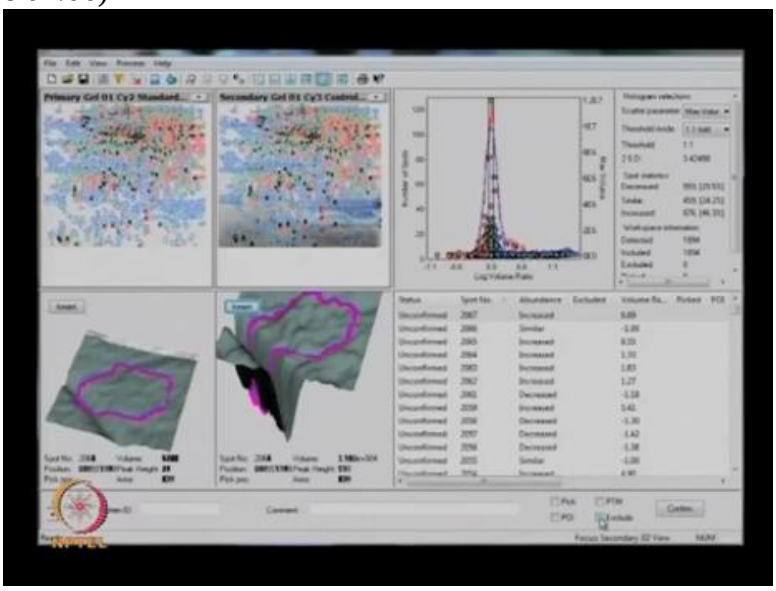
Expert: so there is no spot at all. Still it has detected some background

(Refer Slide Time 04:04)



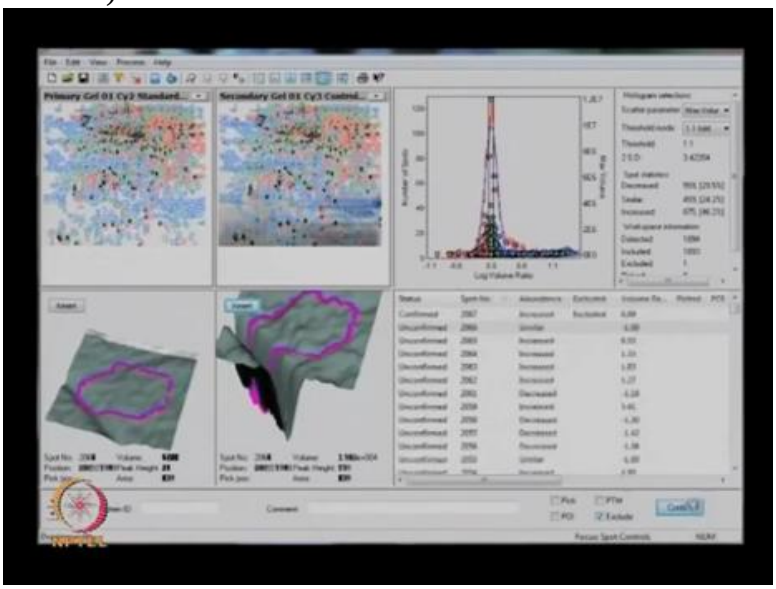
Expert: So you can exclude it

(Refer Slide Time 04:06)



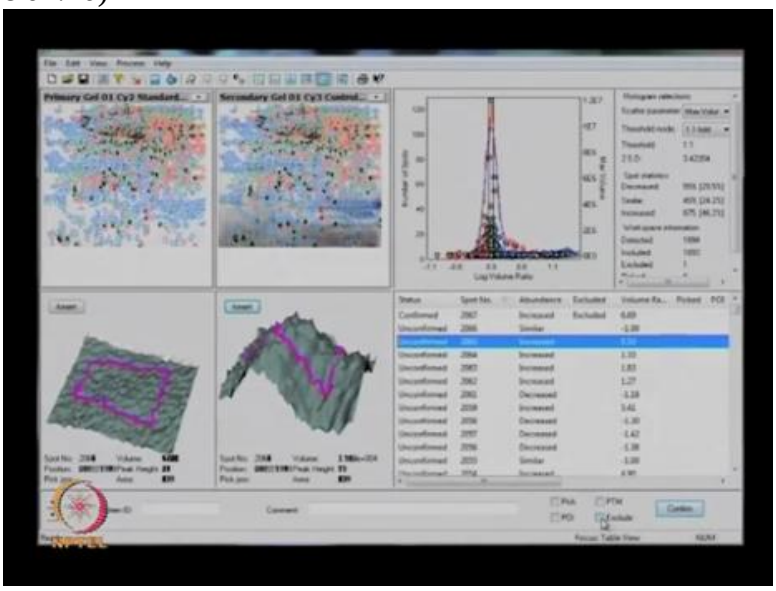
Expert: from here by clicking,

(Refer Slide Time 04:09)



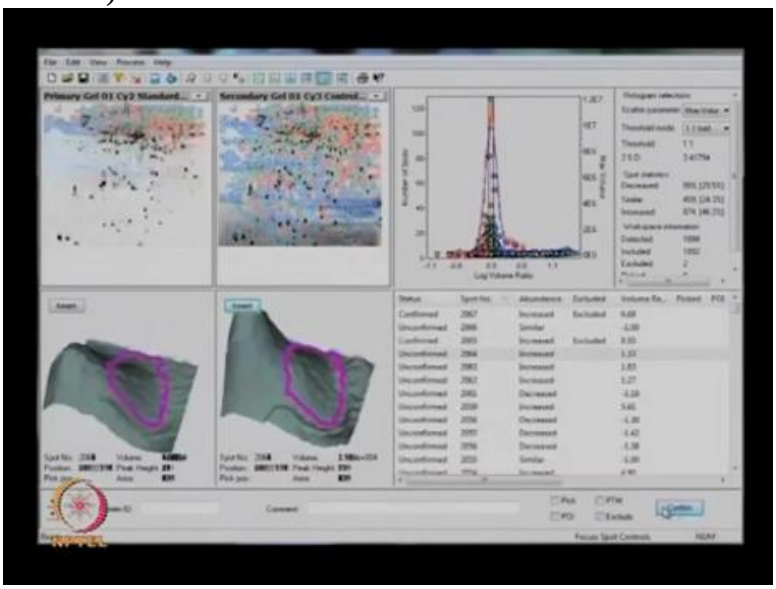
Expert: then confirm it.

(Refer Slide Time 04:20)



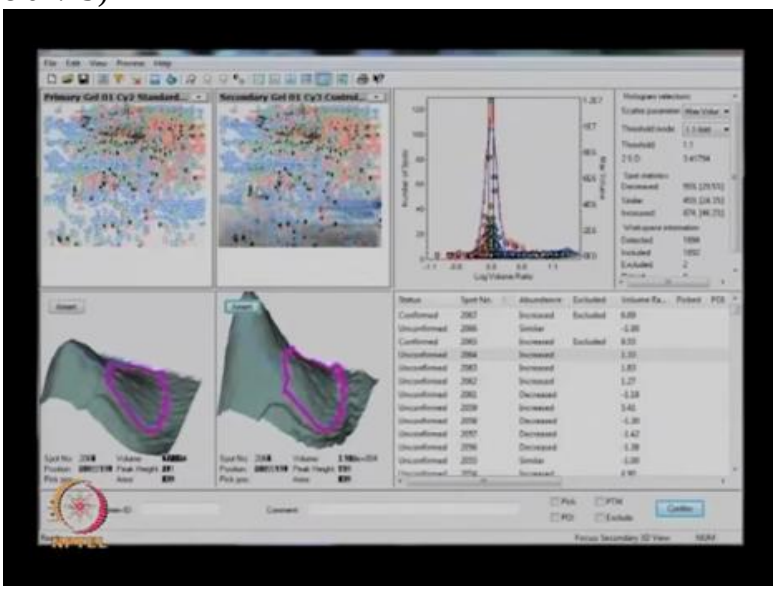
Expert: So this protein has been removed from the gels

(Refer Slide Time 04:22)



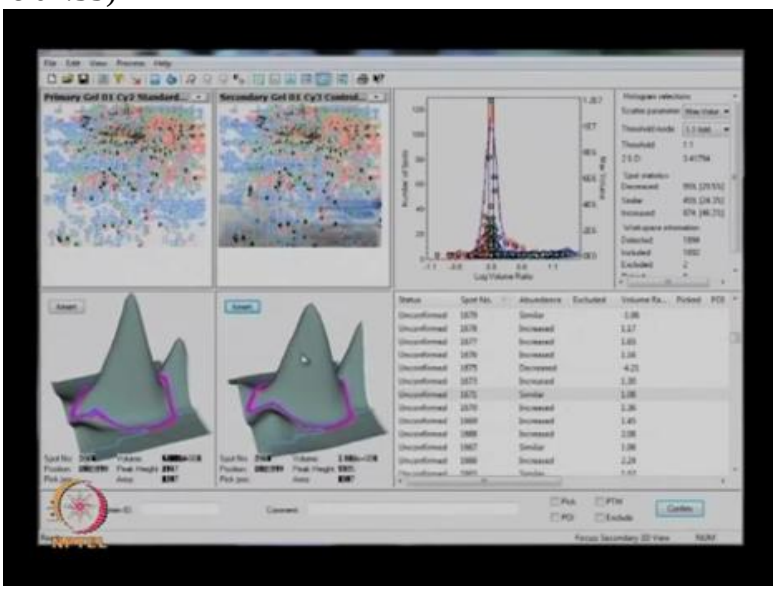
Expert: So the same way, we can go each and individual,

(Refer Slide Time 04:29)



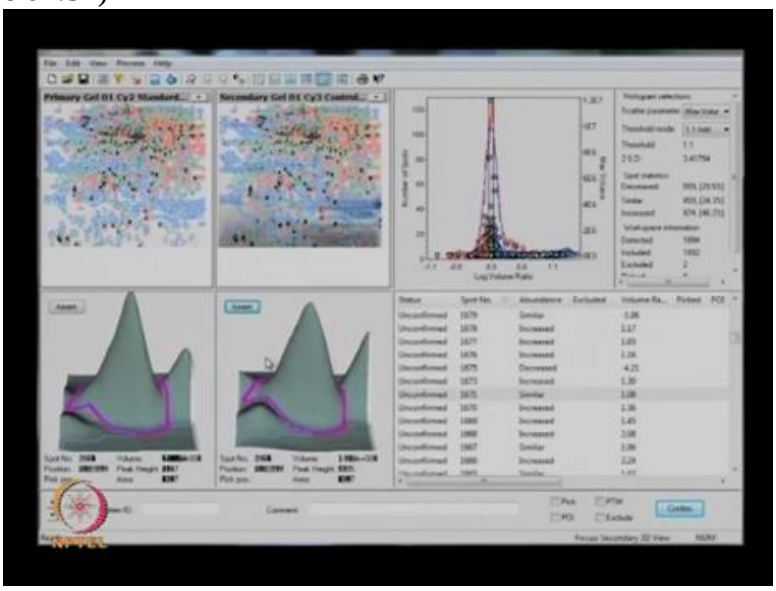
Expert: then exclude it, confirm it. By this way we can check all spots and you can have more accurate data with you like you can see

(Refer Slide Time 04:33)



Expert: how accuracy would be there, like the spot detection

(Refer Slide Time 04:37)



Expert: Now you can see. This is what we will get in DIA.

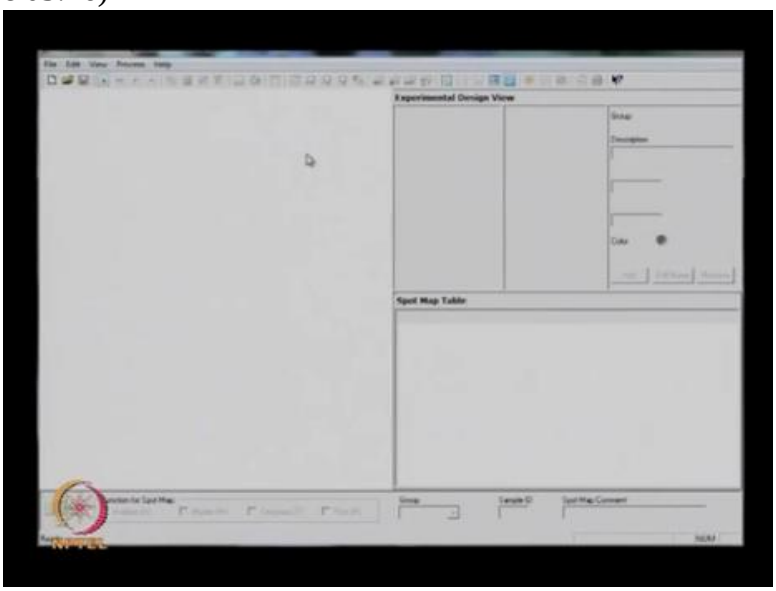
(Refer Slide Time 04:43)

Expert: This DIA creation we finished

(Refer Slide Time 04:47)

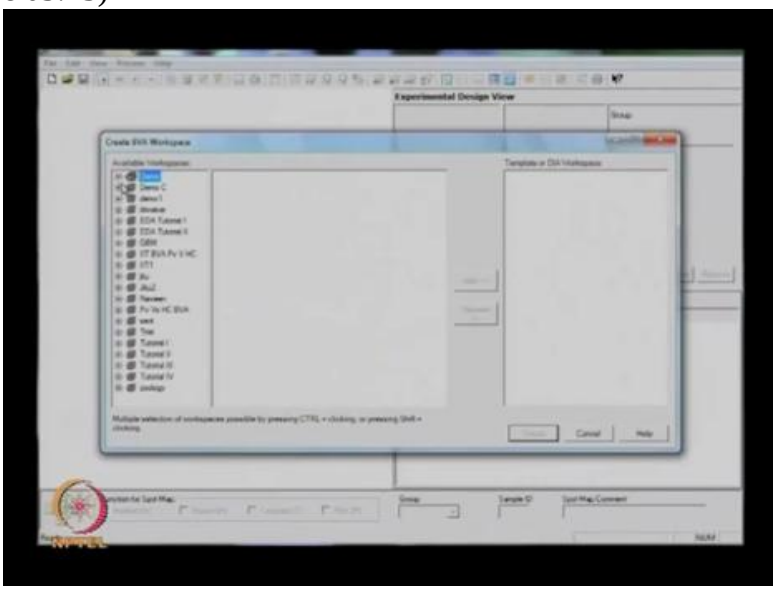
Expert: This is the BVA. BVA is nothing but Biological Variation Analysis One of the Internal Standard image is selected as a master image and all Internal Standard images match into this sample standard spot ratio for each protein. Each sample, then compared giving T-test value, fold changes, ANOVA values for each and individual protein

(Refer Slide Time 05:16)



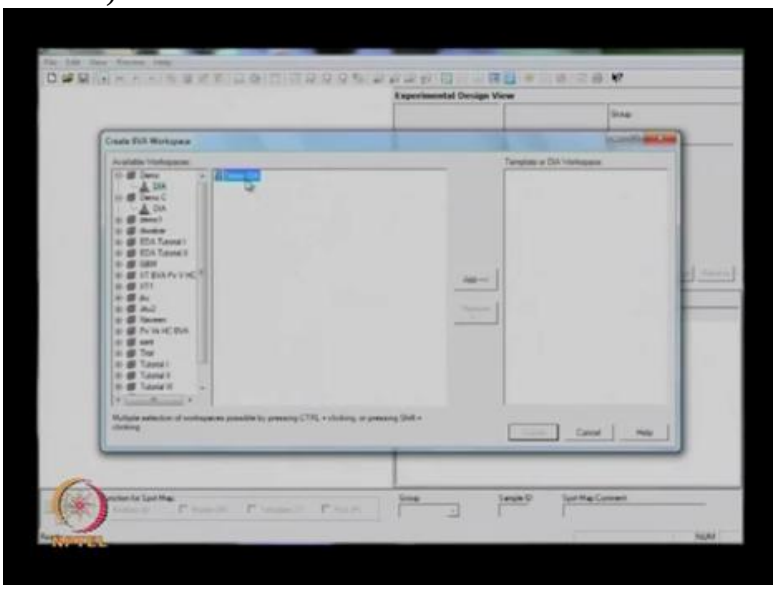
Expert: How to create BVA workspace? You can open the BVA and create BVA workspace

(Refer Slide Time 05:23)



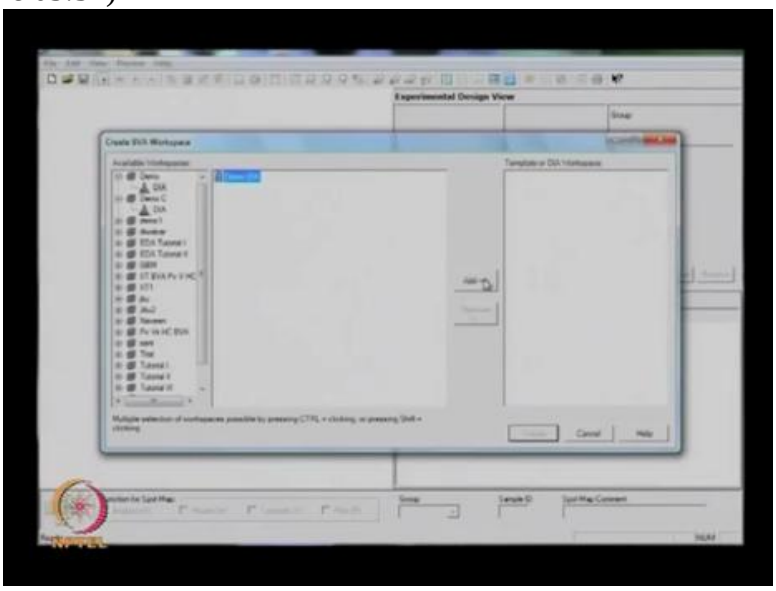
Expert: and go to our DIA workspace where we have our DIAs. From there

(Refer Slide Time 05:33)



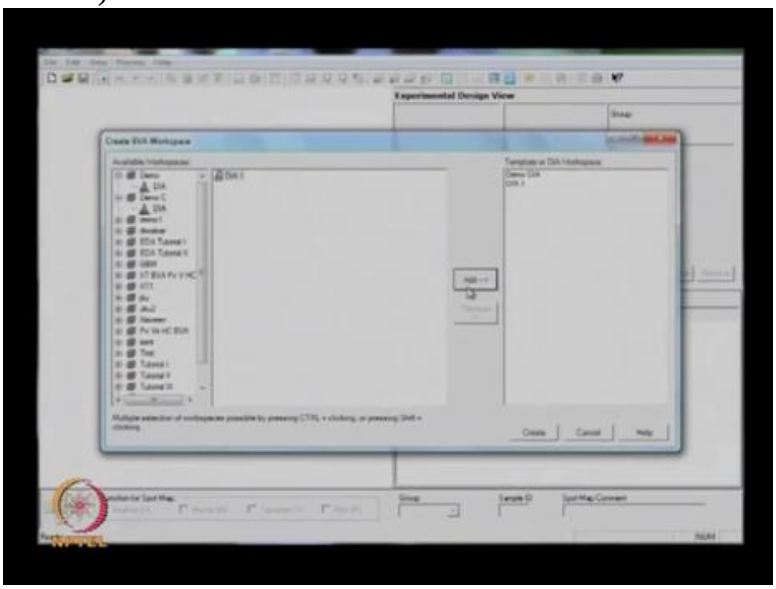
Expert: you can create

(Refer Slide Time 05:34)



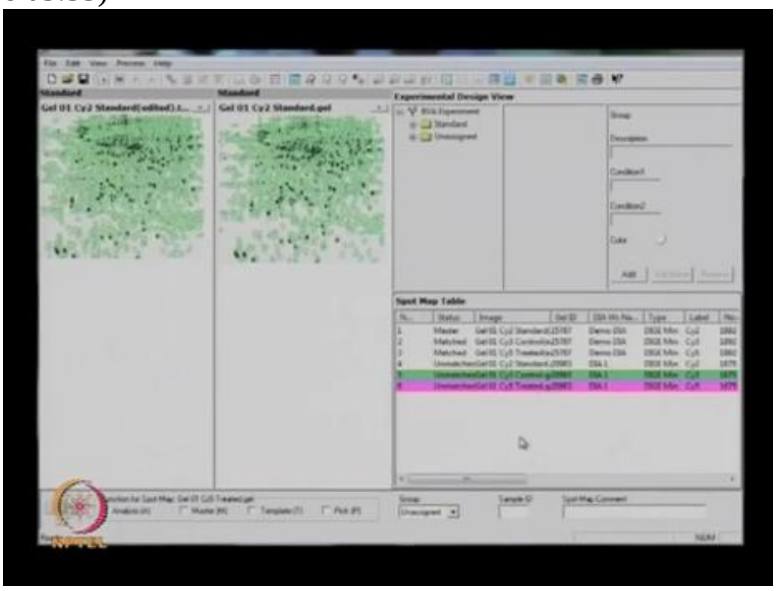
Expert: actually...add it, one DIA I have added and this is another DIA

(Refer Slide Time 05:40)



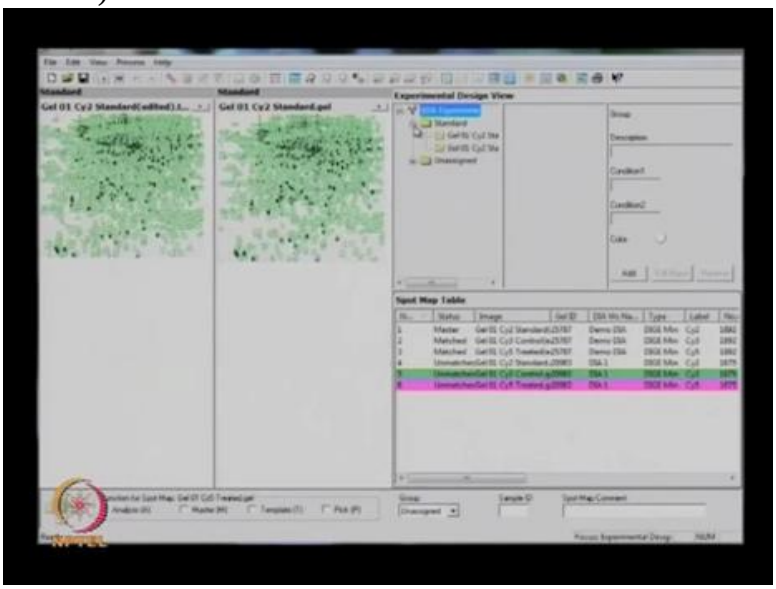
Expert: I am adding here. Minimum 2 DIAs we require for the BVA, so we have 2 DIAs here, so click on create. So it creates a new BVA for you.

(Refer Slide Time 05:55)



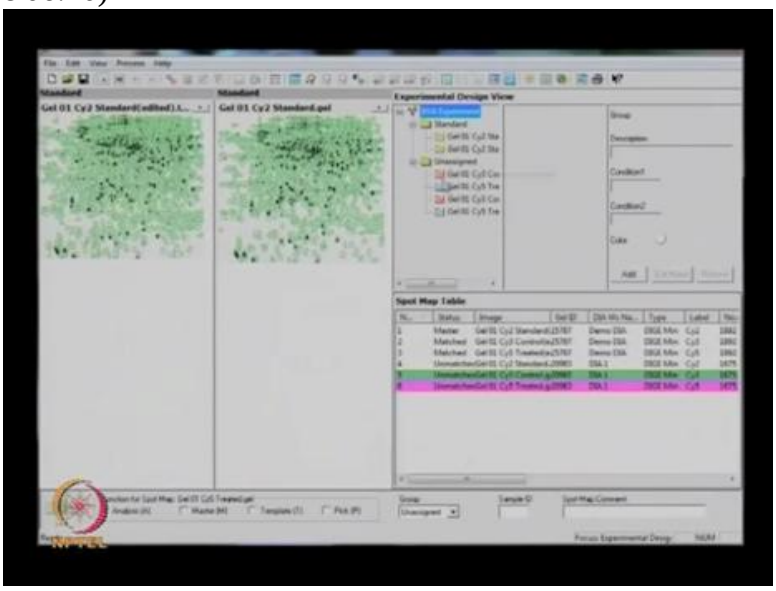
Expert: This is the new BVA. From here, first

(Refer Slide Time 06:02)



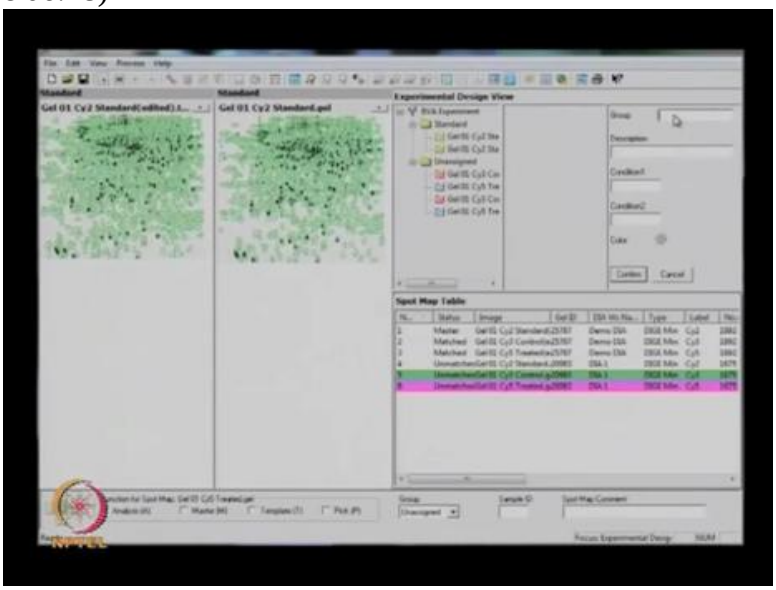
Expert: all Cy2 gels automatically go to Standard folder. There is a Standard folder you can see and

(Refer Slide Time 06:10)



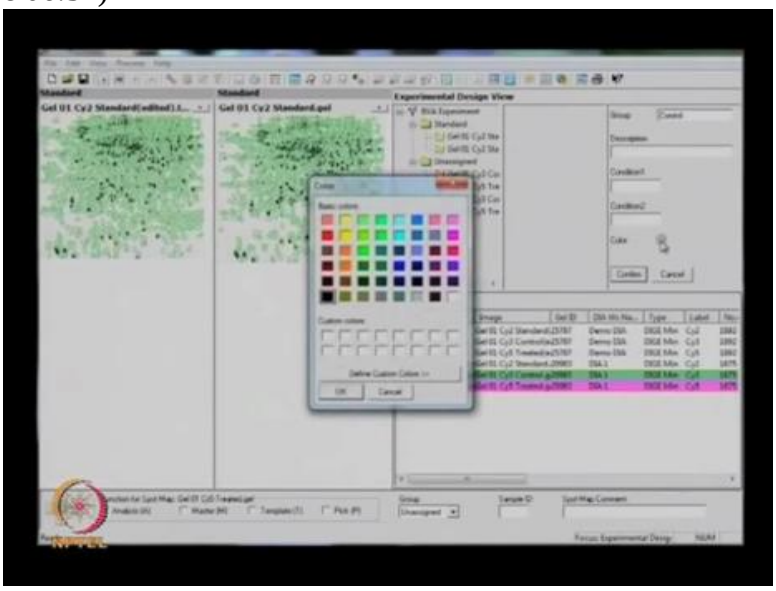
Expert: remaining all gels remain in the unassigned folder where we need to assign these gels as according to the gel type or sample type. Then you have just have to click on Add option,

(Refer Slide Time 06:28)



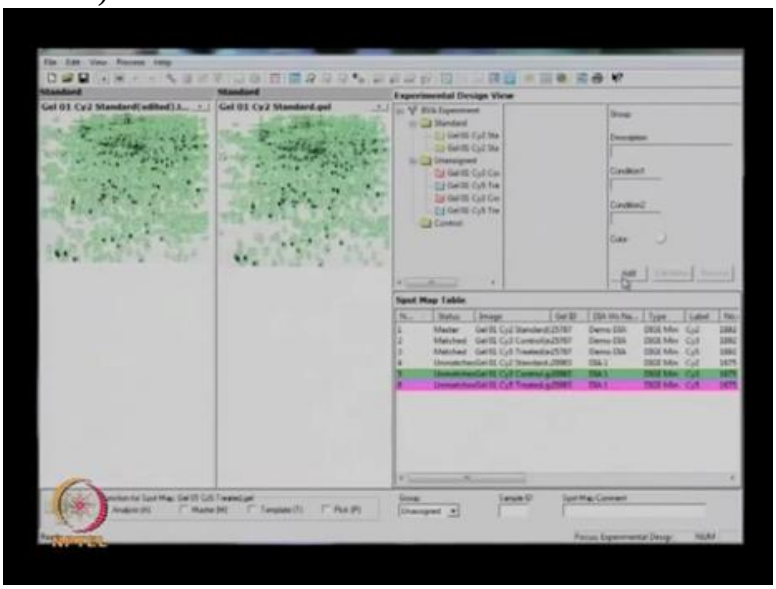
Expert: now create a group, it may be control or treated. If first one is the control, and

(Refer Slide Time 06:37)



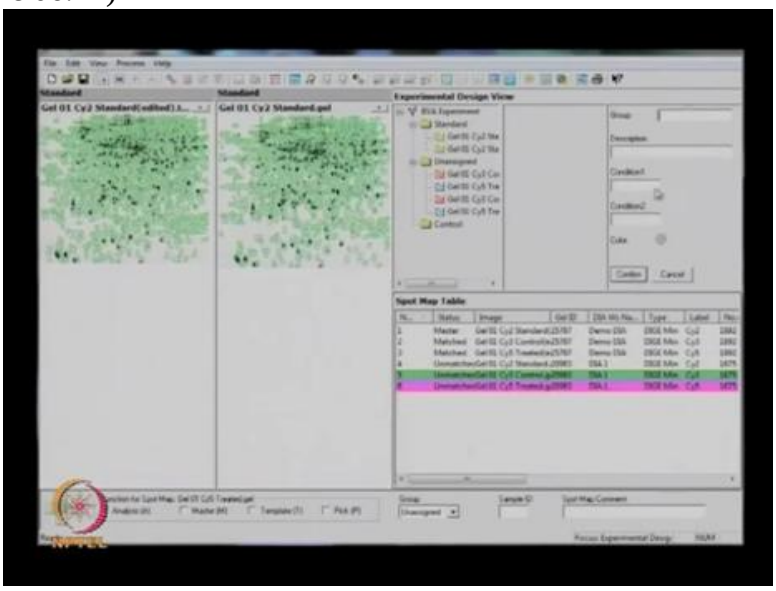
Expert: apply some color draft, confirm it,

(Refer Slide Time 06:45)

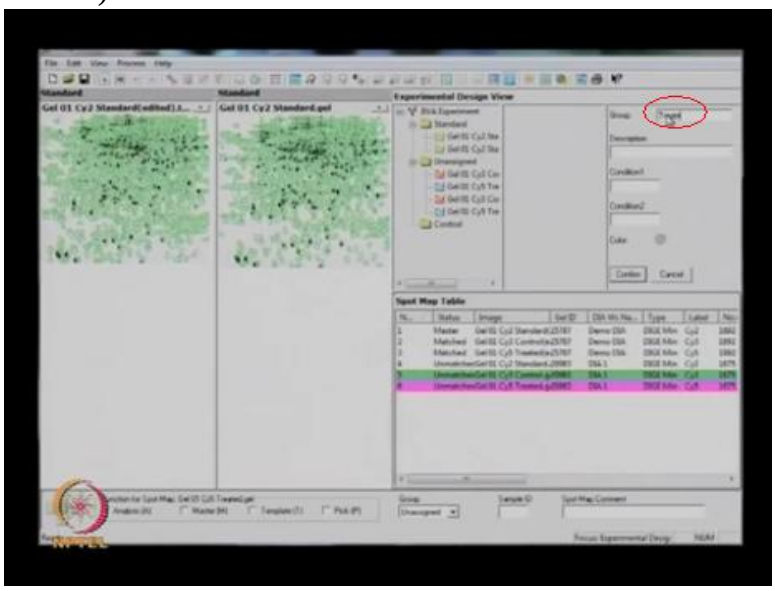


Expert: then another you can create

(Refer Slide Time 06:47)

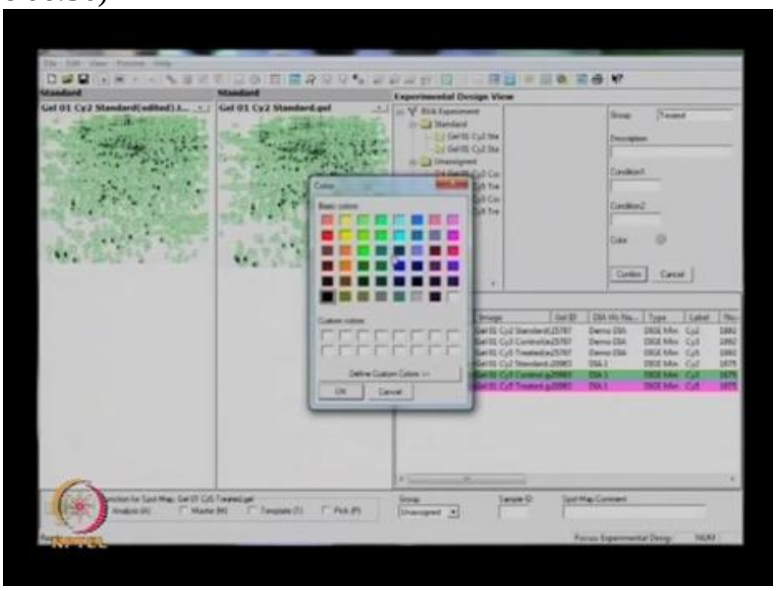


(Refer Slide Time 06:51)



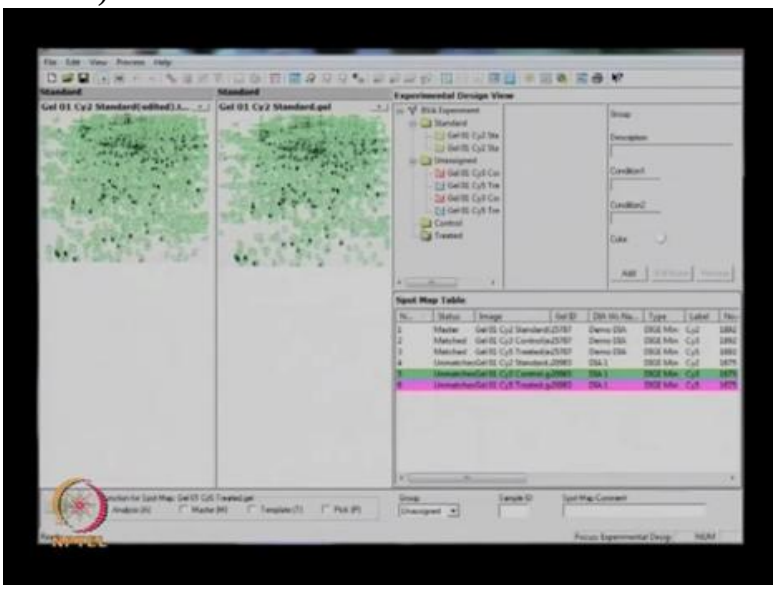
Expert: like treated

(Refer Slide Time 06:56)



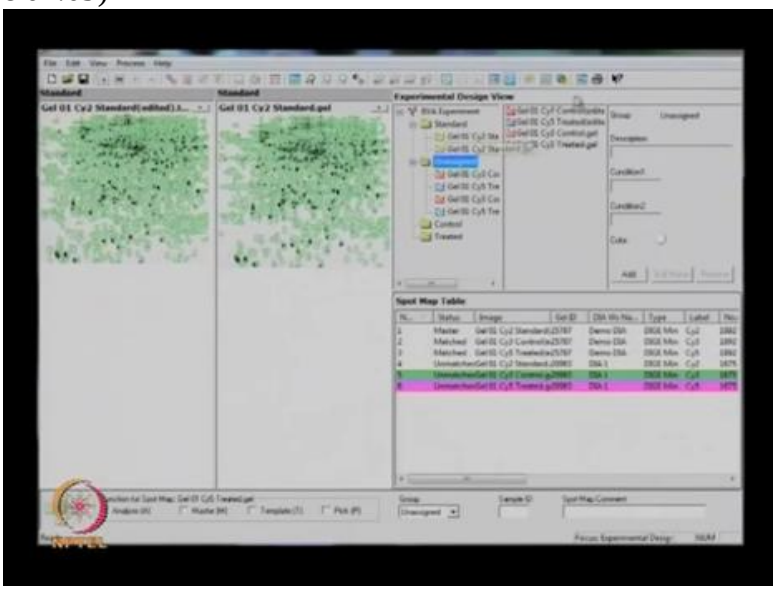
Expert: then give some color draft, confirm it.

(Refer Slide Time 07:03)



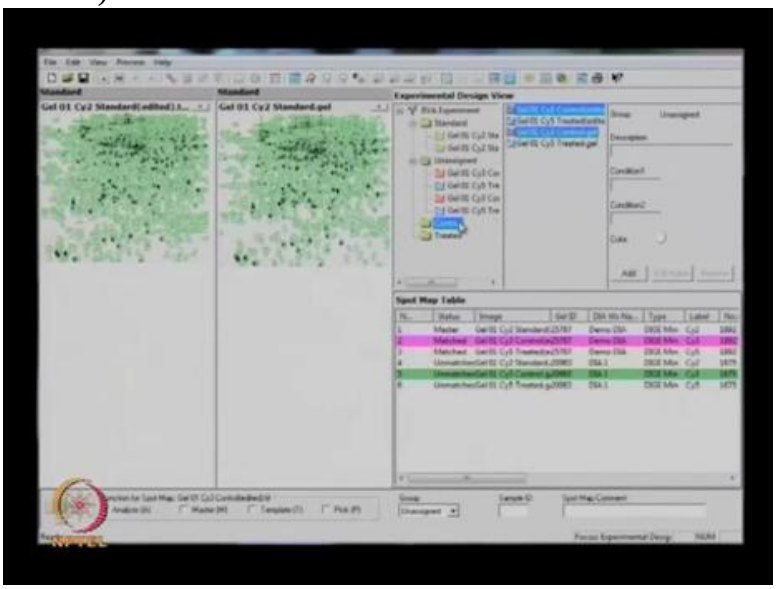
Expert: Now we have 2 folders, confirm and treated. So, as we have in assigned folder

(Refer Slide Time 07:09)



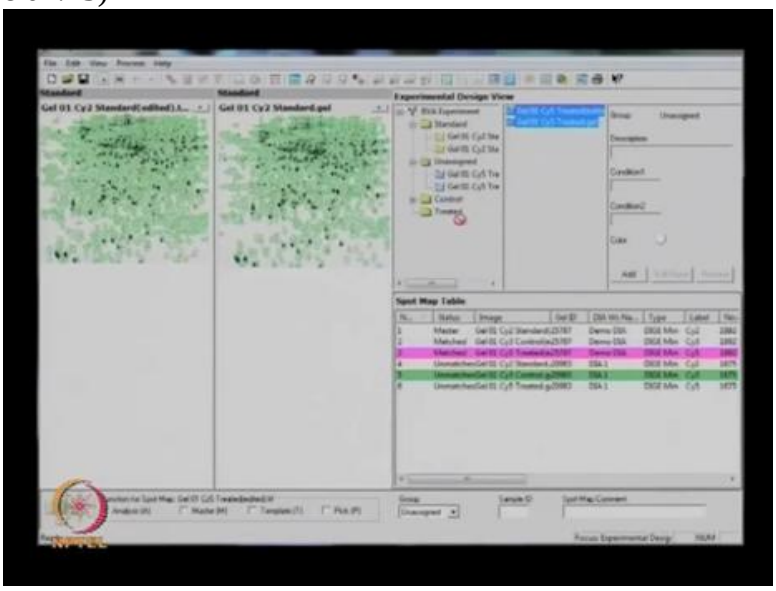
Expert: both control and treated; these control gels we can transfer into Control folder

(Refer Slide Time 07:17)



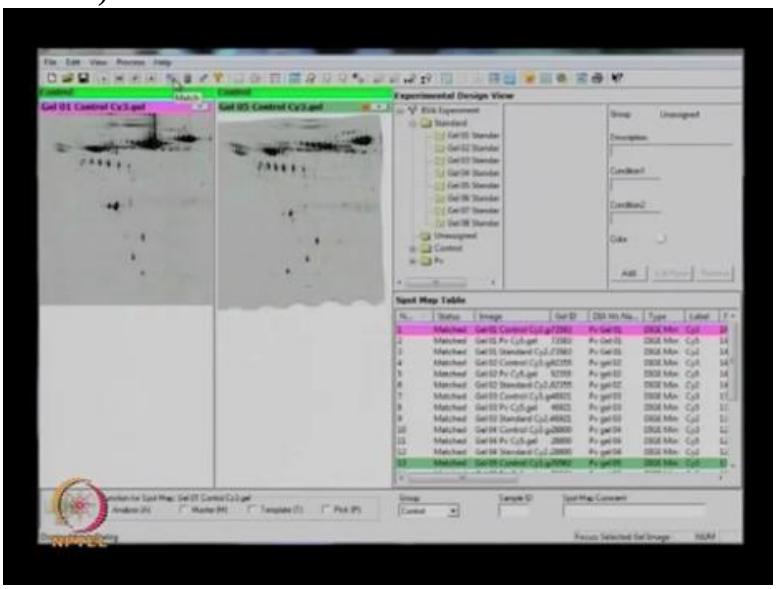
Expert: by dragging those images and treated gels we can transfer to Treated folder by dragging them.

(Refer Slide Time 07:23)



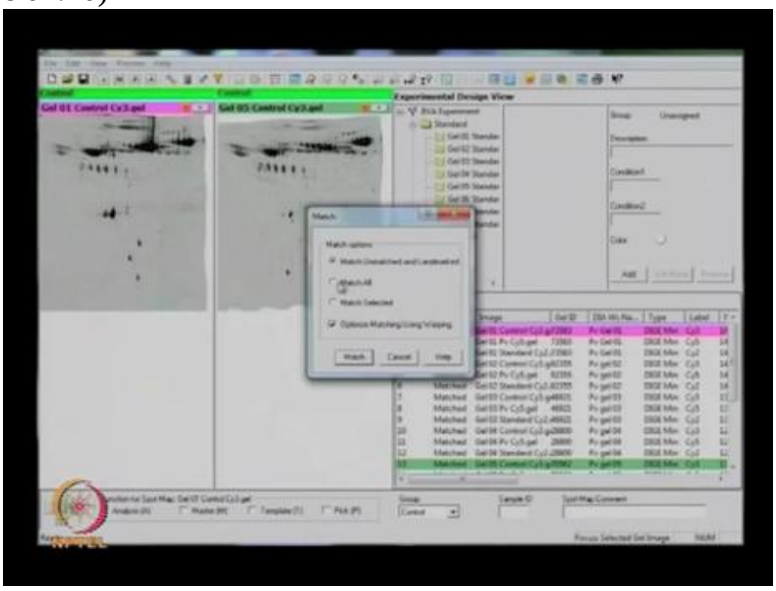
Expert: Now we have our images here.

(Refer Slide Time 07:34)



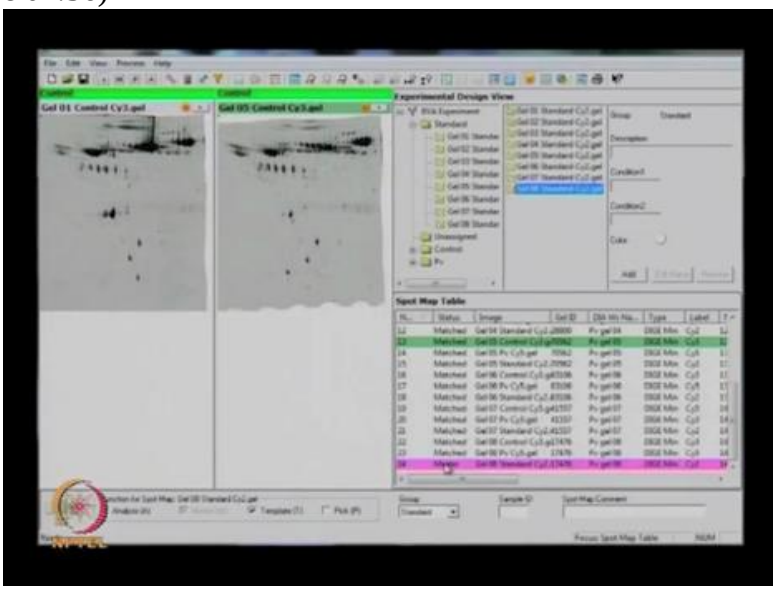
Expert: After shifting control to Control and treated to Treated, we have to match all gels.

(Refer Slide Time 07:40)



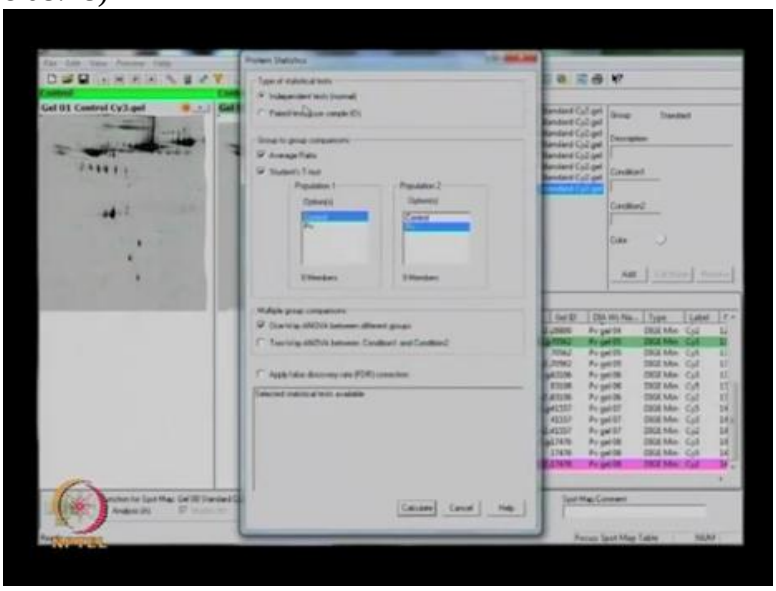
Expert: Just click on match and match all. It's matched. The matching process has been finished. Now as we discussed in out of all standard gels, one gel selected as master image

(Refer Slide Time 07:56)



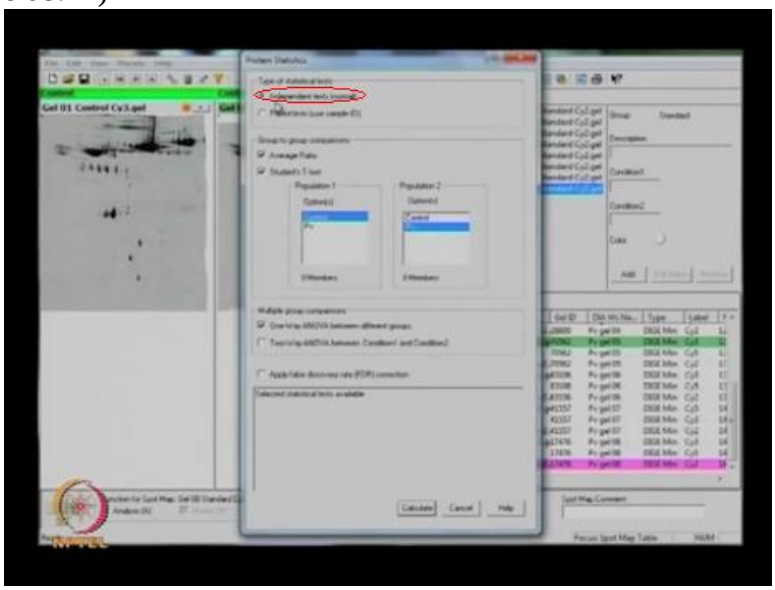
Expert: as you can see the number 24 gel has been selected as the master and it will compare remaining other gels with this master gel. So now we have this comparison data. After that we need to calculate statistical parameters.

(Refer Slide Time 08:18)



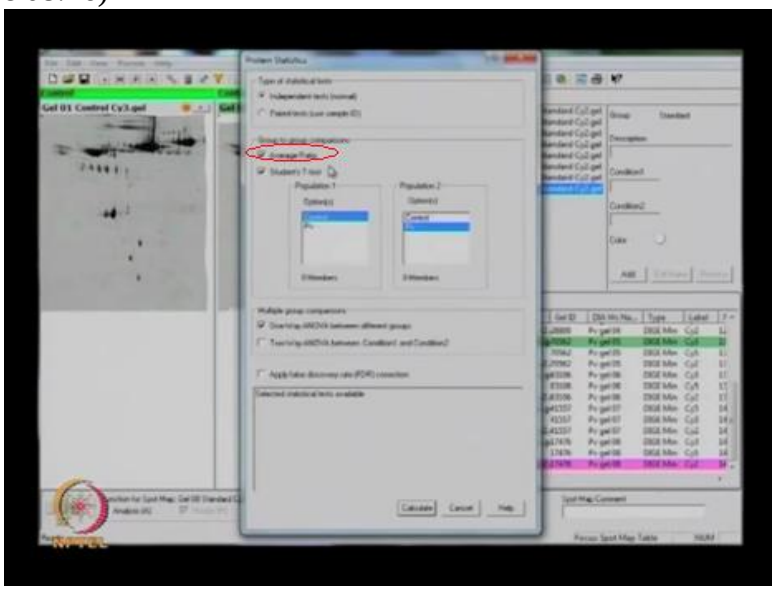
Expert: So click on Statistical Parameter button. See now we have some statistical parameters like

(Refer Slide Time 08:24)



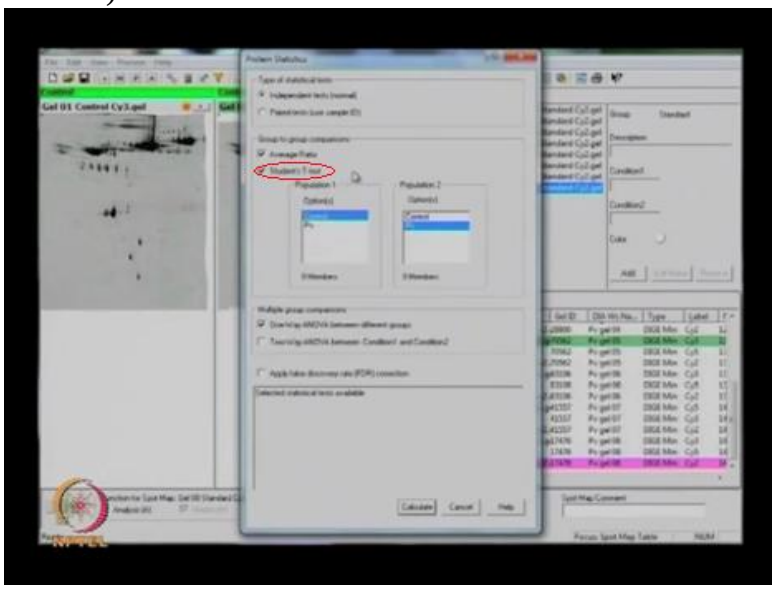
Expert: independent T-test

(Refer Slide Time 08:26)



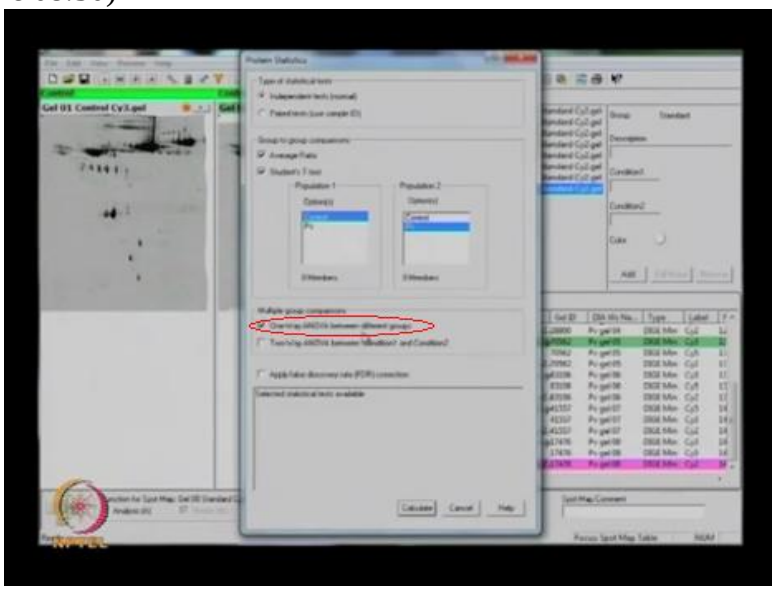
Expert: average ratio,

(Refer Slide Time 08:27)



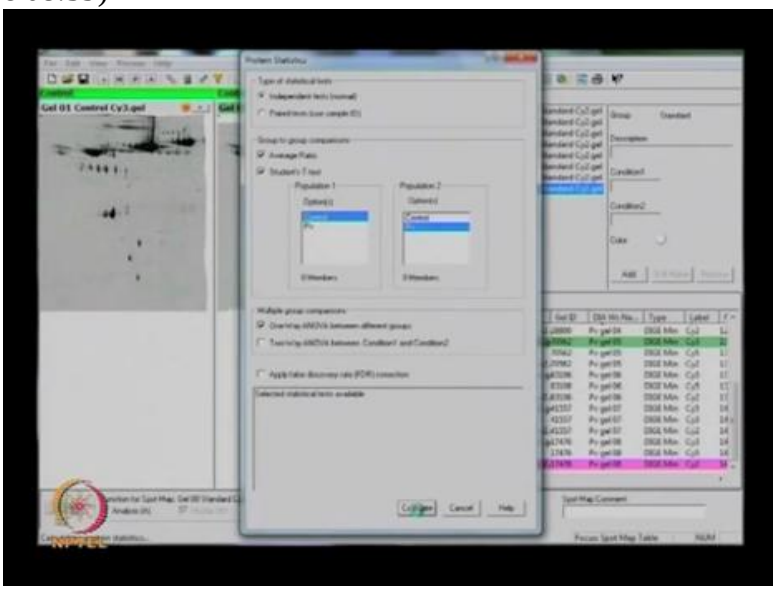
Expert: Student T-test

(Refer Slide Time 08:30)



Expert: one way ANOVA in between different groups We are doing between control and treated. So calculate them.

(Refer Slide Time 08:39)

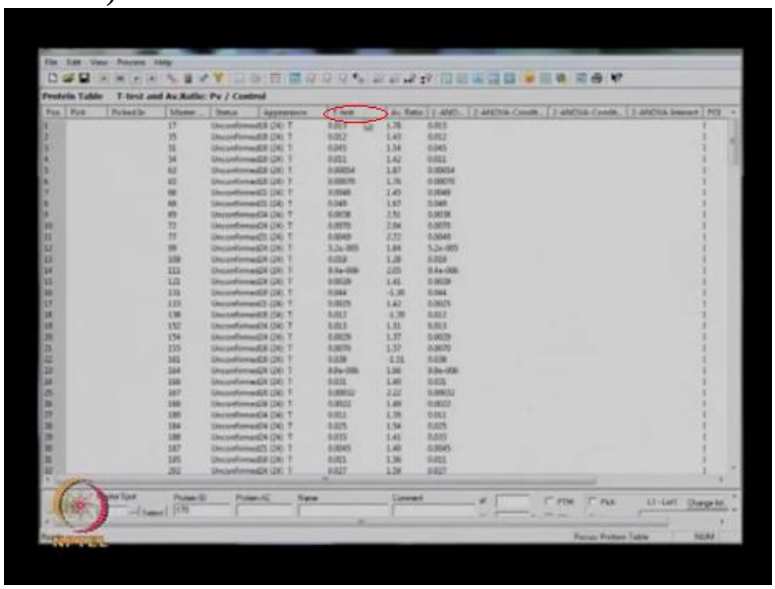


Expert: So statistical parameters has been calculated

(Refer Slide Time 08:45)

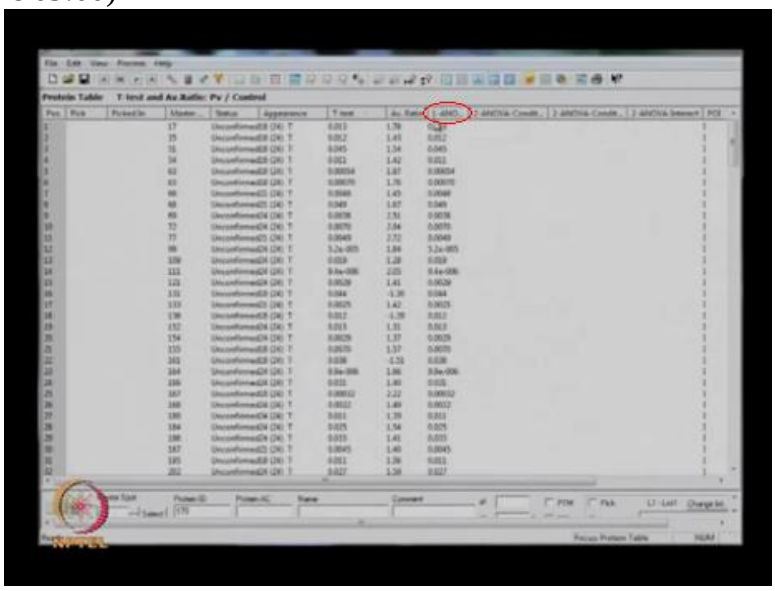
Expert: Now we can see exact results of statistical parameters. If I can go to the table view

(Refer Slide Time 08:56)



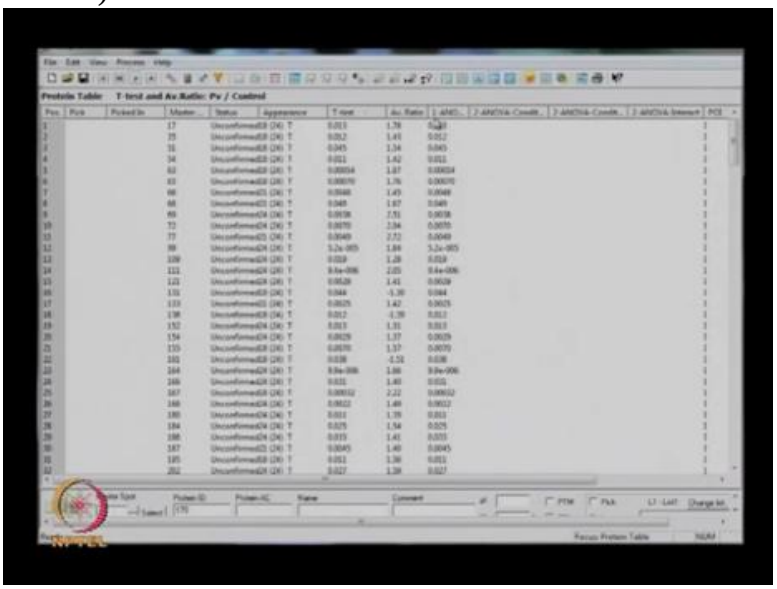
Expert: here you can see T-test

(Refer Slide Time 09:00)



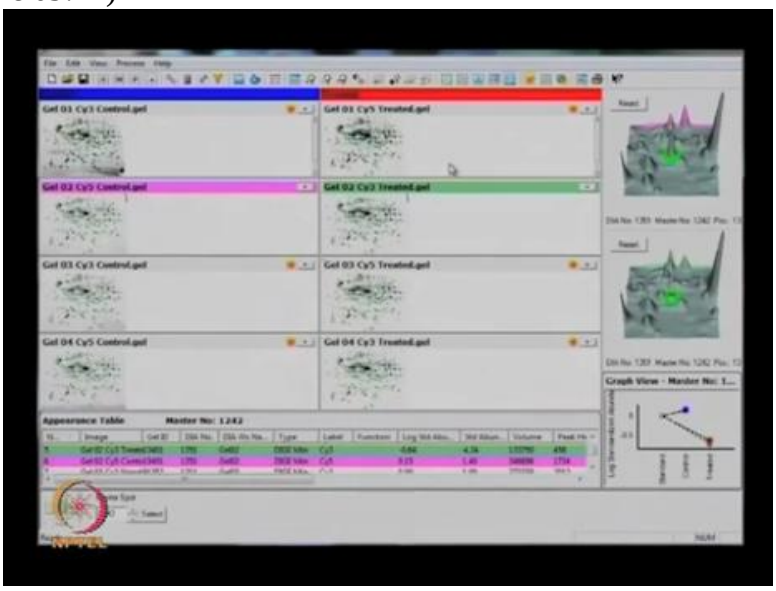
Expert: one way ANOVA, this you can see.

(Refer Slide Time 09:01)



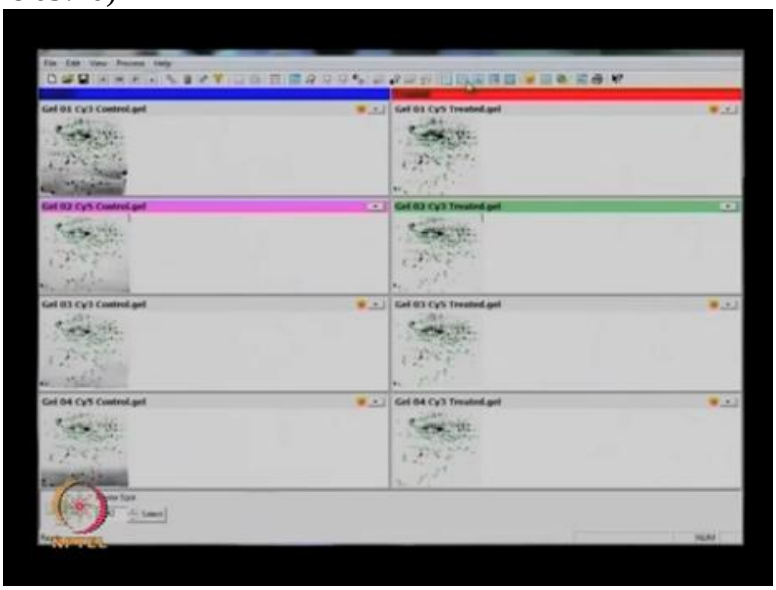
Expert: So we can select from here which are all the statistically significant and which are not significant.

(Refer Slide Time 09:11)



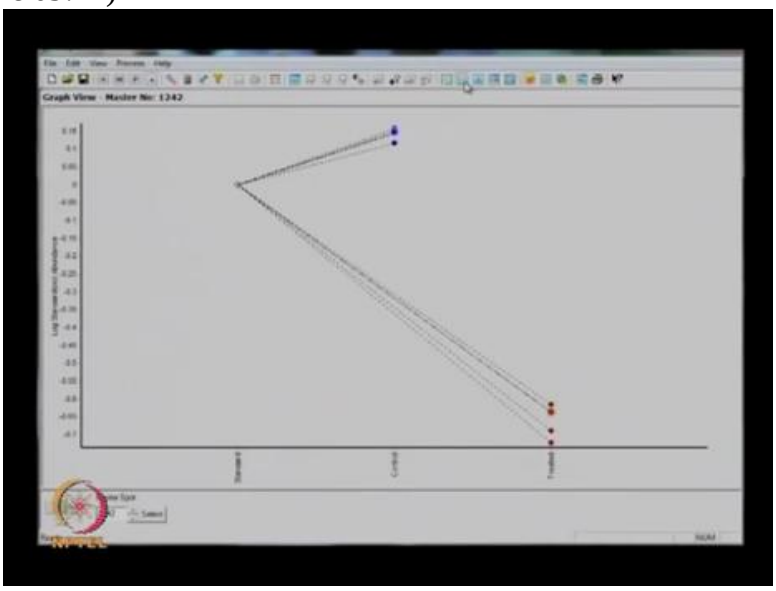
Expert: After analyzing the statistical data, now we can see the complete results here. Here we can see the four views

(Refer Slide Time 09:20)



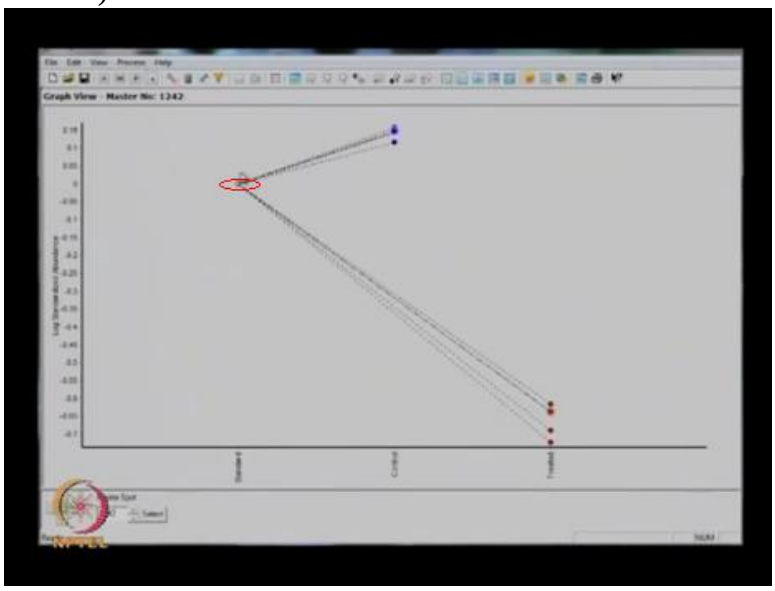
Expert: like this is the image view

(Refer Slide Time 09:22)



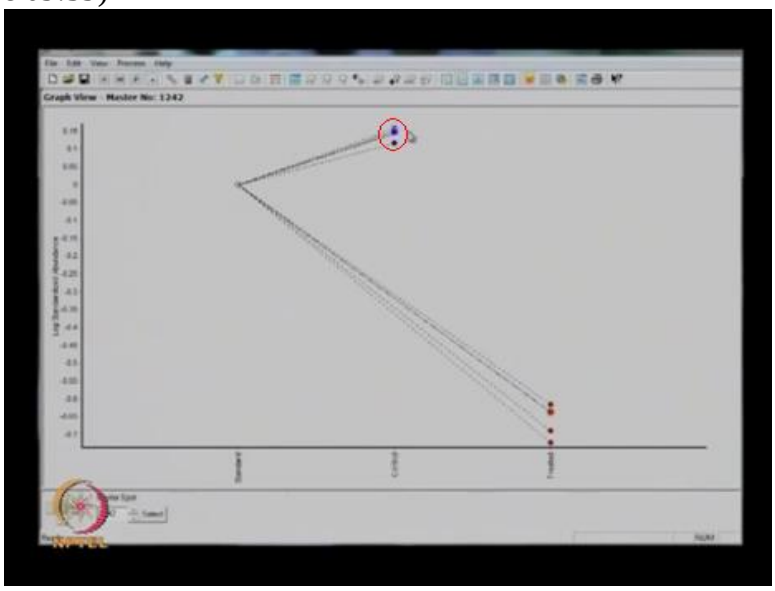
Expert: And this is the histogram view. In histogram view we can see clearly particular protein, how it is behaving throughout control and treated. We can see this is the standard gel, that means this is the mixture of control and treated, this is

(Refer Slide Time 09:39)



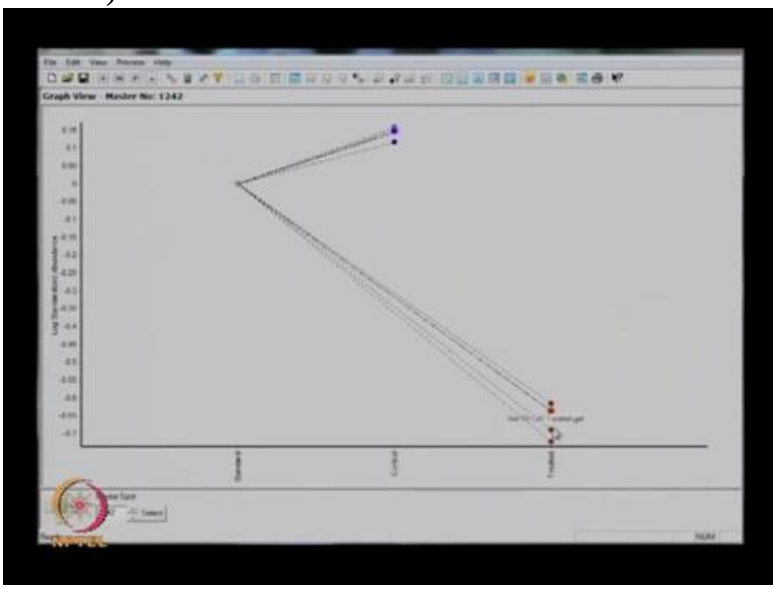
Expert: somewhere zero, we can consider this one. Then control is completely...

(Refer Slide Time 09:39)



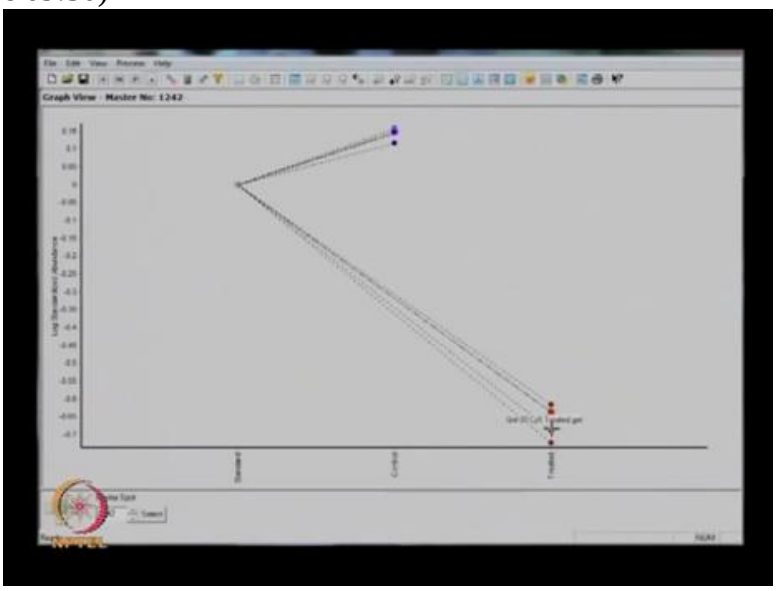
Expert: it is showing up-regulation and

(Refer Slide Time 09:47)



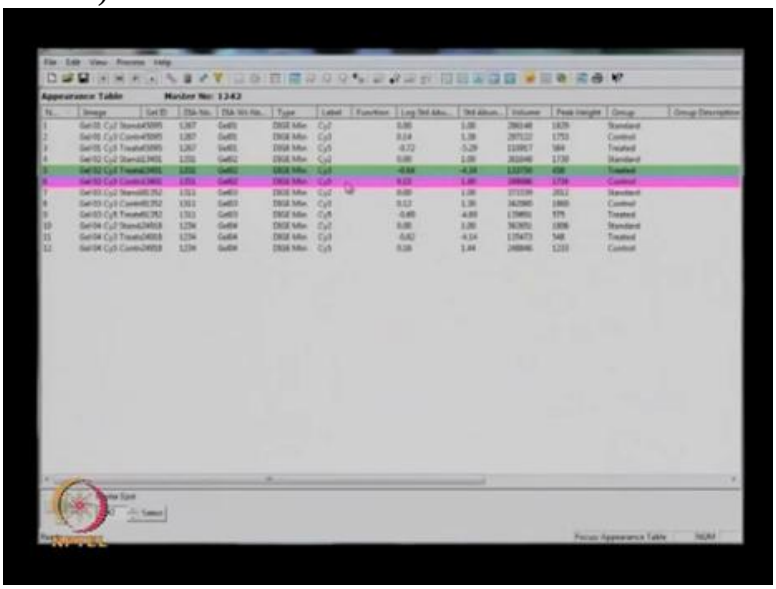
Expert: after giving the particular treatment, it is showing the down-regulation.

(Refer Slide Time 09:50)



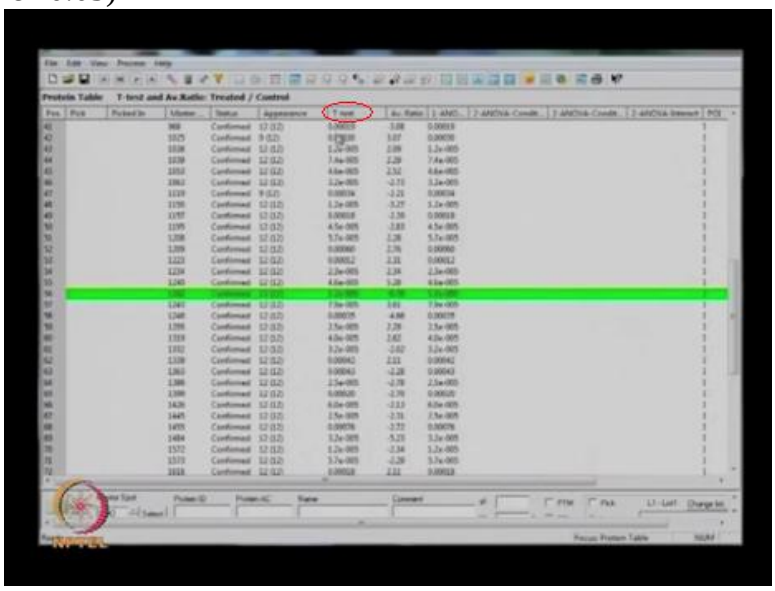
Expert: So this kind of data we can see here, then in the table view

(Refer Slide Time 09:57)



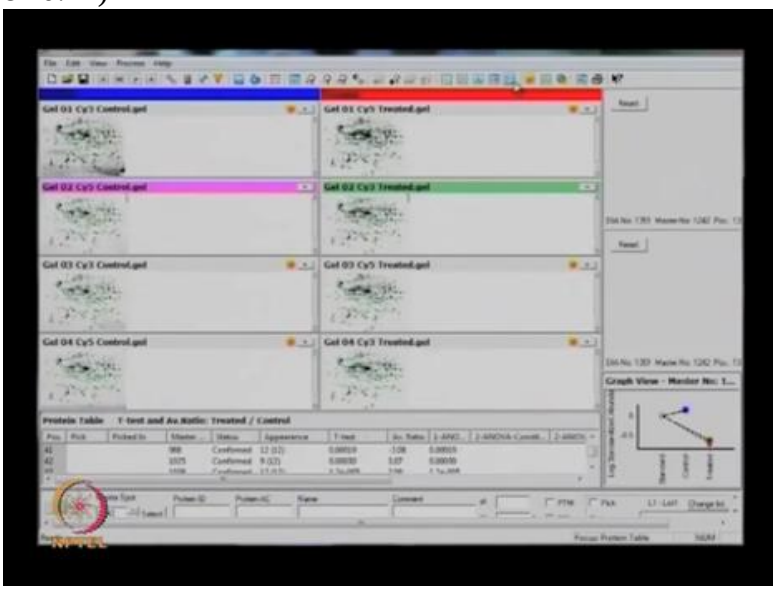
Expert: as we can see the complete protein data

(Refer Slide Time 10:03)



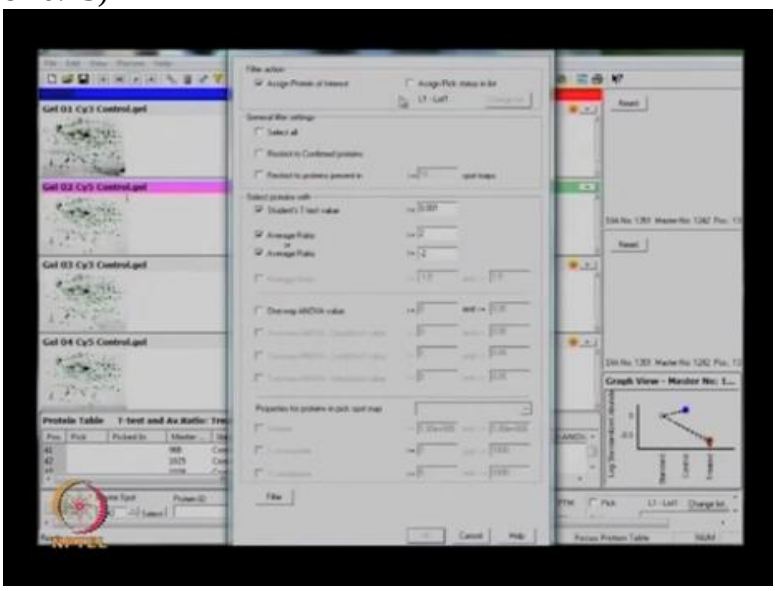
Expert: where the T-test value, average ratio value, one D ANOVA value, these all we can see here in the table view. So the 4 views at a time

(Refer Slide Time 10:14)



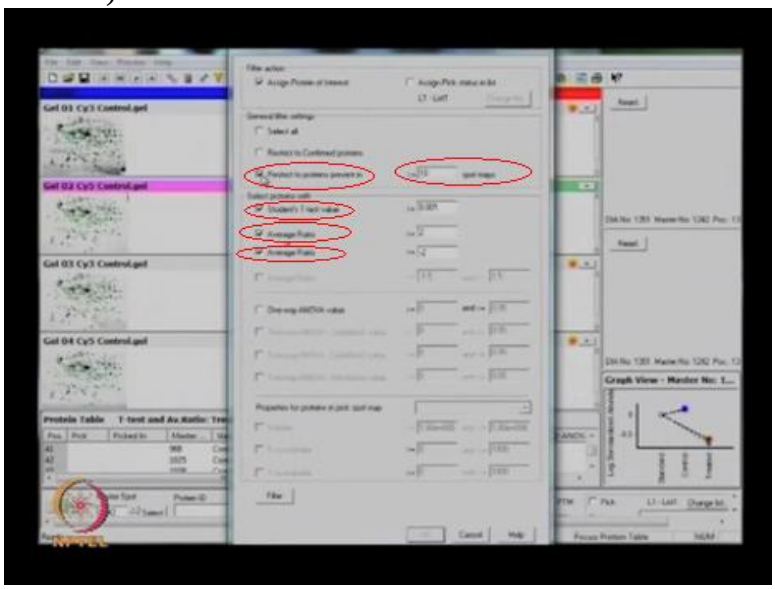
Expert: to see This is the four views we can see here. So after this, we can filter them according to our interest.

(Refer Slide Time 10:23)



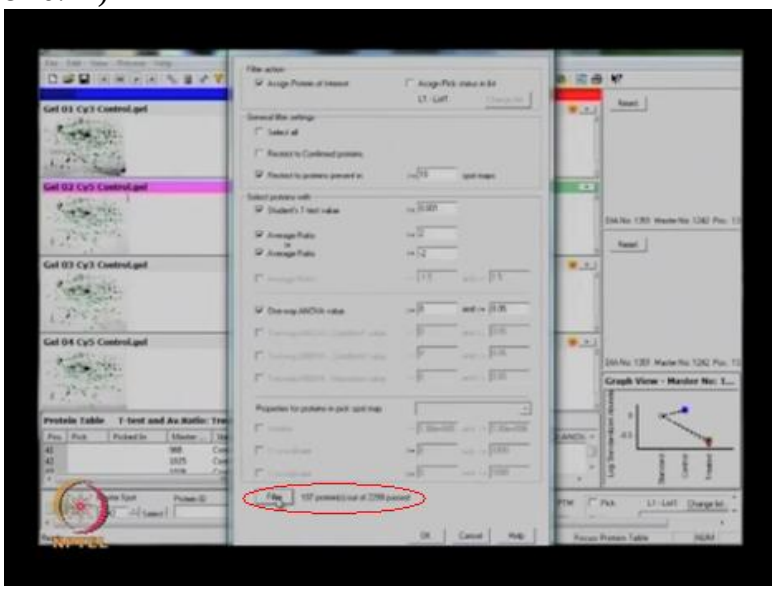
Expert: So select few parameters which are available

(Refer Slide Time 10:28)



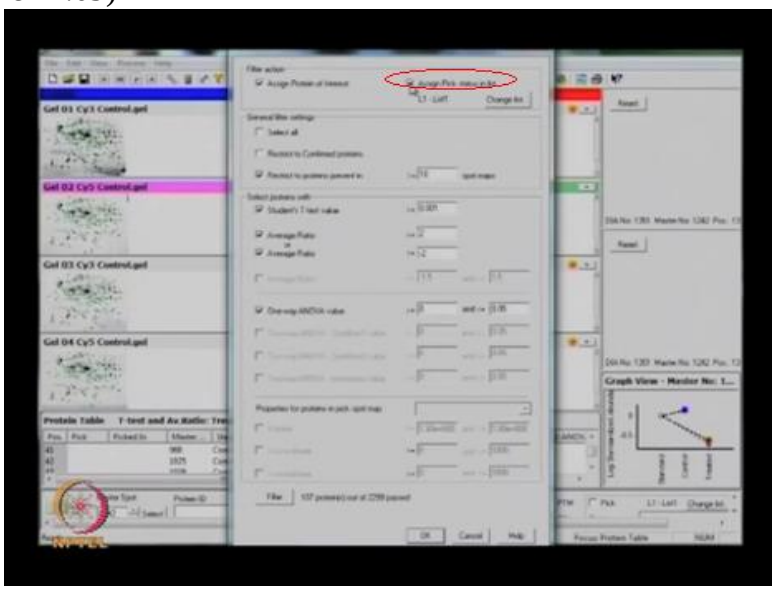
Expert: restrict to 10 gels which are spot maps, which are present. A particular protein should be there and student T-test as well as average ratio, then one way ANOVA value and filter it

(Refer Slide Time 10:47)



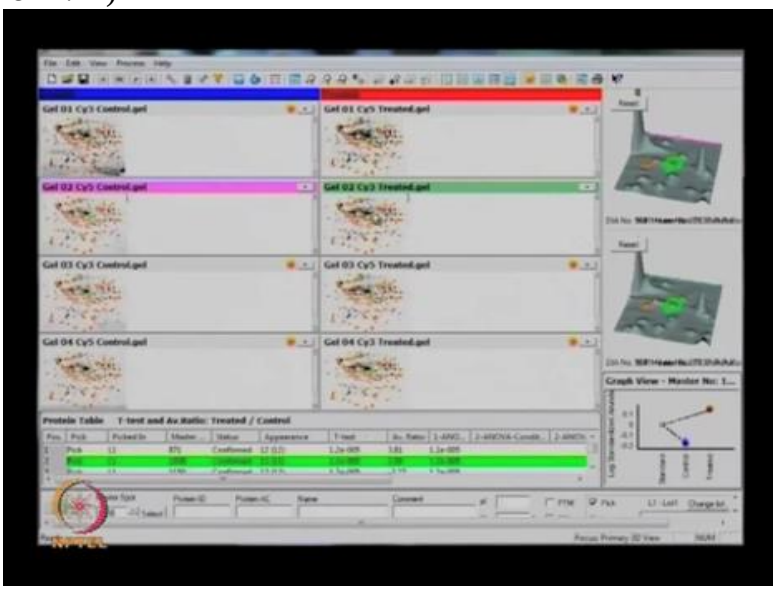
Expert: So there are 2299 spots are available in these all gels but 107 proteins only passed all these parameters. So these parameters we can select as a protein of interest and

(Refer Slide Time 11:09)



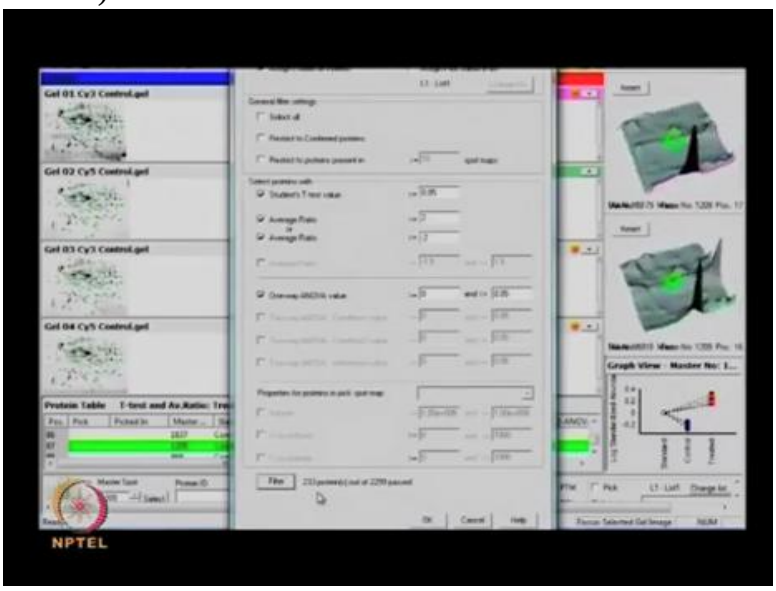
Expert: assign pick list so that these proteins can be saved in a file. This file can be given furtherly to spot picker.

(Refer Slide Time 11:21)



Expert: These are all the things we are enable to identify in BVA. This is very user-friendly. There is no much more manual interference, Ok. This is what this helps you to analyze your DIGE gel.

(Refer Slide Time 11:39)



(Refer Slide Time 11:45)

Professor: So, can you elaborate on what is EDA or Extended Data Analysis? What it can do which we are unable to do in BVA? So there is layers here, right, one is DIA followed by BVA...

Expert: Yeah

Professor: And then ultimately EDA.

Expert: Exactly. Basically what we can do here is we can compare two BVAs together there exactly. Here we are talking about a particular disease or a particular set of data only. There we can analyze different BVAs together in EDA, there you can get majorly Differential Expression again you will get as well as PCA and Discriminant Analysis, these kinds of statistical data you will get in EDA. Very shortly, I will just show briefly....

Professor: If I understood correctly, probably the statistical parameter will be more stringent over there in EDA...

Expert: Yes

Professor: We can have some better biological significant information...

Expert: Exactly

Professor: From dataset

Expert: Exactly.

Professor: Because in lot of clinical data or different types of treatment, people like to do several gels and lot of treatment.

Expert: Yes

Professor: So your number of samples to be analyzed is very large.

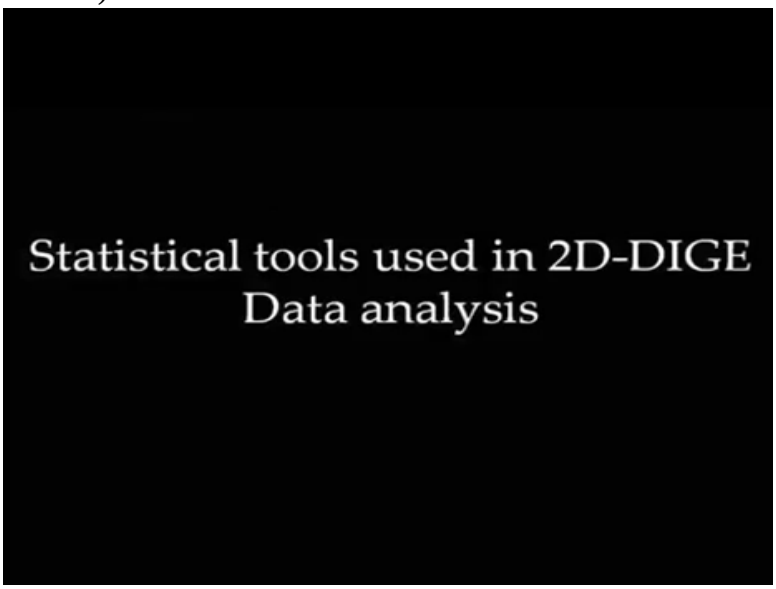
Expert: Exactly

Professor: And really obtaining the meaningful information is the real challenge.

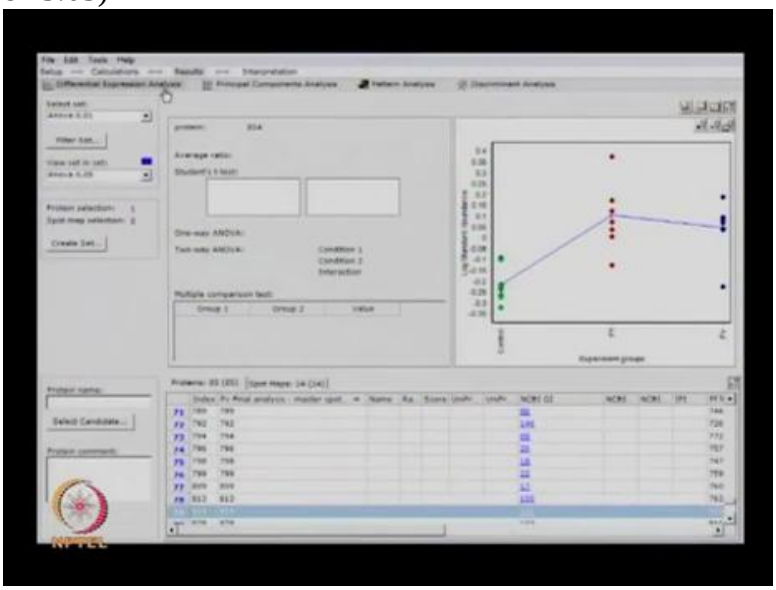
Expert: Exactly

Professor: In all the protein analysis. So I would like to see now EDA.

(Refer Slide Time 12:53)

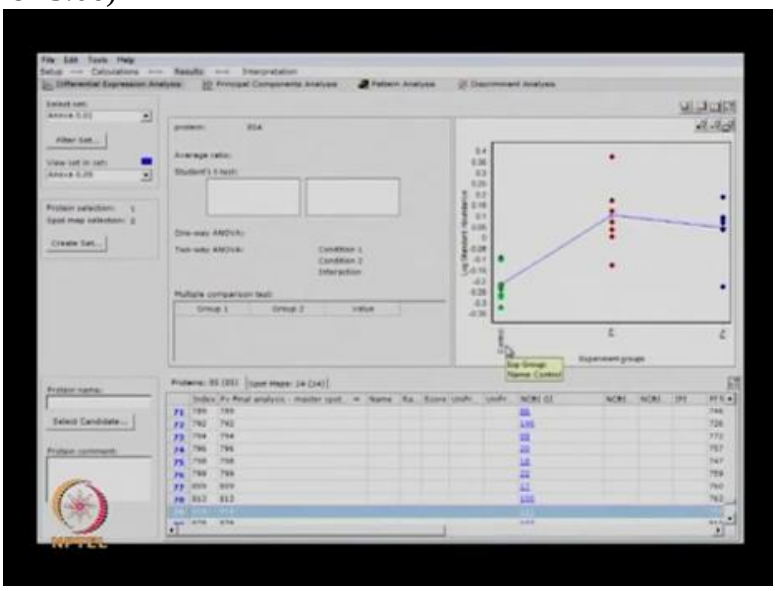


(Refer Slide Time 13:03)



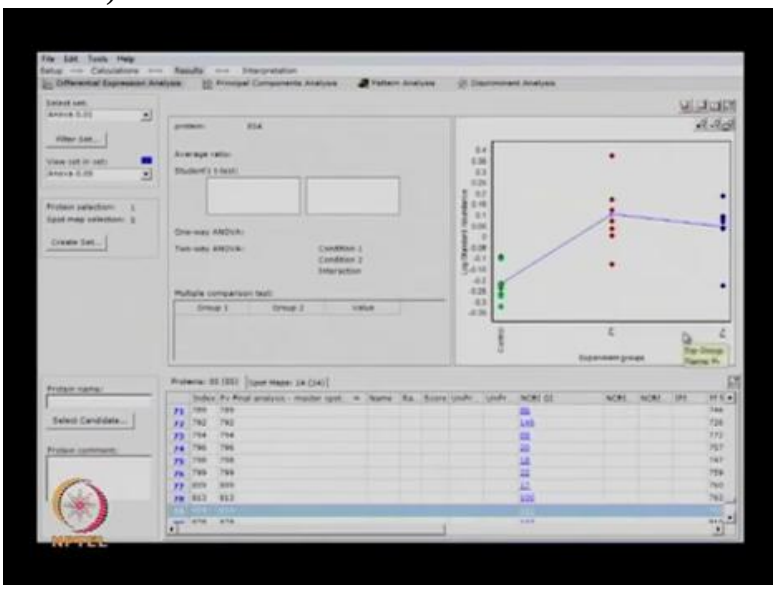
Expert: Thing which we can see here, Differential Expression Analysis in which you can see differences in

(Refer Slide Time 13:06)



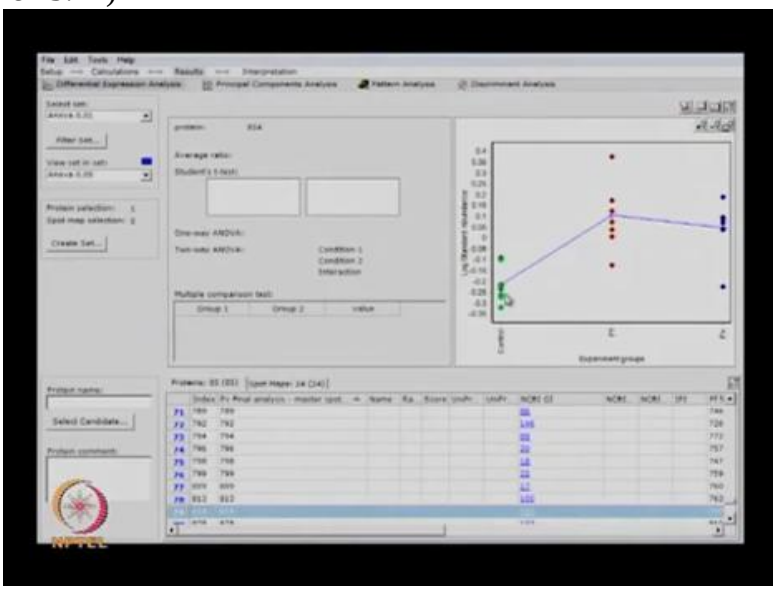
Expert: between control as well as two experimental data

(Refer Slide Time 13:11)



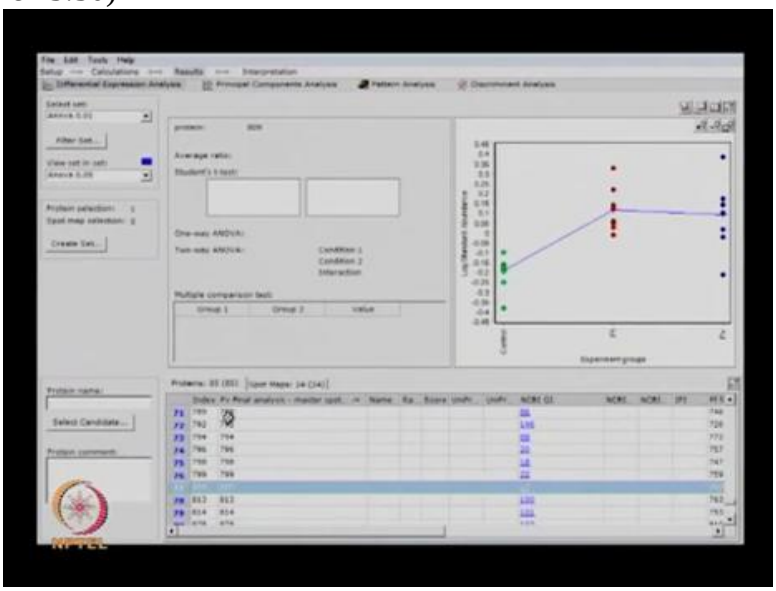
Expert: This is the different treatments which is given Here you can see how the particular protein is ...

(Refer Slide Time 13:14)



Expert: expressing throughout these control as well as this Pf and Pb, this kind of things and you can see this kind of data for each and individual protein here. So that from here, you can see which one is your interest and which is not...

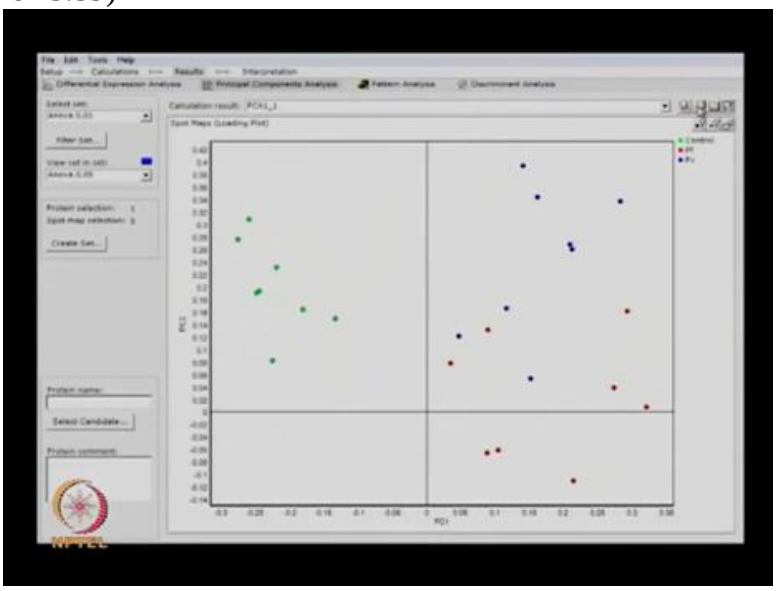
(Refer Slide Time 13:30)



Professor: you are actually...You are actually analyzing the data spot wise now, spot by spot you are looking...

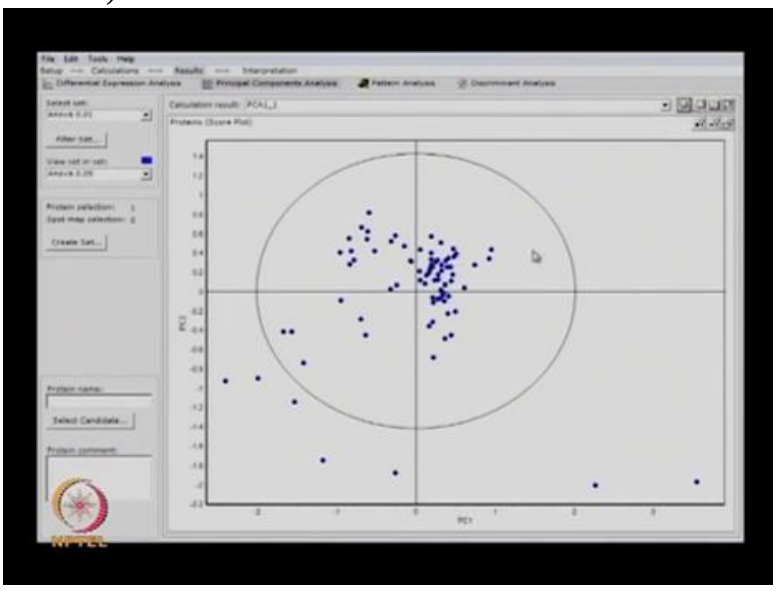
Expert: Exactly here the spot by spot which we are seeing, the number of ...even the index number shows there is a master gel, from the master gel you can see exactly this number. This is what which we are seeing here for each and individual spot, here we can see the results as well as

(Refer Slide Time 13:59)



Expert: we can see the Principal Component Analysis of this data.

(Refer Slide Time 14:03)



Expert: Here there are almost 89 proteins, out of these 89 proteins, you can see, inner the circle, there are proteins, some proteins are present, and out, out layers are there. The inner circle, they are similarly, especially if I can say 95% statistically significant is there, and out layers which you can see are exactly, these can be some non-reproducible spots or else what the major thing is, these are all very highly up-regulated or highly down-regulated. So this can be worked as the marker also. Then we have to go back to our BVA data and we can check the protein, how exact it is regulating. Then we can identify the protein and we can use for further analysis.

Professor: So this is a powerful statistical parameter

Expert: Exactly

Professor: by using which you can identify some outlets

Expert: Exactly

Professor: which could be the potential discriminator between the control…?

Expert: Control and treatment.

Professor: And once you identify those proteins, you can go back to your original data for the BVA

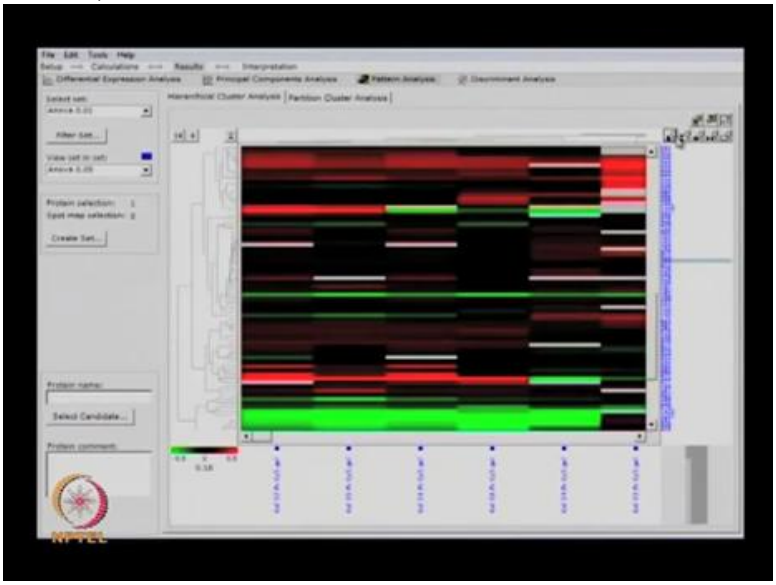
Expert: BVA

Professor: And data analysis is done

Expert: Exactly

Professor: This is very interesting.

(Refer Slide Time 15:22)

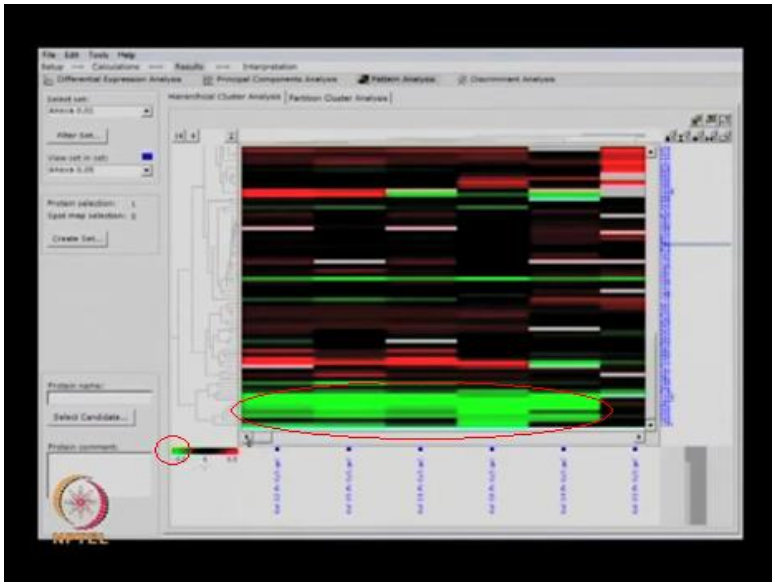


Expert: And next pattern analysis is we can see the whole proteomes and how there are difference from each other...

Professor: So this is the heat map

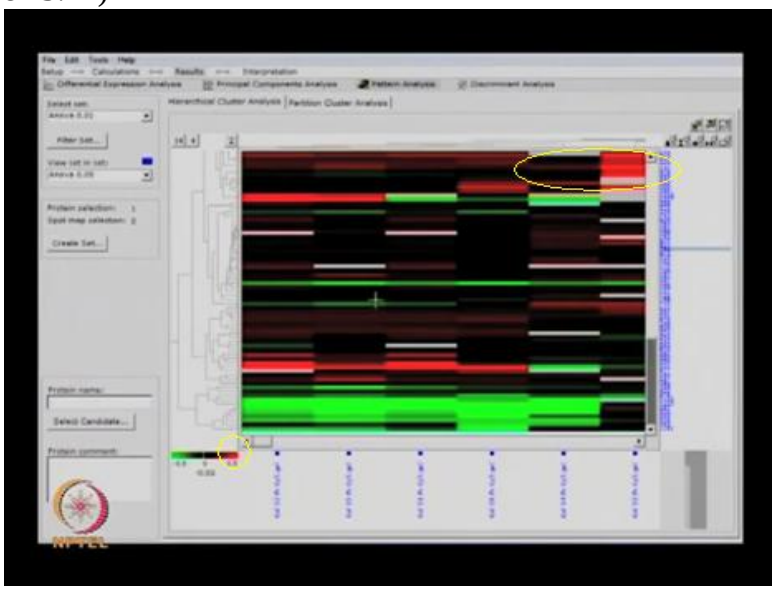
Expert: This is the heat map of the total 82 proteins which are taking into consideration, then how, in which area they are up-regulated, we can see the blue area,

(Refer Slide Time 15:36)



Expert: sorry the green area exactly we can see is completely down-regulated area

(Refer Slide Time 15:42)



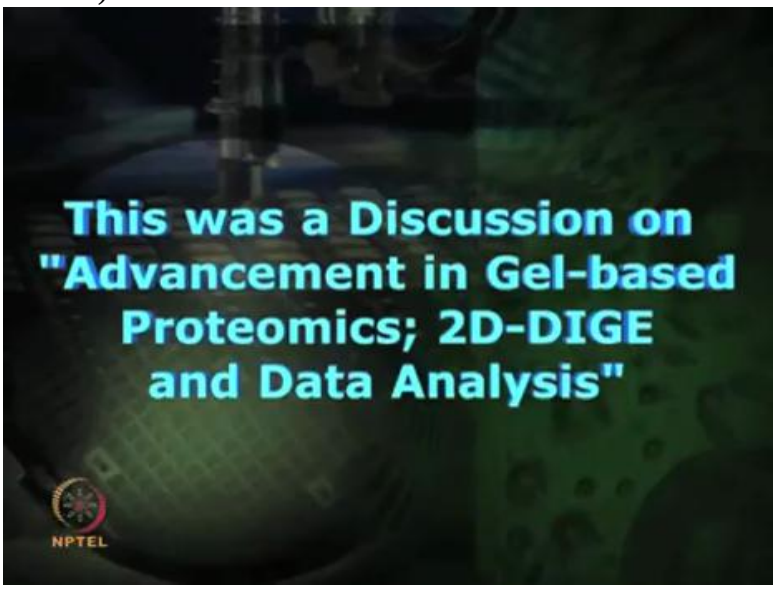
Expert: and the red color portion which you can see, that is the up-regulated portion and the remaining black color which you can see, those proteins are similarly regulated. This is what you can see here. This kind of data will help you to represent your complete, whole analysis.

(Refer Slide Time 15:57)

Professor: Doctor Srinivas, it was very useful to have you here and to get an overview of DIGE technology, how people can use this type of software and analyze their data by using DIA, BVA and EDA and although there was not enough time but you gave a very good demonstration in a very short time to give a glimpse of the processes involved in doing this analysis as well as how different types of statistical parameters can be applied to get some very powerful statistical information from our biological data. So thank you very much for coming here and giving this very good introduction about DIGE technology

Professor - expert conversation ends

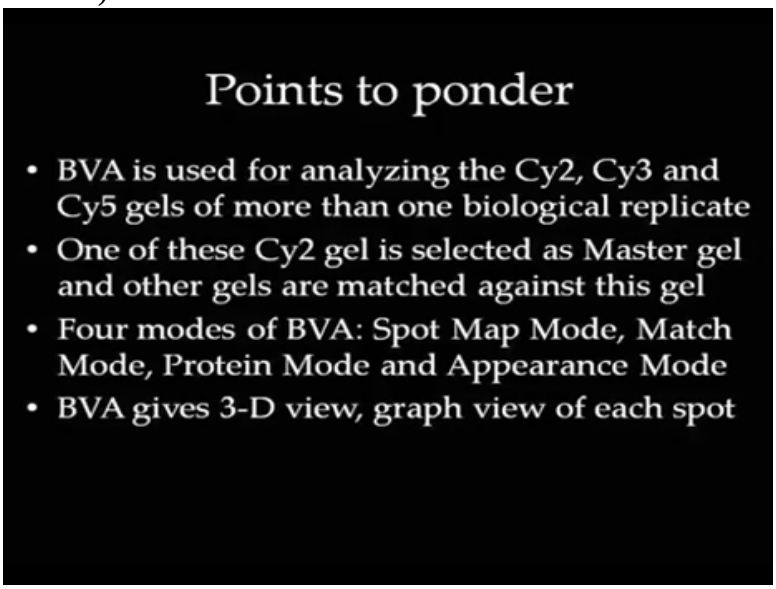
(Refer Slide Time 16:40)



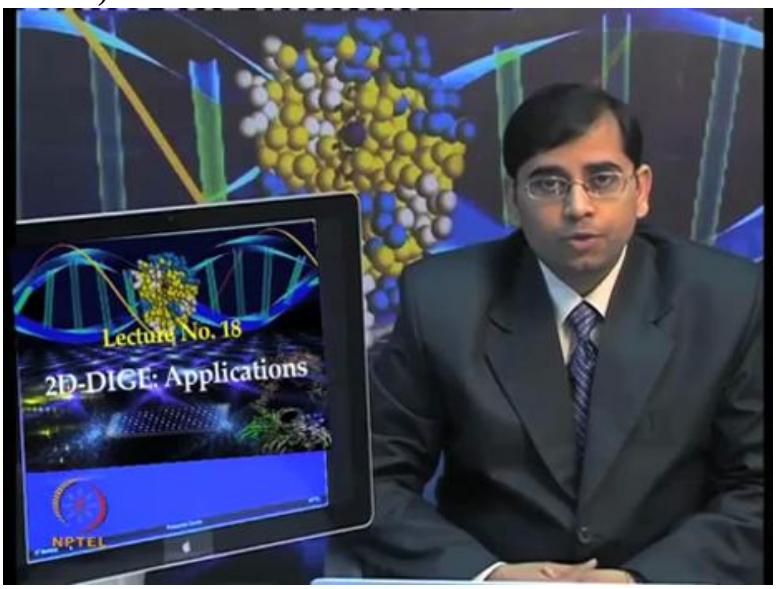
So I hope our discussion with Doctor Srinivas was useful and now you can perform these analyses by using specialized software and obtain some very useful biological information from your data set.

Probably you must appreciate there are lots of meticulous steps involved in performing these experiments but at the end this provides very useful, quantitative, multiplexing approach to separate proteins and to analyses different types of variations.

(Refer Slide Time 17:30)



(Refer Slide Time 17:41)



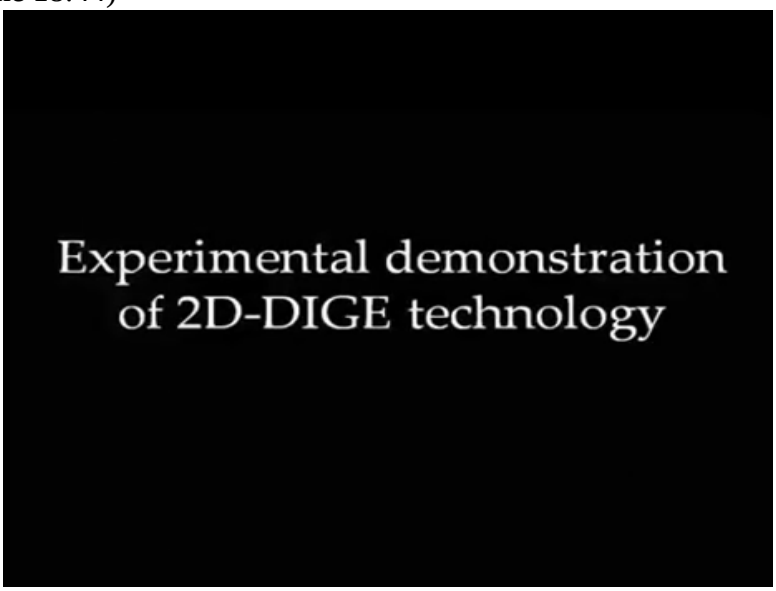
I hope at the end of this module and lecture you will be able to perform gel-based proteomics experiment. But please keep in mind these protocols and methods are only giving you a feel for performing these experiments.

Each experiment, each sample type, each biological question brings its own unique challenges and depending upon those conditions and your sample type you need to optimize

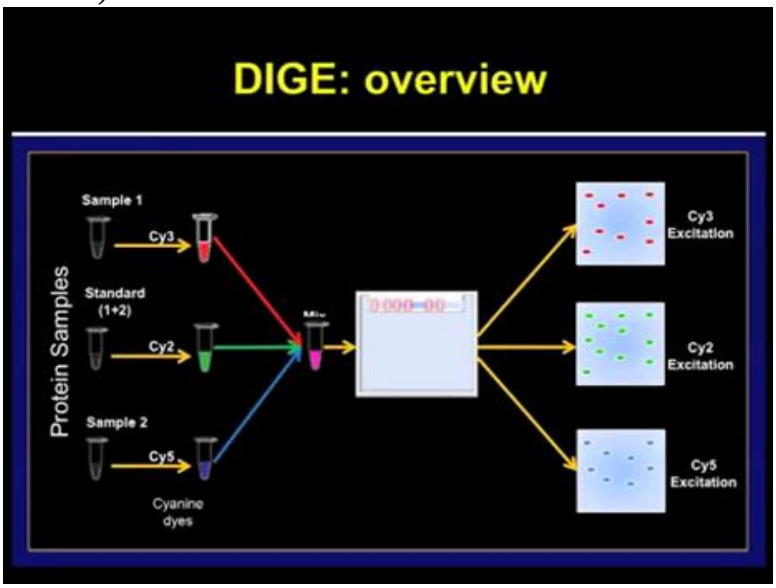
these methods. There is no one technology which can answer all of your questions but it is good idea for you to know; what are different methods that are available for you to use.

So I hope by taking this module on gel-based proteomics, now you are familiar with different types of gel-based techniques. These are only few, there are many other methods as well available but these are the most commonly used methods which people are applying in the field of proteomics.

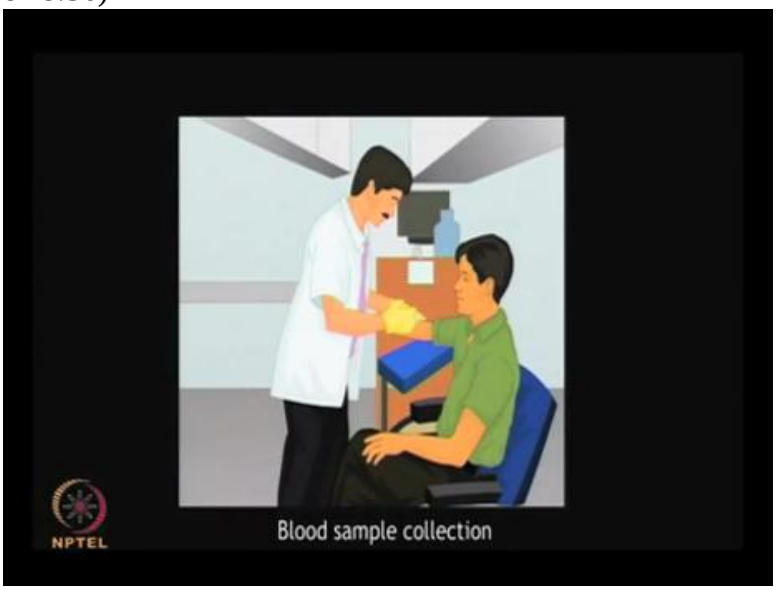
(Refer Slide Time 18:44)



(Refer Slide Time 18:51)



(Refer Slide Time 18:56)

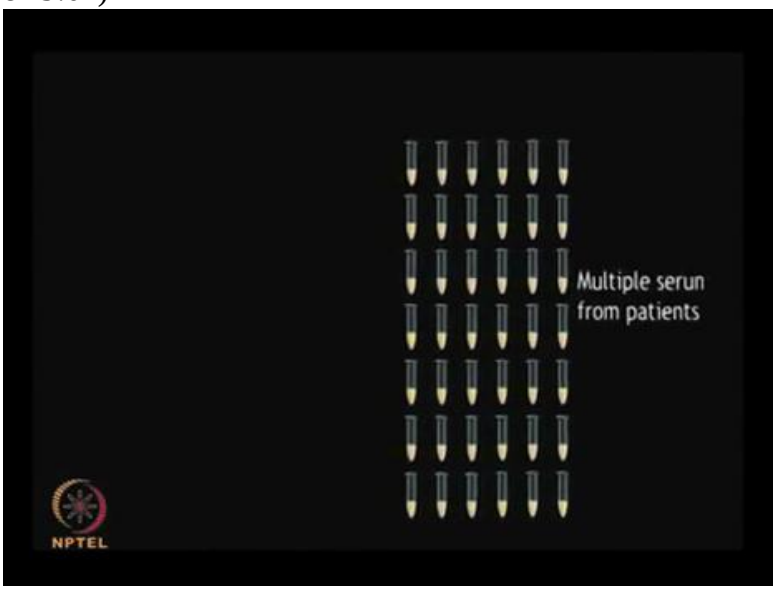


Say among 2DE and DIGE, which of these two techniques will be better to

(Refer Slide Time 19:00)

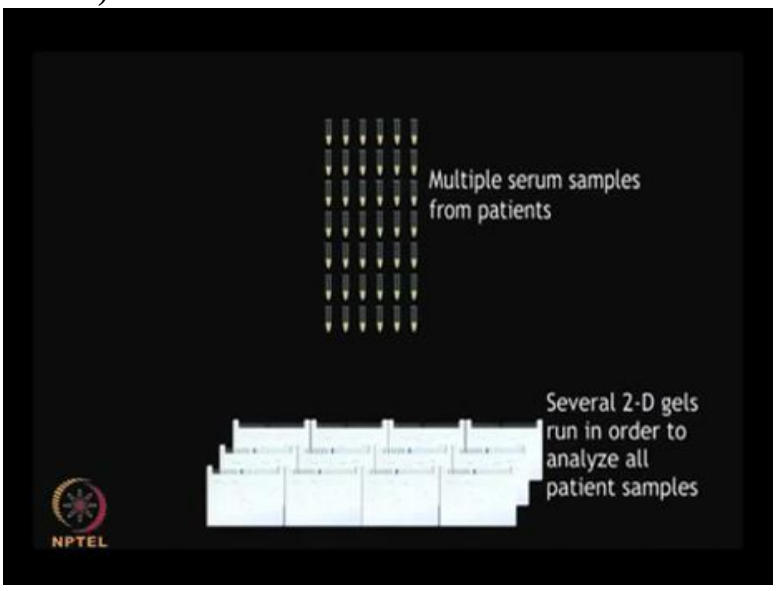
separate serum protein samples obtained from large number of patients in a clinical trial?

(Refer Slide Time 19:07)



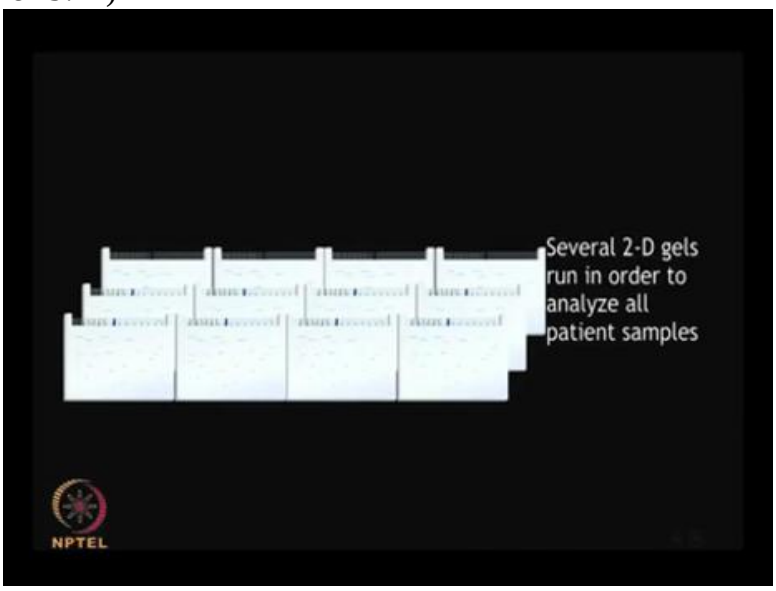
If I have multiple serum samples

(Refer Slide Time 19:10)



from patients, two-dimensional electrophoresis although a very useful technique,

(Refer Slide Time 19:17)

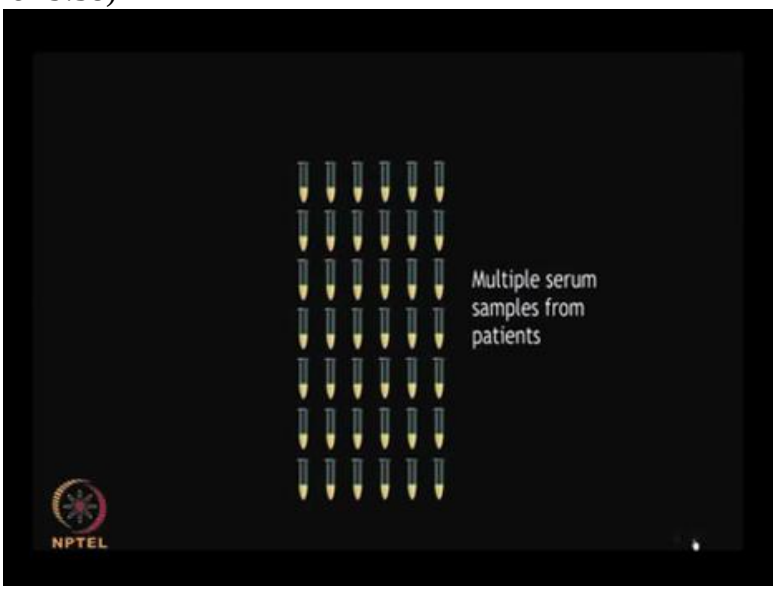


but it may not be the best option in this case to analyze serum proteins from large number of patients.

(Refer Slide Time 19:32)

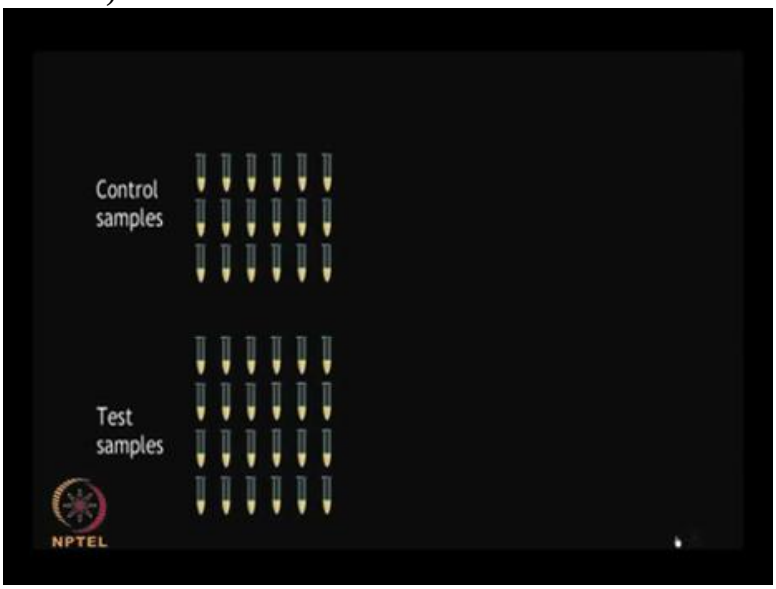
In this case DIGE will be extremely valuable tool for analysis of large number of samples

(Refer Slide Time 19:38)



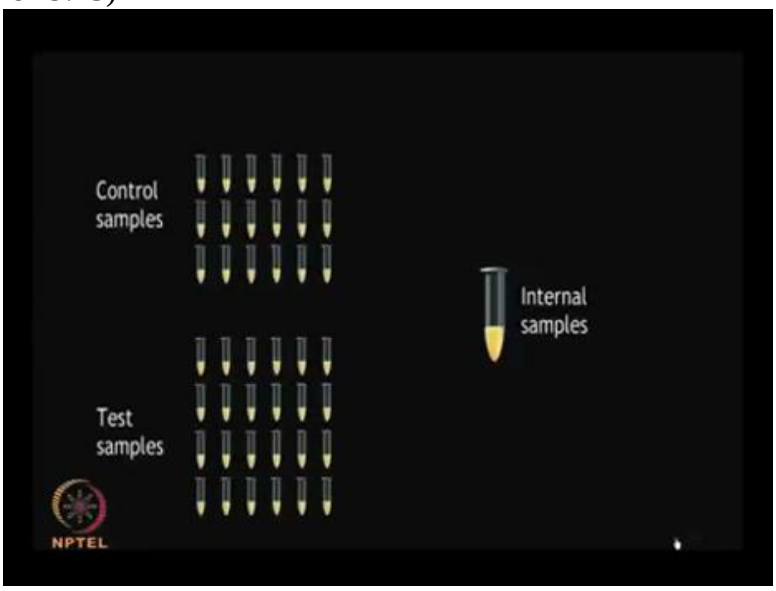
simultaneously without having to overcome

(Refer Slide Time 19:43)



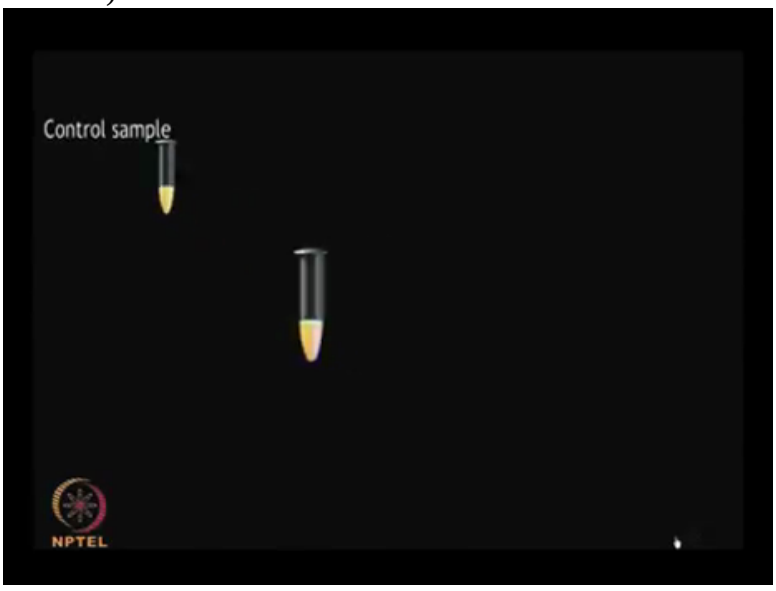
the problem of

(Refer Slide Time 19:45)



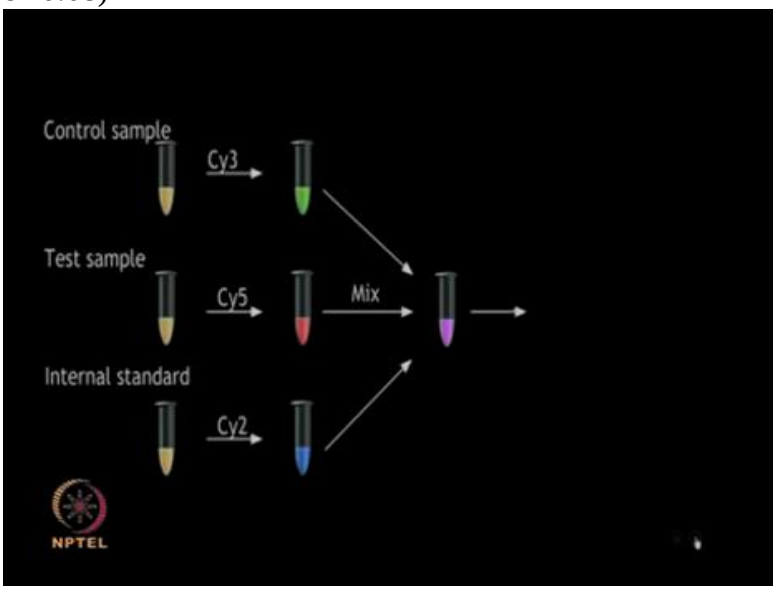
Gel-to-gel variations

(Refer Slide Time 19:48)



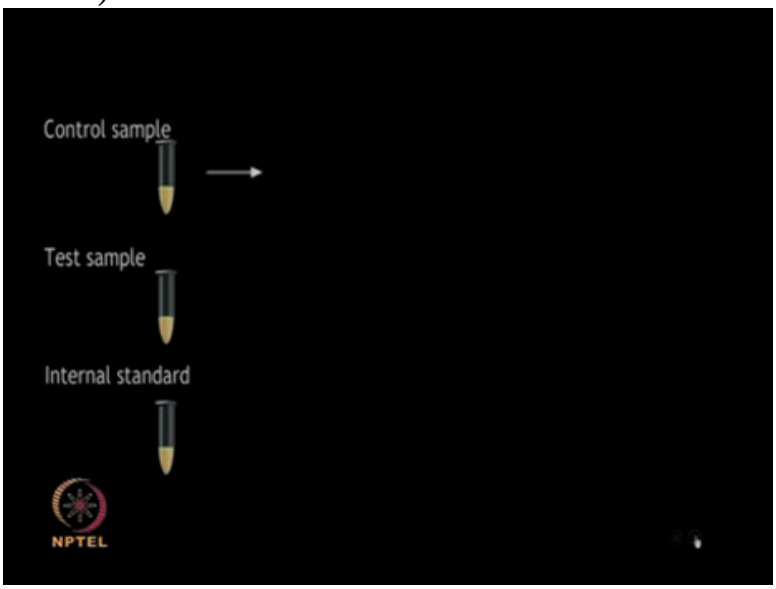
In DIGE gels the control and the samples can be differentially labeled by using the cyanine dyes and then run on the single gel.

(Refer Slide Time 20:08)



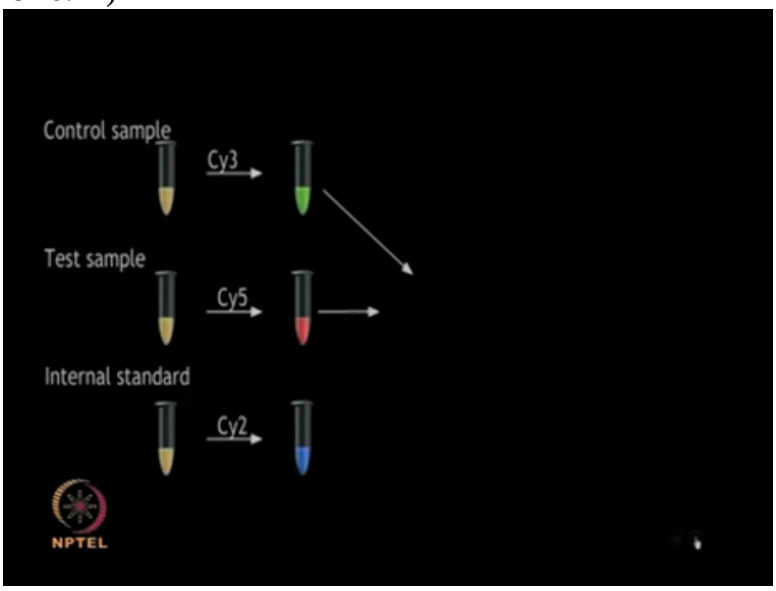
The pooled internal standard

(Refer Slide Time 20:10)



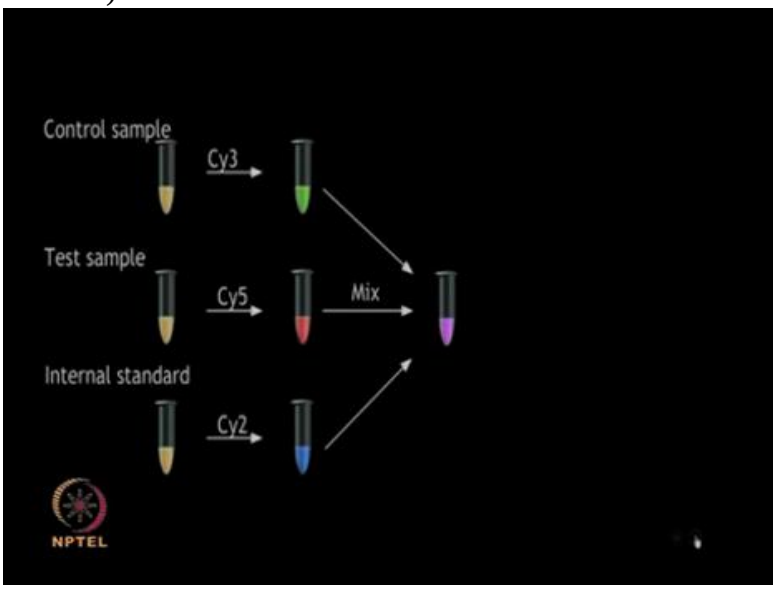
for DIGE

(Refer Slide Time 20:11)



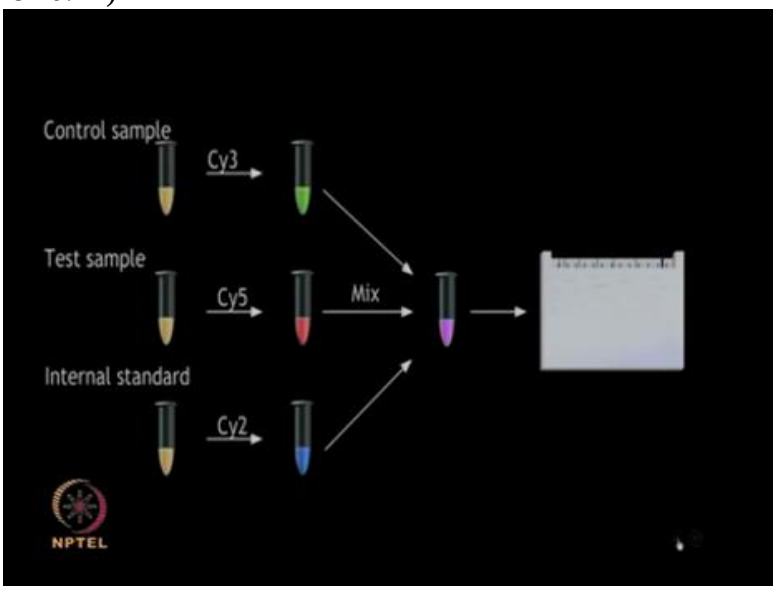
is prepared

(Refer Slide Time 20:12)



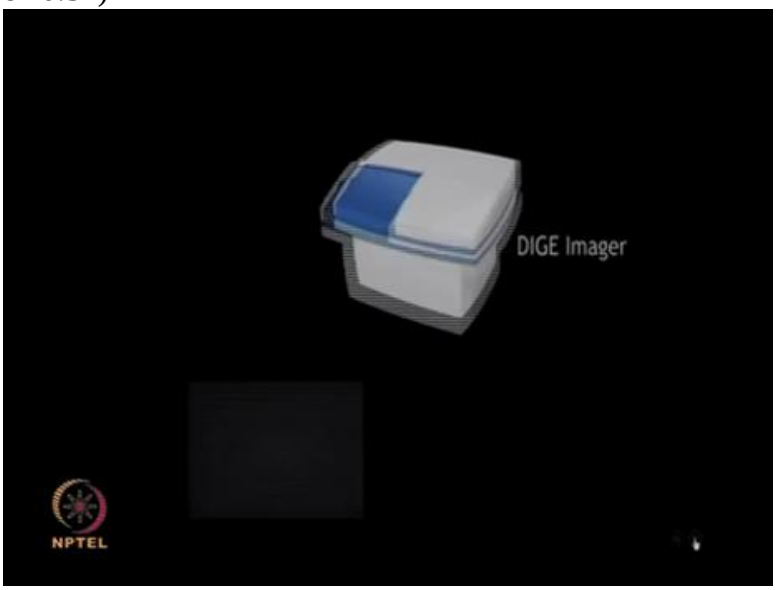
by mixing

(Refer Slide Time 20:14)

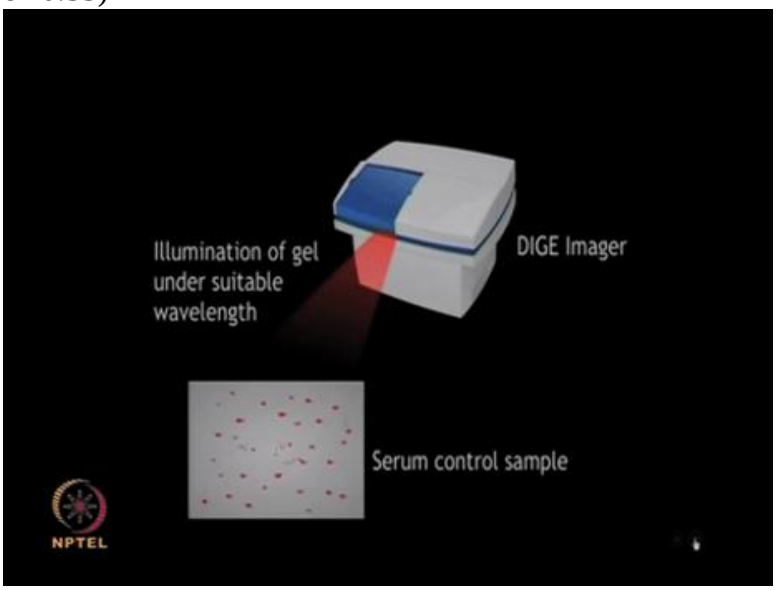


equal amounts of all the samples that need to run on the gel and this prevents the problem of gel to gel variations.

(Refer Slide Time 20:31)

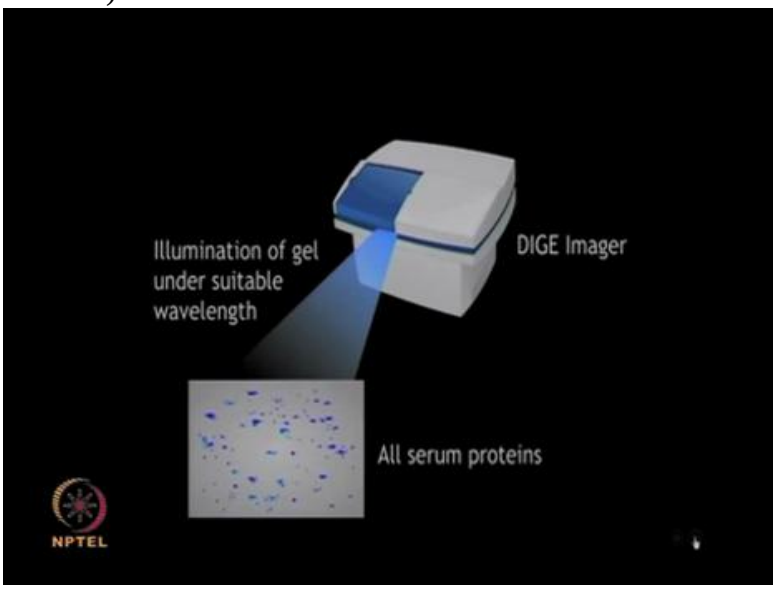


(Refer Slide Time 20:33)



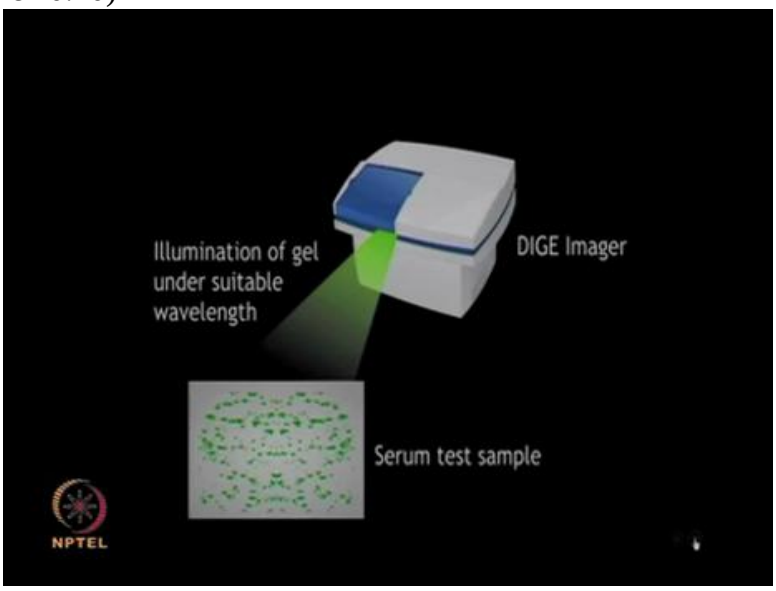
From the same gel, three different images can be obtained for Cy2,

(Refer Slide Time 20:37)



Cy3…

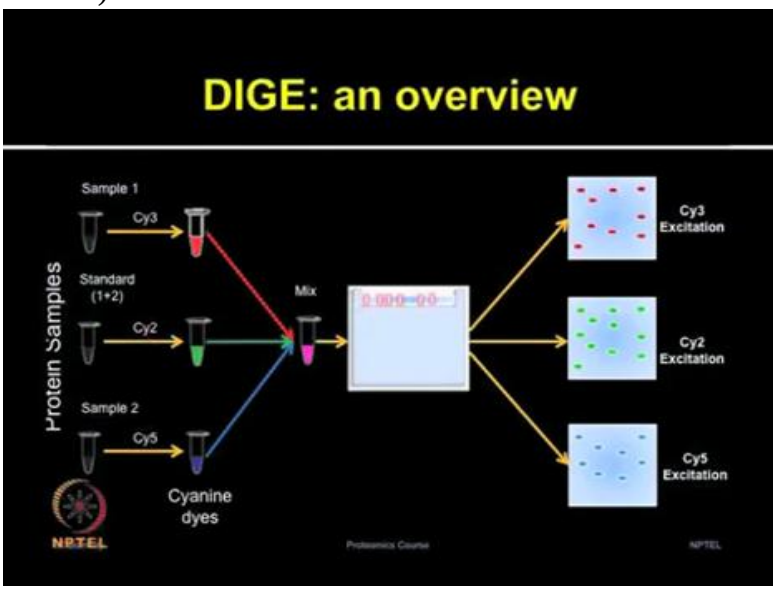
(Refer Slide Time 20:40)



and Cy5, therefore there would be no reproducibility issue and various artifacts can be eliminated for the clinical or the large number of sample masses.

The main aim for development of Difference in Gel electrophoresis was to overcome the inherently poor reproducibility of conventional two-dimensional electrophoresis.

(Refer Slide Time 21:06)

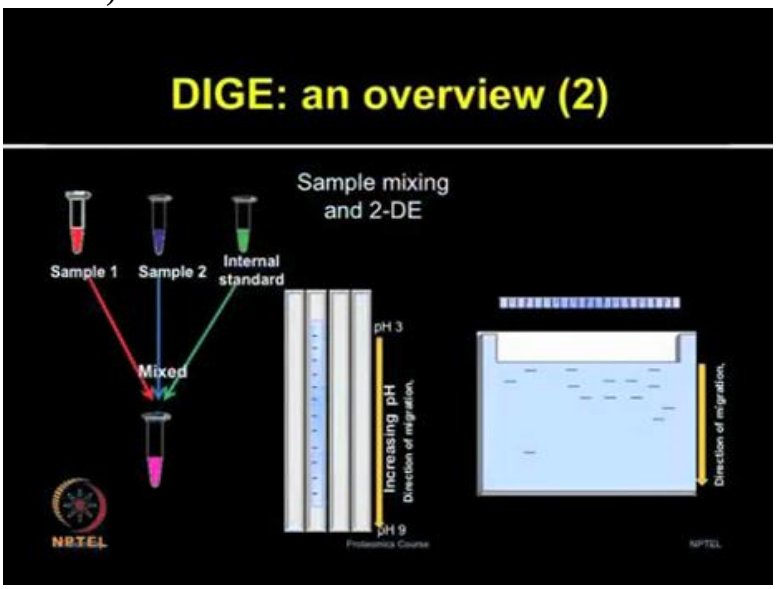


The DIGE is quite sensitive technique with less than one femtomolar of proteins which can be deducted, and it can enable the linear detection of very broad dynamic range of the proteins.

So, as you can see in this slide the protein samples directly have control, and treatment those are labeled with two different dyes Cy3 and Cy5,but a small aliquot of both of these samples is mixed together to make a internal pool.

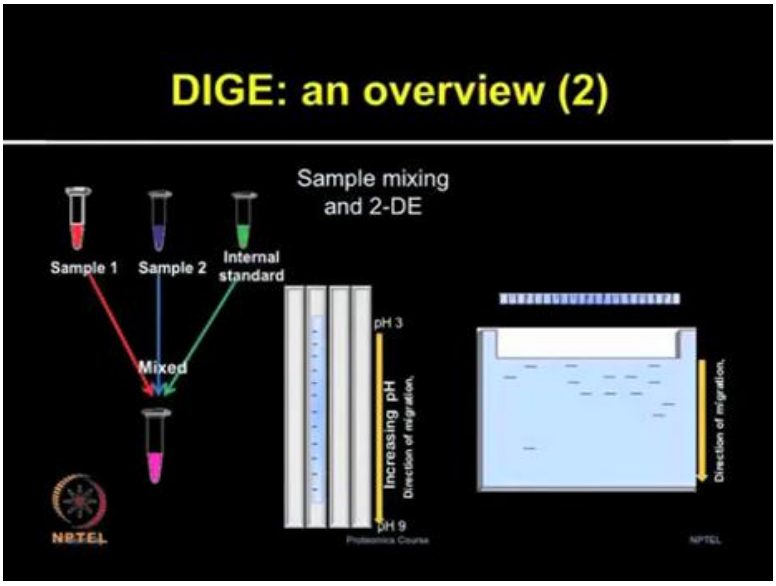
That internal pool is labeled with third dye Cy2.All these three protein samples are mixed together in one tube, which contains both control treatment, as well as the reference spots from the internal pool. All these protein mixture are separated in the first dimension on the same strip, and then the same gel can be scanned with the three different wavelengths to obtain the images for the Cy2, Cy3 and Cy5.

(Refer Slide Time 22:20)



So, in the conventional 2 dimensional electrophoresis the gel-to-gel variations which come from the acrylamide polymerization, electrical pH and thermal fluctuations in different gels that can be overcome in the DIGE gels, because all the protein separation is going to happen on the same gel. So, all those artifacts can be minimized by using DIGE approach.

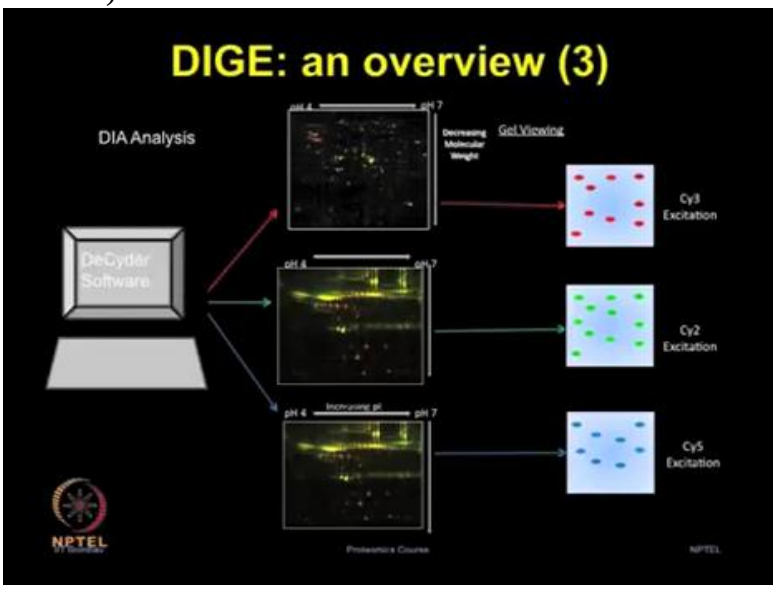
(Refer Slide Time 22:54)



In the slide it is shown that the three samples are mixed, and then isoelectric focusing is performed from the pooled sample, and then this strip is placed on the SDS page gel for the protein separation in the second dimension.

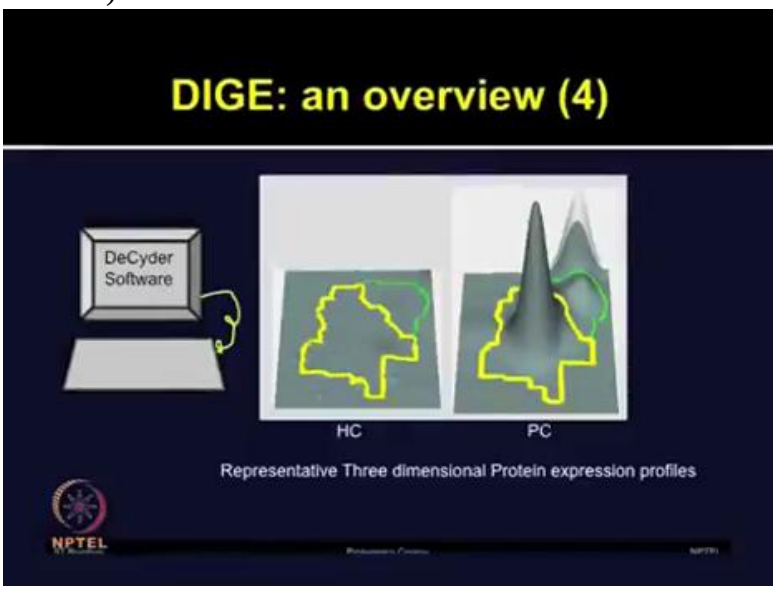
So, overall DIGE provides very uniform staining from gel to gel, and shows high sensitivity and linear dynamic range of detection for the expression profiling of complex biological samples. See, for aim is to resolve thousands of proteins, and cover comprehensive proteome coverage, then DIGE is a very good platform.

(Refer Slide Time 23:45)



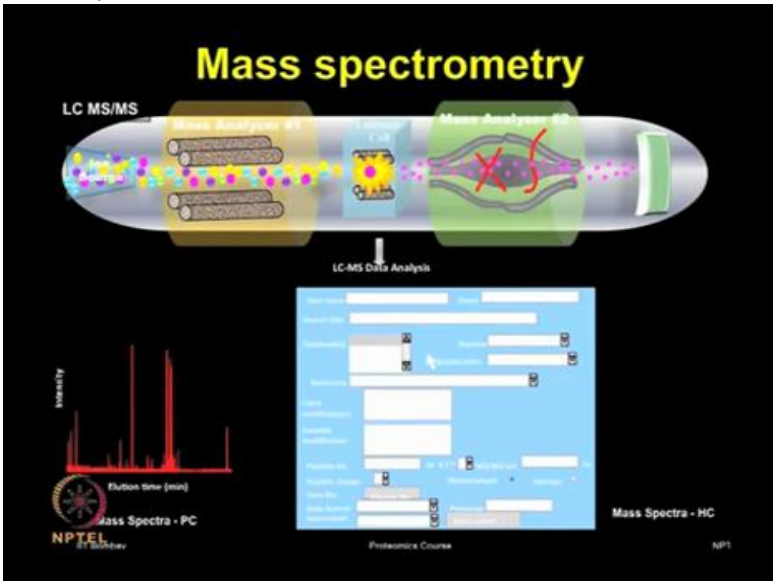
Especially, if you want to do the comparative or differential proteomic analysis, because your gel to gel variations and other variations will be minimized, and DIGE will provide the very high sensitivity

(Refer Slide Time 23:59)



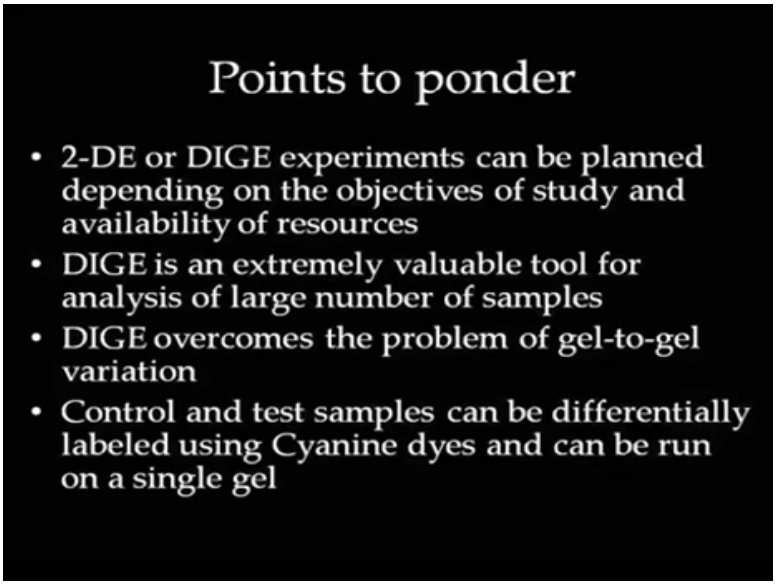
So, once you have run these gels, now from the same gel the images can be obtained, three images of your control and the treatment, and these can analyzed from different software, such as DeCyder software, and then by looking at 3-dimensional views and the statistical data, then these proteins can be considered as interesting for further investigations.

(Refer Slide Time 24:28)



Once the spots are analyzed and excised from the gels, then the same tradition you have to follow, you can use any of the mass spectrometry platforms, and then obtain the MS spectra for further analysis using different type of bioinformatics tools, such as Mascot.

(Refer Slide Time 25:00)



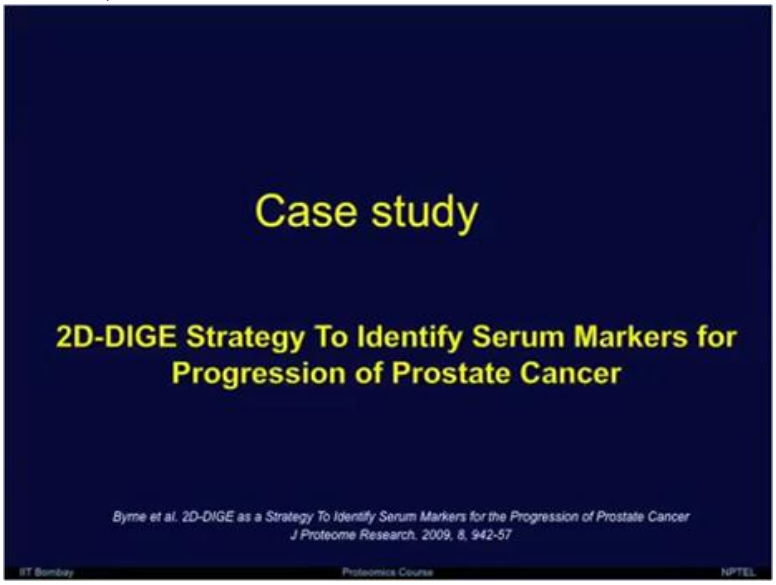
(Refer Slide Time 25:05)

So, overall the DIGE method is far more superior in terms of the reproducibility as compared to the conventional two-dimensional electrophoresis, and for the quantitative accuracy.

(Refer Slide Time 25:27)

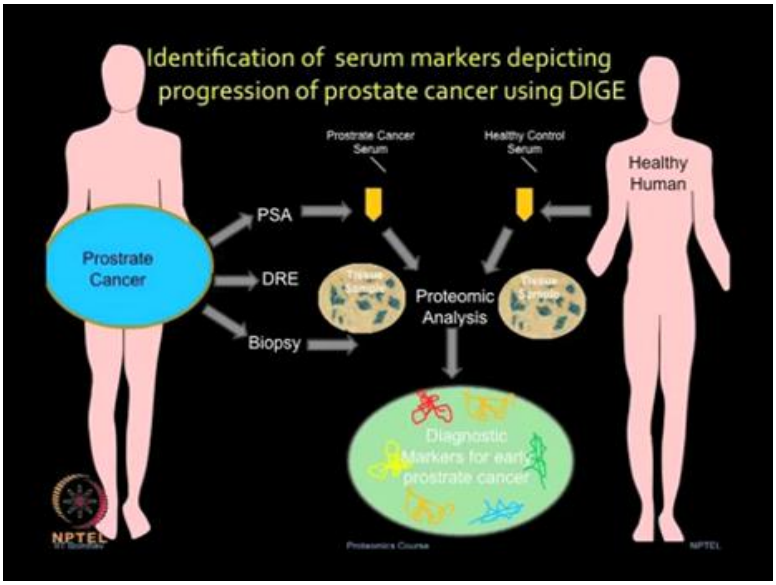
Therefore, applications of 2D DIGE can be found in virtually all major biological research areas. If you see the recent publication, you will appreciate there are several papers on each of the biological system for different, different type of applications, whether its cell signaling, looking at developmental biology, looking at plant proteomic analysis, neurosciences, clinical studies, different type of diseases including cancer, you will find there are hundreds of publications available, which have employed the power of 2D DIGE technique.

(Refer Slide Time 26:10)



Let us talk now a new case study, case study 3 on 2D-DIGE as a strategy to identify serum markers for the progression of prostate cancer. Study by Byme et al, published in 2009. So, this study authors aimed for identification of serum markers by depicting the progression of prostate cancer, by using difference in gel electrophoresis technique.

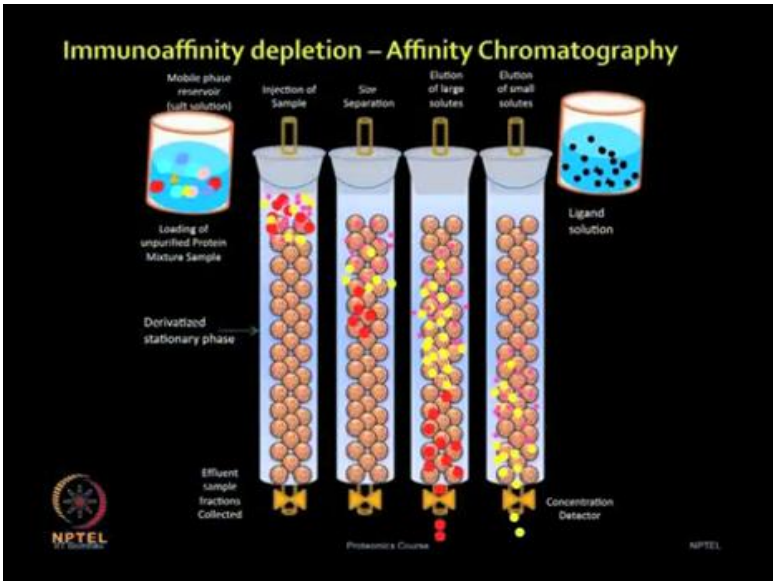
(Refer Slide Time 26:45)



So, prostate cancer is recognized as a significant problem in older male population. The prostate cancer screening relies heavily upon testing for the higher level of Prostate Specific Antigen, also known as PSA within the peripheral circulations.

So, PSA is very sensitive marker, but the lot of discussion on reliability and specificity of PSA for the prostate cancer. Reason being that the level of PSA is also high in benign prostatic hyperplasia or prostatitis. So therefore, there is lot of discussion whether one should rely on only PSA for detection of the prostate cancer. So, this study aims to identify some new markers in the prostate cancer by studying the serum proteome analysis.

(Refer Slide Time 27:52)

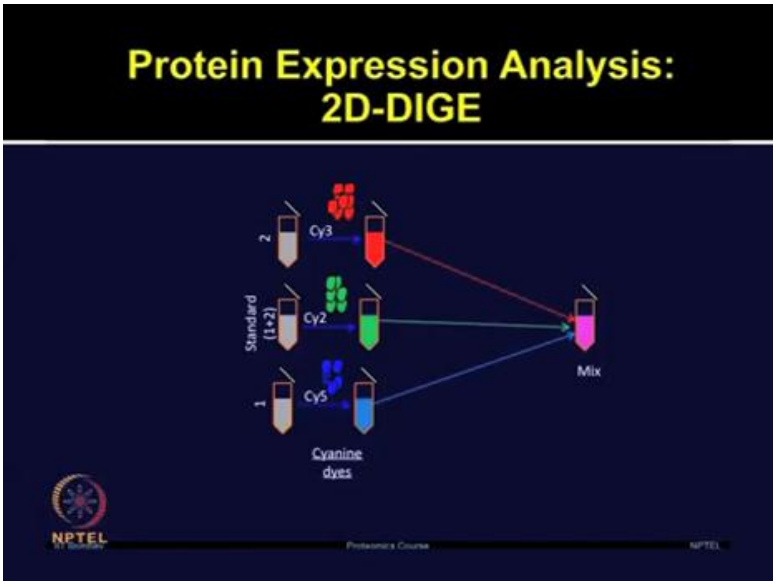


As you are aware, and in fact we have discussed the protein preparation from the serum earlier, so each of the biological sample poses lot of technical challenges, and serum is one among them where presence of highly abundant proteins, such as albumin and immunoglobulin, they result into the masking of low abundant proteins.

So, to eliminate those high abundant proteins, authors used multiple affinity removal system from the Agilent technologies, and they removed most highly abundant proteins from the serum sample including albumin IGG antitrypsin IGA transferrin and haptoglobins. So, after the abundant proteins were depleted from the serum sample, then authors moved for the protein extraction and further analysis.

So, the differential proteomic analysis was performed in the two different cohorts of histologically confirmed prostate cancer, with different grades of the disease.

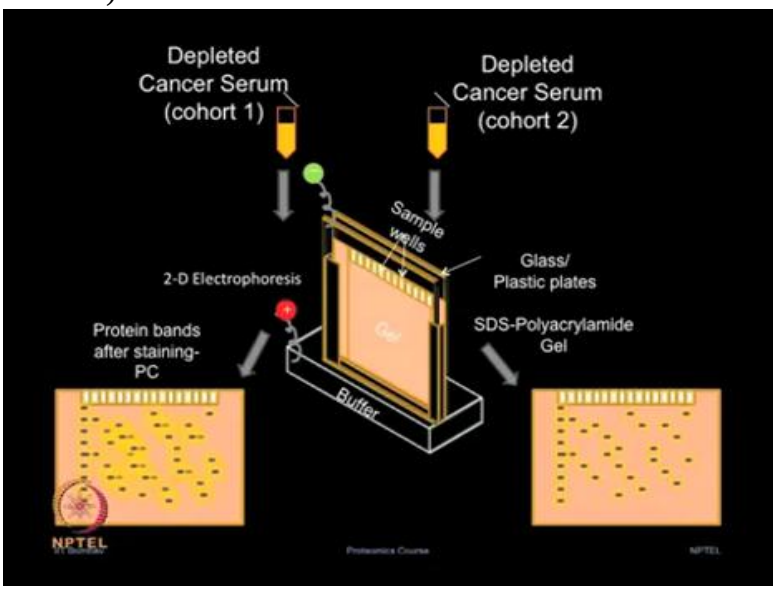
(Refer Slide Time 29:14)



They used the patients with two different grading system based on Gleason grading. So, the Gleason grading system that is used to help and evaluate the prognosis of men with the prostate cancer The depleted serum samples obtained from then patients with Gleason score 5 and Gleason score 7 were used for comparison and further analysis.

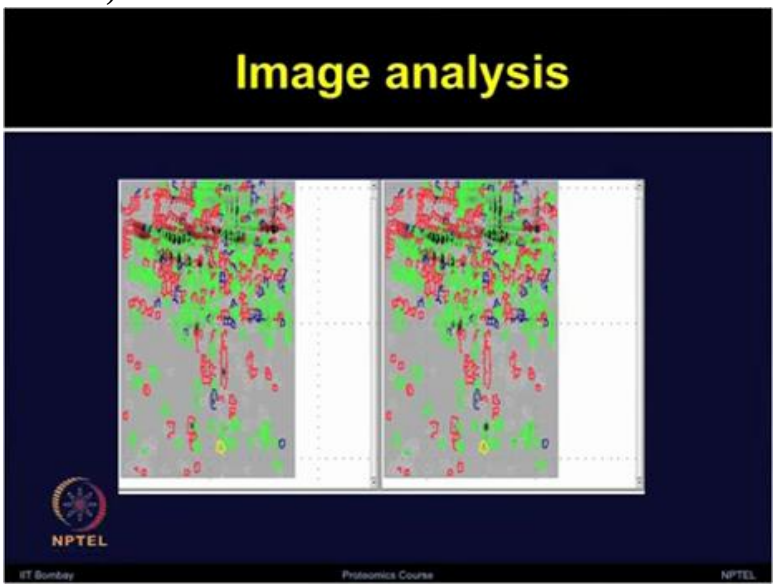
As you can see in the slide these samples were first labeled with the Cy3, Cy5 and also the internal reference pools were made which were labeled with the Cy2 dyes. These samples were then further mixed, the depleted cancer serum from first cohort of Gleason score 5,

(Refer Slide Time 30:14)



and second cohort of Gleason score7, those were mixed, separated in the first dimension and followed by protein separation in the second dimension.

(Refer Slide Time 30:27)



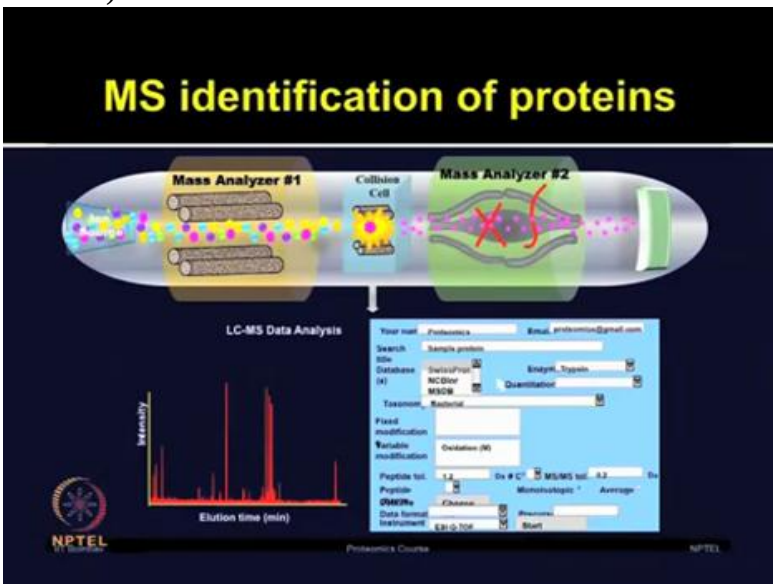
Now, when authors analyzed these DIGE images, they found at 63 protein spots were differentially expressed between the Gleason score 5 and 7 cohorts, and 13 of these proteins were statistically significant among these two populations.

So, as you know analysis of these gels is always challenging, especially if you are looking at the conventional 2D gels, where you have separate gels obtained from each of this groups. But, analysis in the DIGE gel is more automated, so if you remember our previous discussion in the DIGE gel analysis.

This analysis is more automated and more straightforward, but still we have to through individual spots, and we have look for the how real, how significant those changes are, and you have to look at the 3D views of those spots to ensure that it is the reproducible among various control and treatment groups.

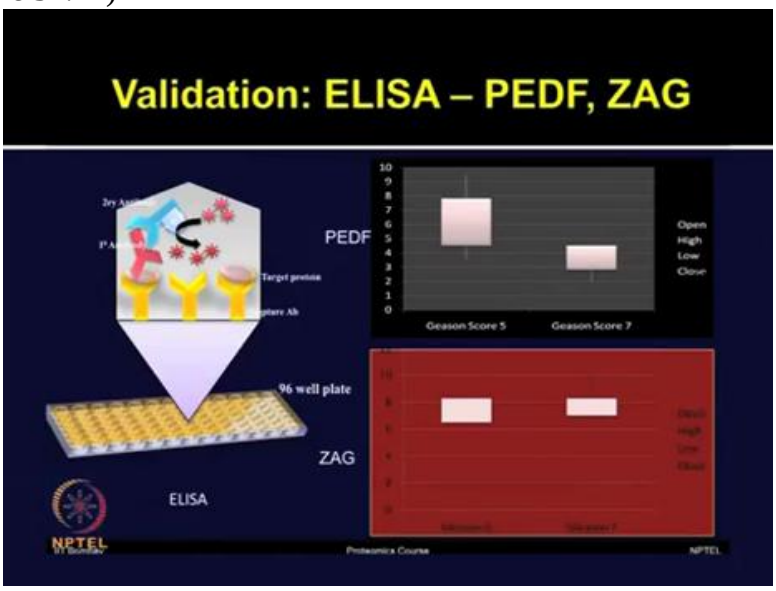
There are different levels of analysis performed which we have talked earlier, but this just shows the final output that 63 spots after all the analysis steps were considered significant

(Refer Slide Time 31:48)



After2D-DIGE gel image analysis, authors excise those spots, and subjected for the mass spectrometry identification of proteins, so the proteins excised from the gels were analyzed by using Finnigan LTQ mass spectrometer, and data from these MS/MS experiments were analyzed by using Bioworks Browser by using SEQUEST program.

(Refer Slide Time 32:27)

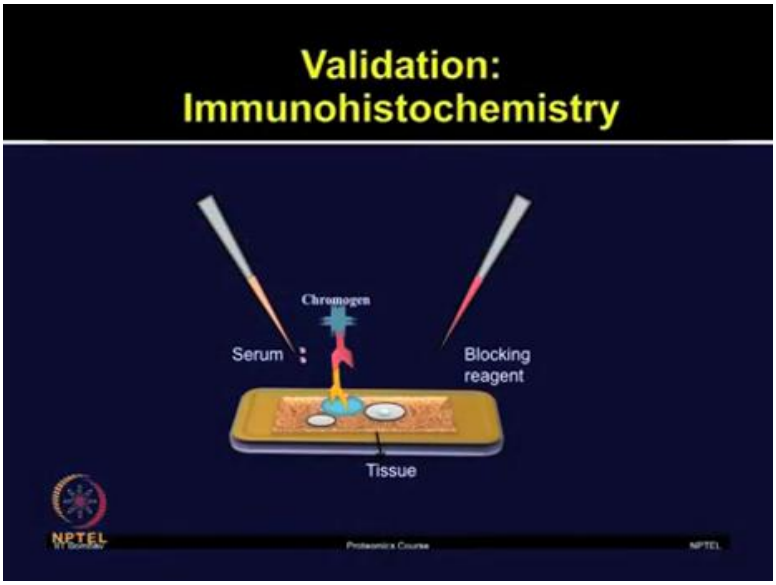


For validation, authors employed various techniques. including Western Blots, enzyme linked immunosorbent assay, ELISA and also immunohistochemistry, Pigment Epithelium-Derived Factor PEDF and Zinc Alpha 2-Glycoprotein often known as ZAG; those proteins were further validated by the ELISA technique.

So, the PEDF levels were quantified by using ELISA kit, and results demonstrated as you can see in the slide that the statistically significant decrease in the PEDF in the Gleason score 7 depleted serum groups.

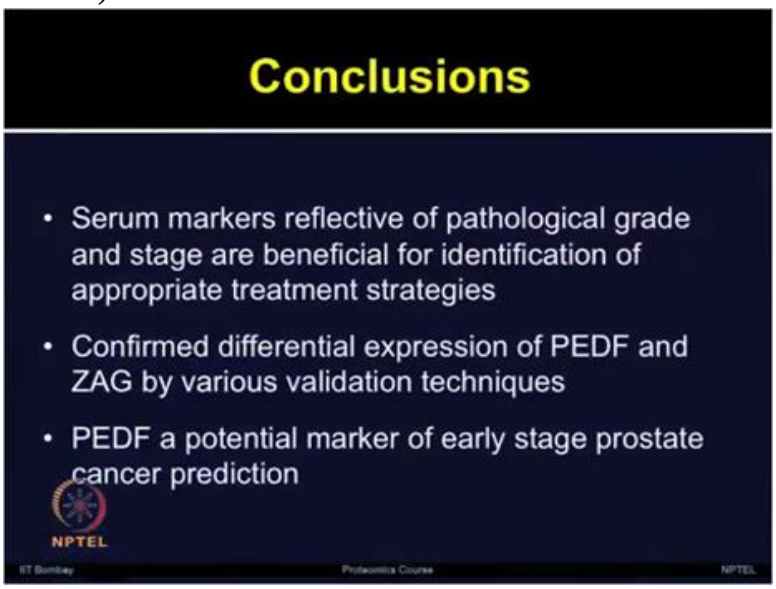
Whereas, the results for Zinc Alpha 2-Glycoprotein ELISA analysis, which is shown in the red in the bottom panel that indicated 1.4 fold increase in the Zinc Alpha 2-Glycoprotein absorbance in the Gleason score 7 group. So, these studies, this ELISA validation confirm their findings from the2D-DIGE experiments

(Refer Slide Time 33:39)



Authors also employed immunohistochemistry, or IHCs for validating the pigment epithelium derived factor PEDF and zinc alpha 2 glycoprotein, so that they are very confident that the proteins which they have identified from the proteomic profiling, those are real, and they also tested those on the independent tissue samples.

(Refer Slide Time 34:25)



So, from this paper the major conclusions were that serum markers, which are reflective of the pathological grade and stage could be beneficial for the identification of appropriate treatment strategies.

Authors confirmed that differential expression of PEDF and ZAG can be performed by using various techniques, such as Western Blot, ELISA and immune histochemistry.

Based on the studies and the follow up experiments, they concluded that PEDF could be a potential marker of early stage prostate cancer prediction. However, more studies and follow up required on the large number of patients before it can be established a good biomarker

You may appreciate that there is lot of power of these techniques and these can be employed for any biological application. You pick an application of your choice and I am sure you will be able to answer those by employing 2D or 2D DIGE techniques. Thank you.

(Refer Slide Time 35:28)