Proteins and Gel-Based Proteomics
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Mod 04 Lecture Number 14

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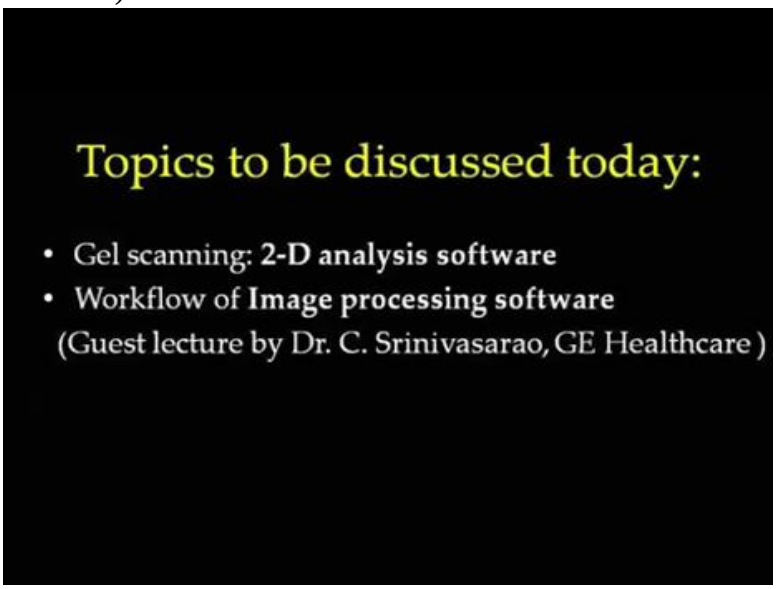

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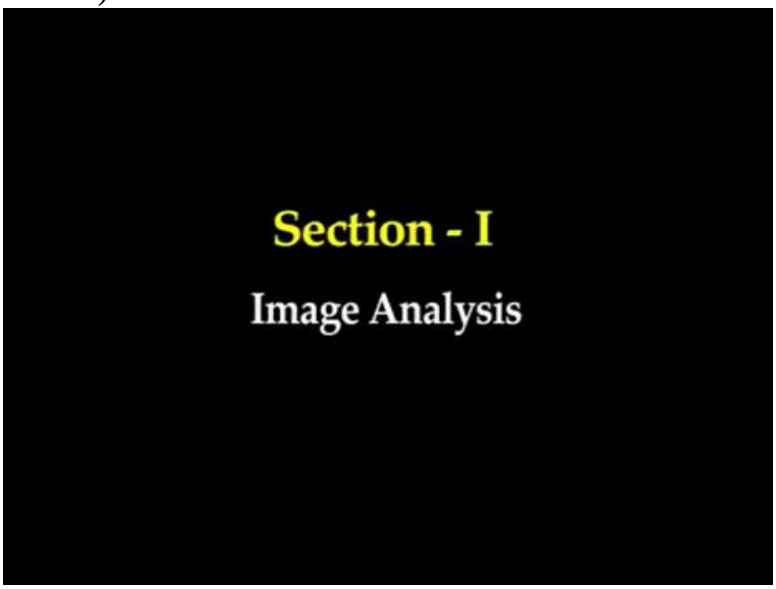

Image analysis is another one of the very important aspect of the two-dimensional electrophoresis workflow.

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There are different types of images, scanners available from different vendors such as one I have shown here Molecular Imager Densitometer, other the Typhoon Variable Mode Imager. There are many staining image scanners available. So now, how to analyze these images, do you want to do the things manually?

So can you take your gel patterns, and sit two of you together and say, OK this is my protein and control; this is your protein and treatment. Now I am going to look at each spot manually and then going to size the spot based on this comparison.

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So that is going to be very, very tedious work and you will not have any information about the spots having any statistical significance or not, how reproducible those are. So you need to scan it by using good scanners and then finally you need to analyze your image from different softwares which are available.

So commercially there are many softwares which are available for doing f two-dimensional gel analysis. I will give you a comparative table at the end …

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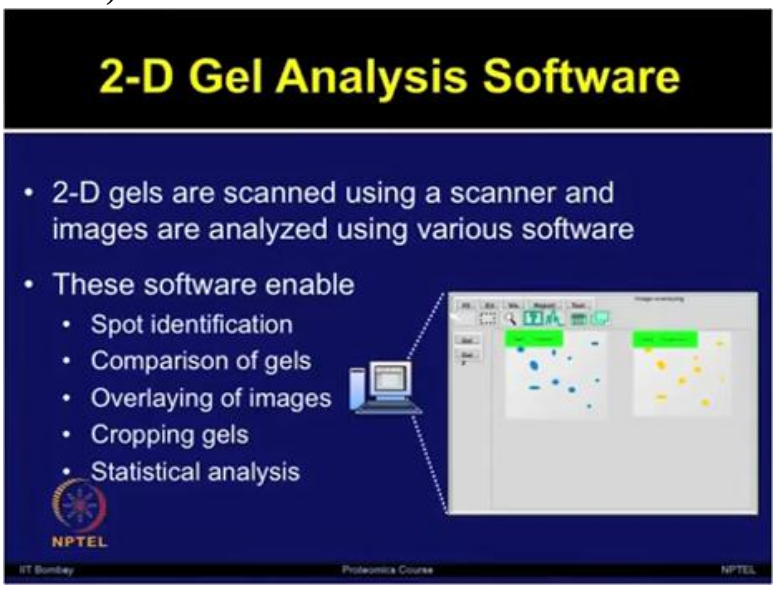

...but almost all of these softwares, they take the scanned images and analyze your gels by using various steps. So all this software enable the spot identification, comparison of the gels, overlaying of the images from your control and your treatment, cropping the gel, the part of which you want to compare and further doing the statistical analysis.

So the crop tool, that is the first part which you would like to use. If in your gel, you have some extra regions which...where you do not have any of the spot of interest, probably you would like to crop those regions and crop both your control and treatment uniformly.

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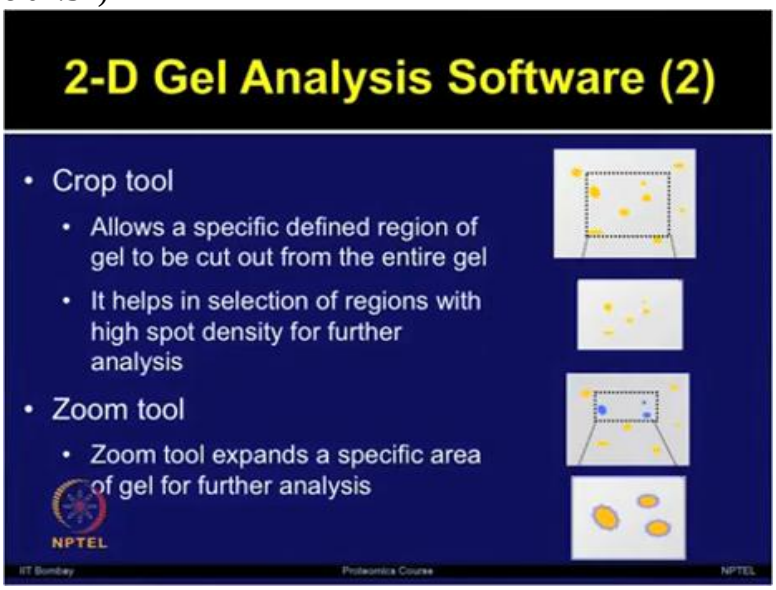

So this crop tool allows for a very specific defined region of the gel to be cut from the entire region. It helps to select the region having high spot density which can be used for doing further gel analysis. Next, you would like to see your spots in more detail. So you would like to use zoom tool which can expand a specific area of the gel for doing further analysis.

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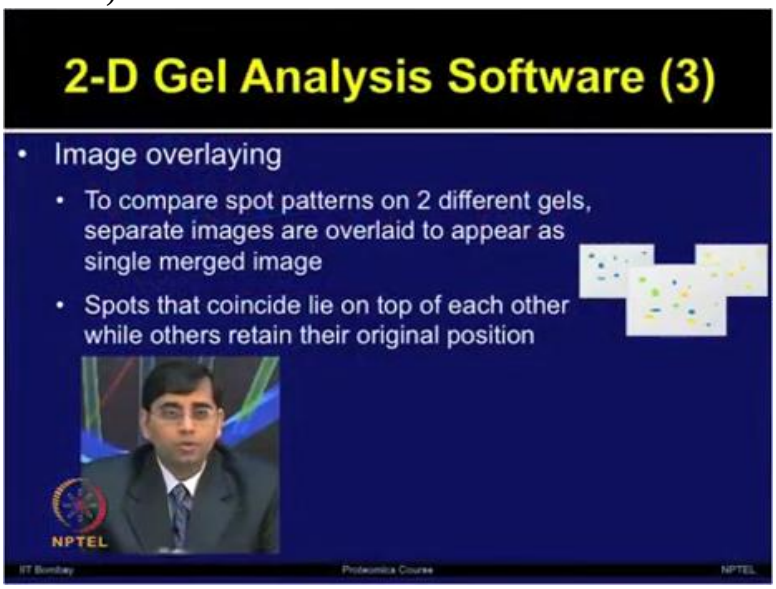

Now image overlaying is very important because if you comparing two gels; on one you have control, on other you have treatment, you would like to overlay those images together to compare the spot pattern on two different gels.

Because you have acquired two separate images, now you need to overlay those so that it can appear as a single merged image. Now spots which are going to coincide on top of each other, where as you can also locate their original position from the each of the individual images.

So image overlaying is important aspect where you can merge your control and your treatment gels. Then you would like to do the spot analysis where it is possible that you can …

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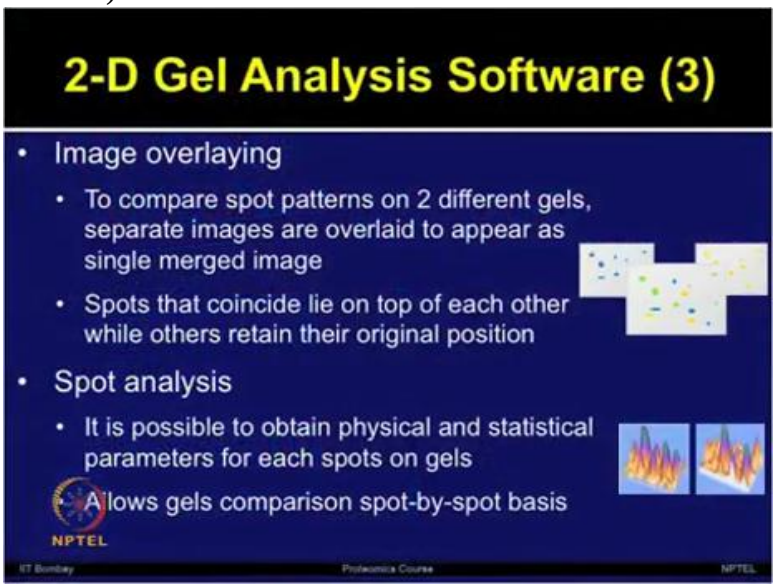

…obtain physical and statistical parameters for each spot which is present on the gel. You can look at the 3 dimensional views of each of the spots, how they are different from the control to the treatment and then you allow the comparison of the gel spot-by-spot basis.

So, often running a gel or acquiring images and generating lot of data is much straight forward as compared to doing the analysis which is more tedious step. One has to really sit and go through the gels usually spot by spot to analyze the gels. Now I am showing you a gel pattern of control and its comparison with the treatment gel.

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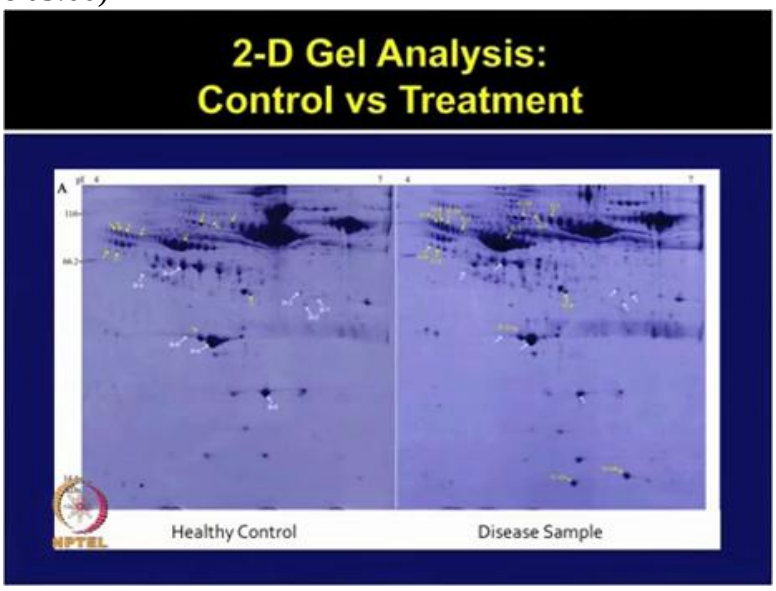

These are taken from one of the healthy control and disease sample and each of the spot is compared from the control to the treatments and one can look at, from different healthy controls and different disease sample, what is happening to each of the spot and if there statistical significance for their overall change if it is going up or going down, is that uniform in all the gels and how significant that is.

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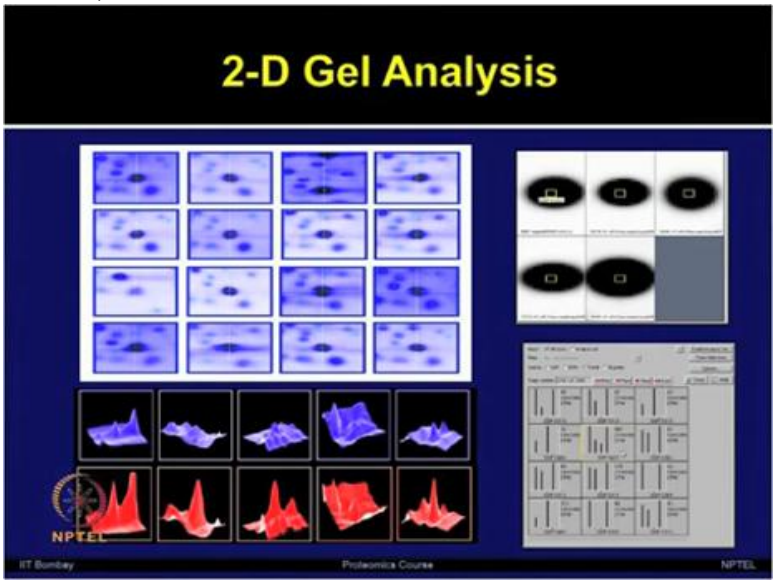

All other analysis can be performed by using different software. As you can see in this image, I am showing you one spot which you would like to compare across 16 gels. Now you have zoomed into that particular region and further you are looking at the 3 dimensional

pattern of each of the spots, how they are different from the control shown in the black and the blue spots and the treatment which is shown on the black background and the red spot.

So after looking at 3 dimensional views of this particular protein you can confidently say that this protein expression is changing and is going higher amount in the treatment. Now, one can look each of these spot intensity in much detail. And then followed by plot, different types of parameters for percentage volume or spot intensity to compare their values and do the statistical comparison.

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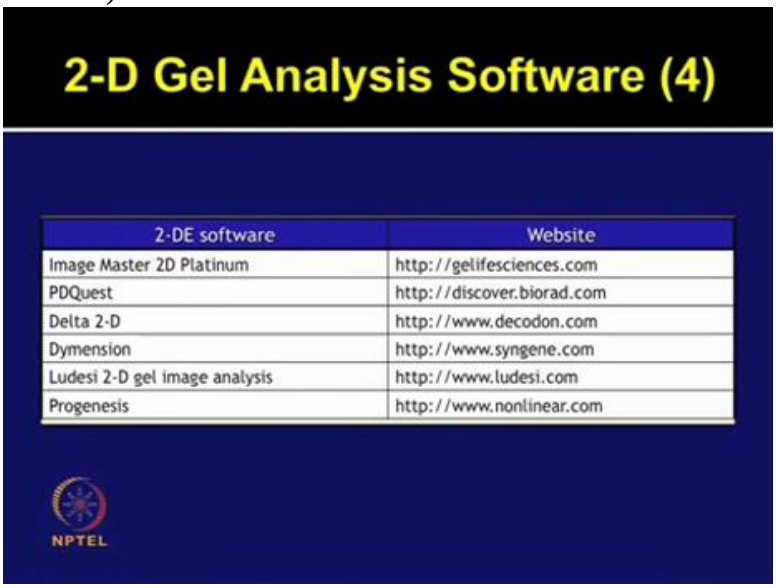

There are various commercial softwares which are available for comparing the 2D gels such as Image Master 2D Platinum from GE Life Sciences, PDQuest from Biorad, Delta2D from Decodon, Dymension from Syngene, Ludesi 2-D gel image analysis software, Progenesis from Nonlinear.

These are just a very few number of software which I have mentioned. These are which are very commonly used but there are many other good software also available which one can use to analyze these gels.

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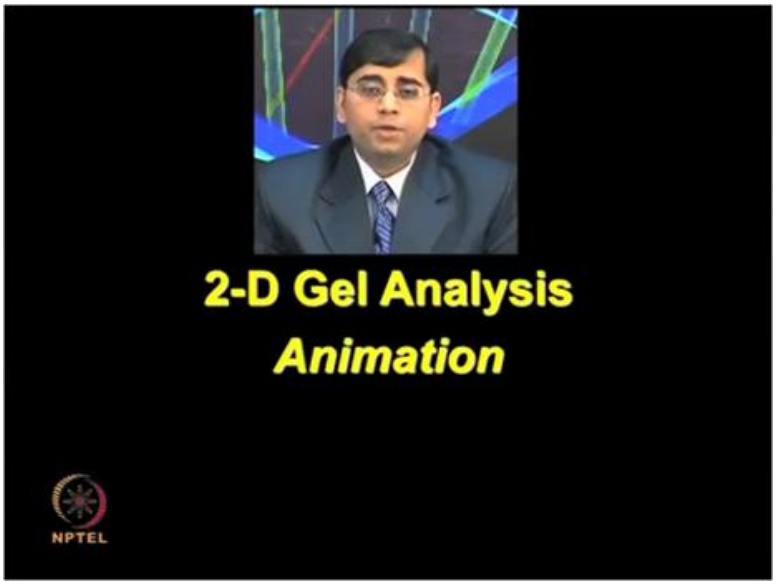

Now let me show you animation for performing two-dimensional gel analysis how to go step-by-step to analyze your gel

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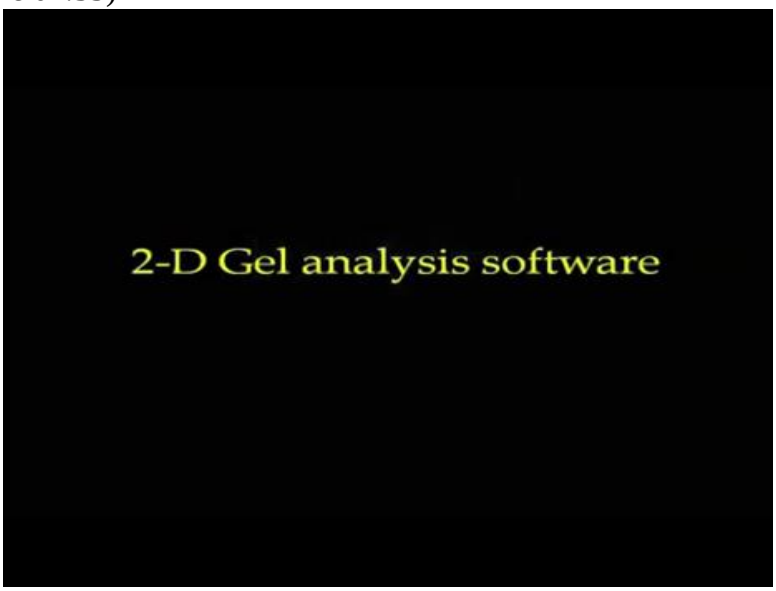

2D gel analysis software, in this animation, I will describe you how to analyze the 2D gel images by using a generic software layout.

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So first, we need to load the gel image. It is possible to load …

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… either a single or multiple gel images simultaneously. This can be done by means of …

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… the load option in the file menu

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You can save the gel images and then you can crop the area depending upon what area you want to analyze. There are several tools which are available for the analysis of gel. It is possible to crop the gel by selecting ….

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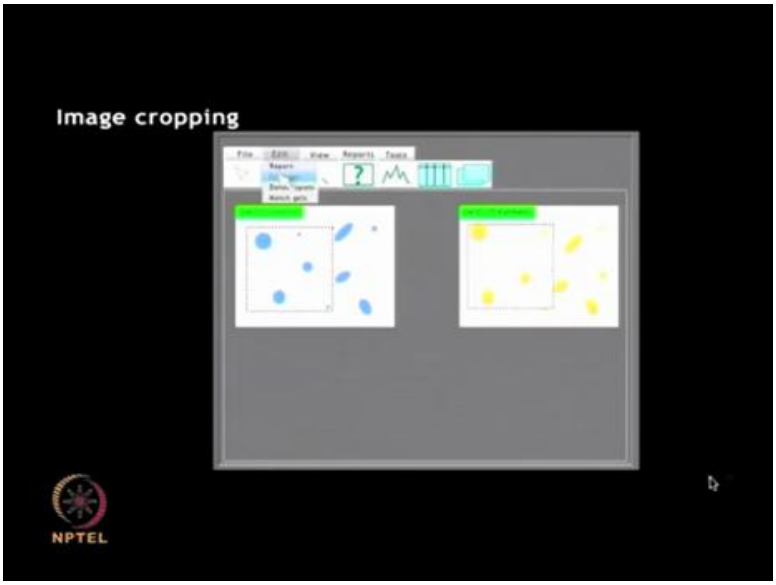

… a specific region …

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… that is to be studied and then…

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… selecting the crop gel function.

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Cropping gel helps in selection of region with high spot density or to reduce the regions which contain high background stains with no spot. Zooming into a selected region… if you want to expand a specific region …

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…you can use zoom tool.

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Specific selected region of the gel can be zoomed into for viewing the spot more closely and for comparison of a spot between two gels. This is particularly useful for gels with large numbers of spots. Once you have seen the area, you would like to overlay the images. Overlaying of images is a particularly useful tool for the comparison of two gels.

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The gels are overlaid such that they appear merged …

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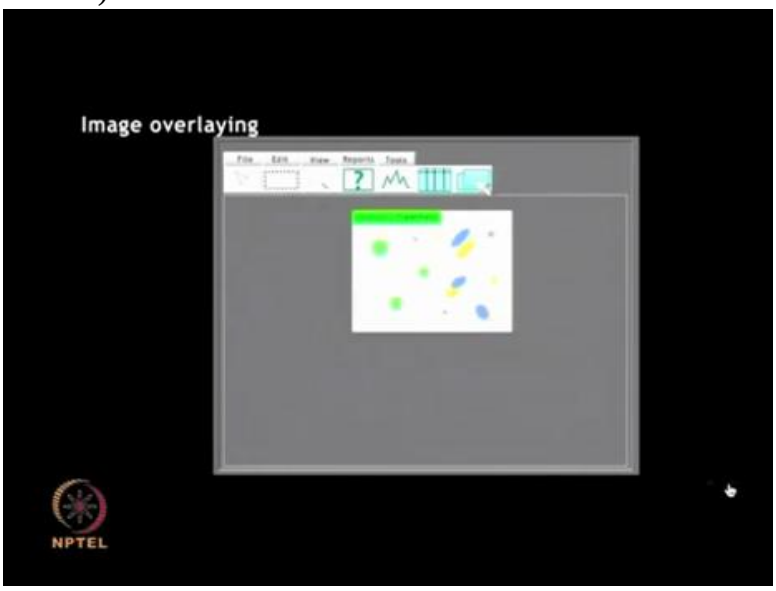

… and spots that coincide will overlap with each other. This is extremely helpful while comparing the large clinical samples of controls and treatments so that you can obtain the clear indication of the proteins which are differentially expressed.

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Now after the analysis, one can look at …

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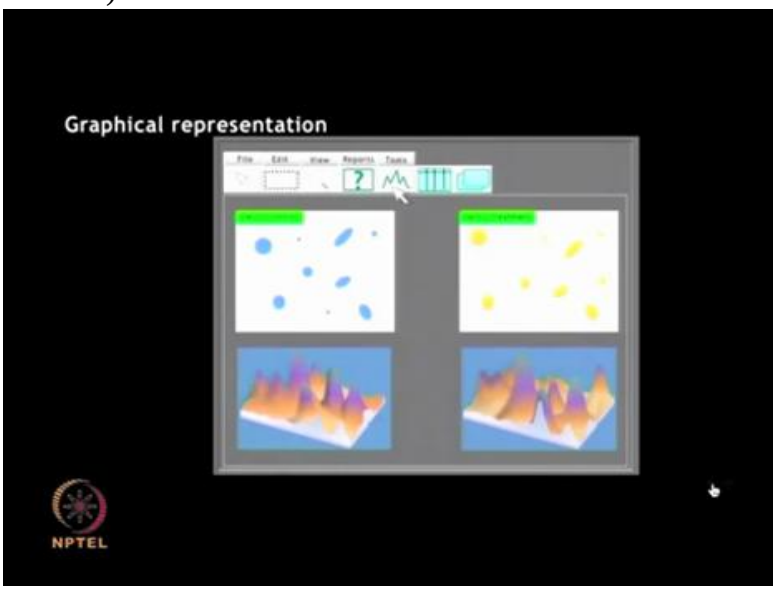

… the graphical representations of these three dimensional view of the spots. The spots on the gels can be displayed as three dimensional graph. Either the entire gel can be chosen or a particular region can be selected for this representation. The peak obtained in the graphical representation is directly related to the spot intensity. Next we talk about spot analysis.

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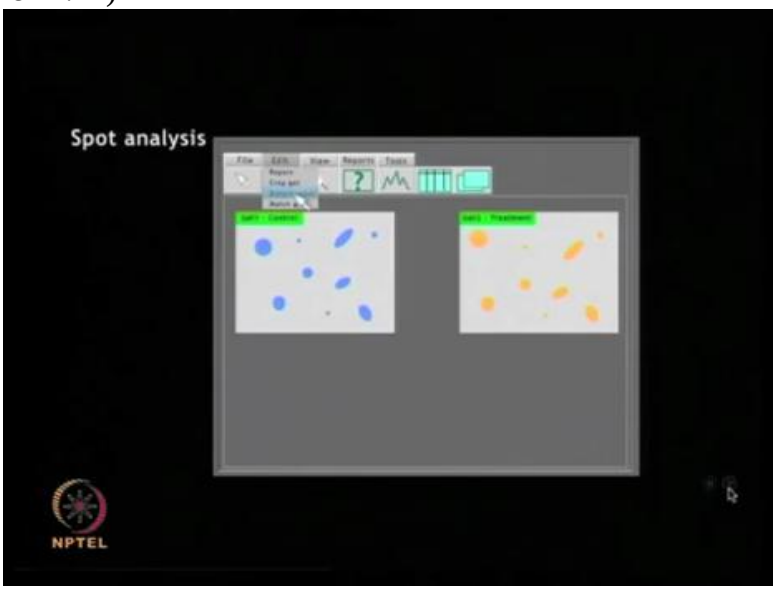

Every spot on the gel can be detected by selecting the Detect Spot option.

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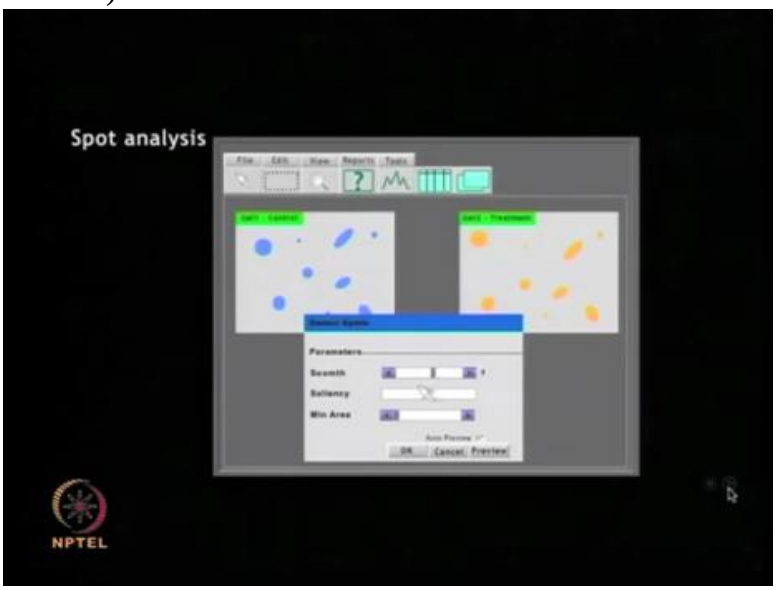

Various parameters such as smoothness, saliency and minimum area must be suitably adjusted for maximum clarity. Once this is done, each spot will either be encircled with a mark or a cross, depends upon the setting along with the mark numbers.

Now I will describe you the Gel Matching. The software facilitates the interpretation of the gel images by matching two different gel images which are obtained in your experiment

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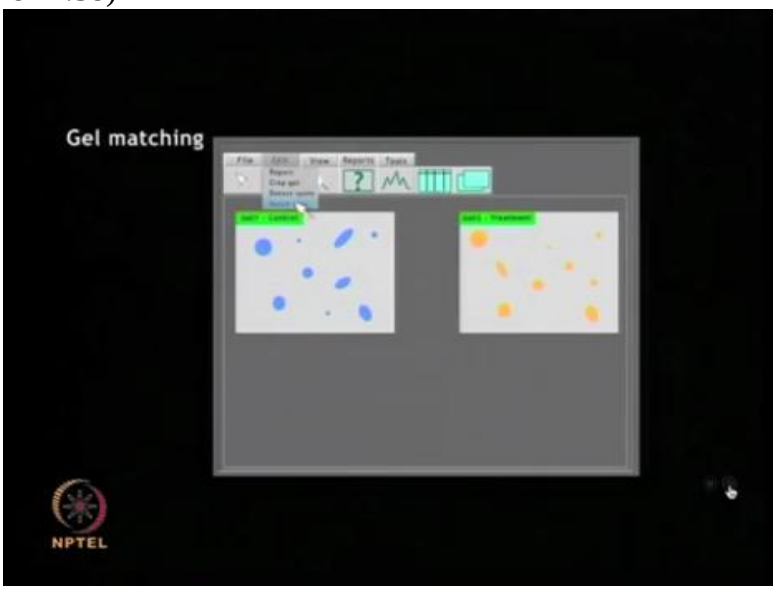

The matching spots are marked …

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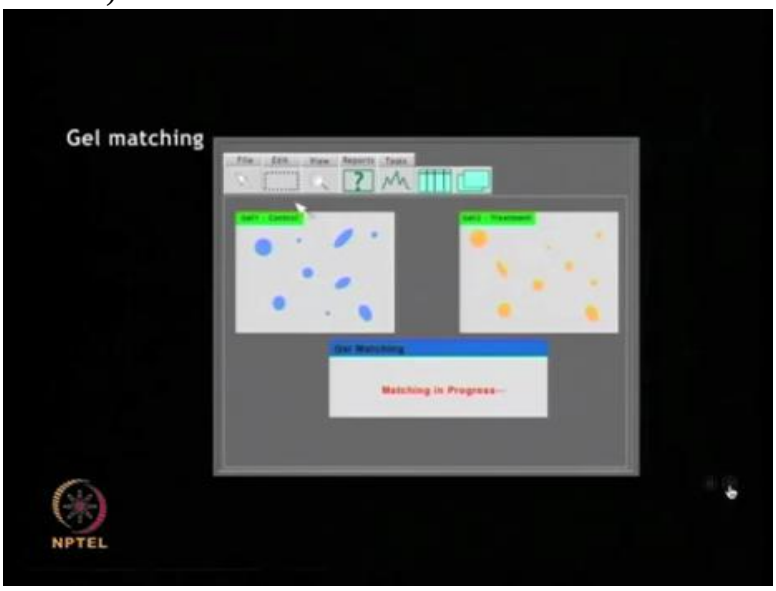

…and after matching is done

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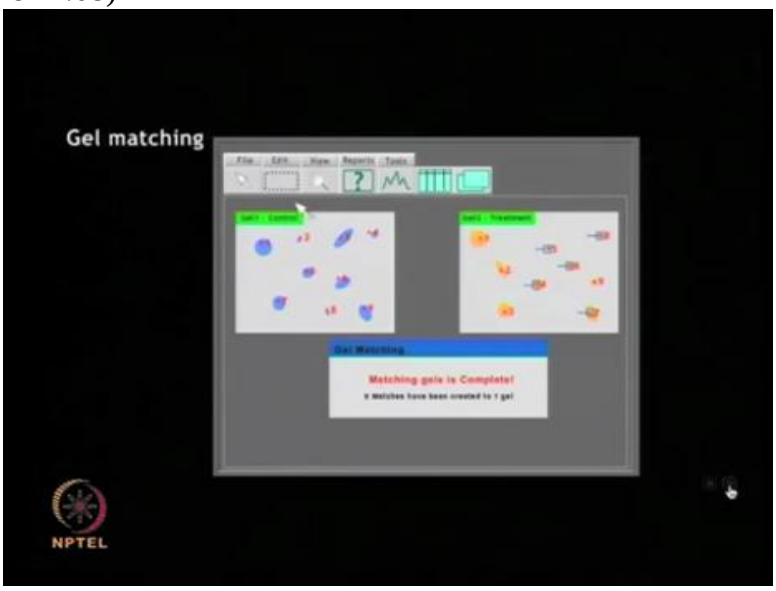

… any variation is spot intensity; spot positions can be indicated by the blue lines as shown in the animation. This provides an understanding about the reproducibility across the gels.

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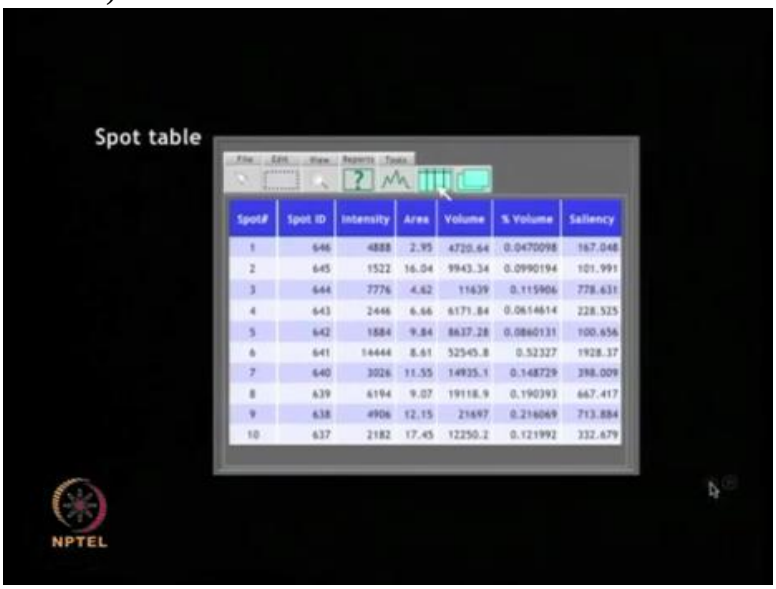

Once you have analyzed the gel, you can obtain the detailed information for the spots from the spot table. Information regarding various physical parameters of each spot can be obtained by this spot table which provides spot number, intensity, area and volume of the spot as well as the saliency of the spot. These parameters help to judge the quality of the gel.

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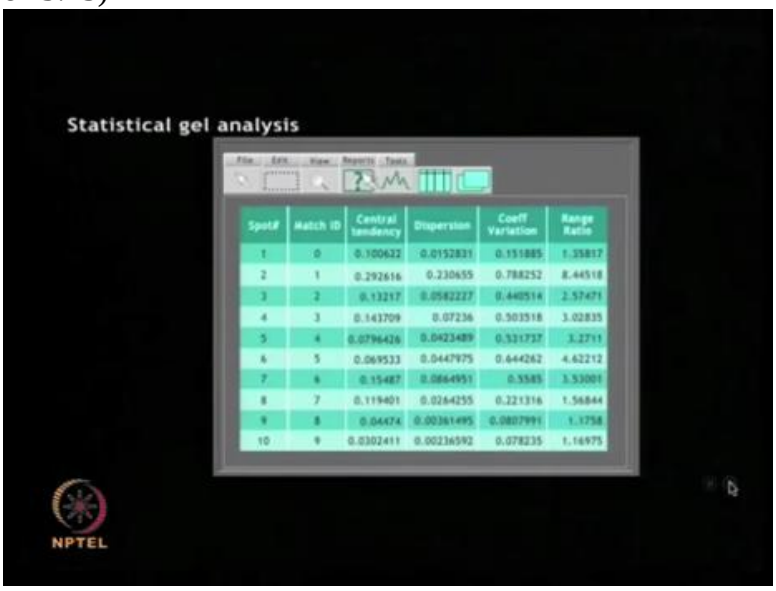

In addition to the physical parameters various statistical parameters can also be computed for each gel …

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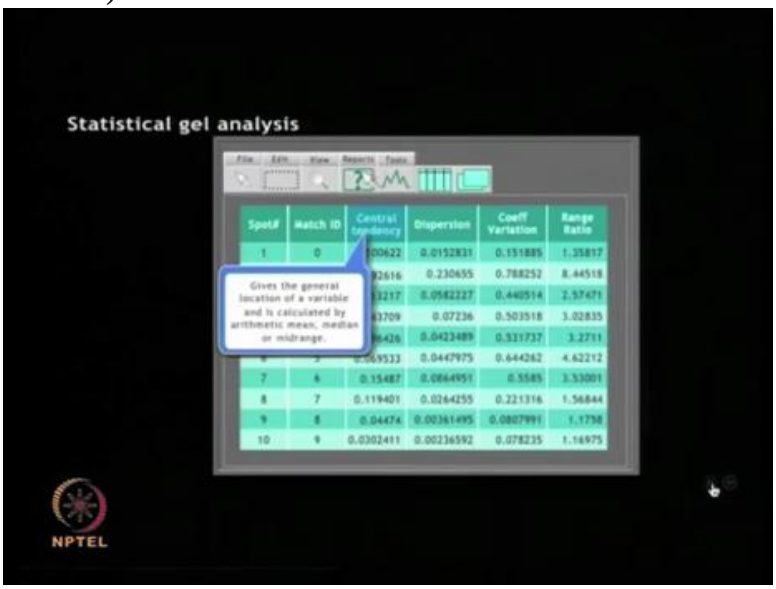

…and each spot on the gel such as central tendency, mean, median…

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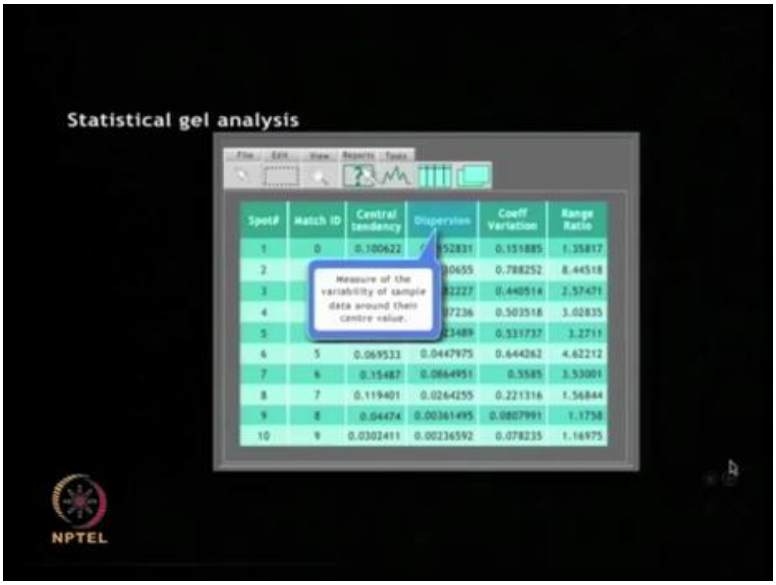

… dispersion, coefficient of variation, standard deviation …

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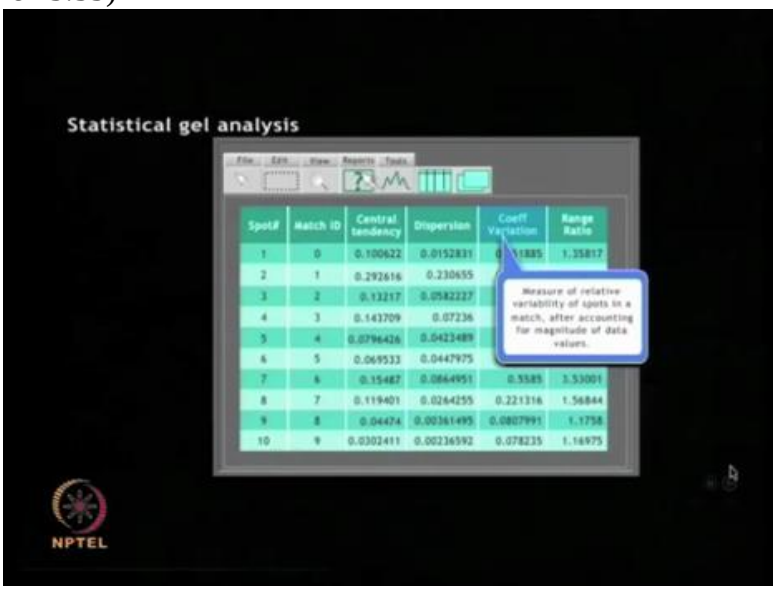

…or other statistical parameters.

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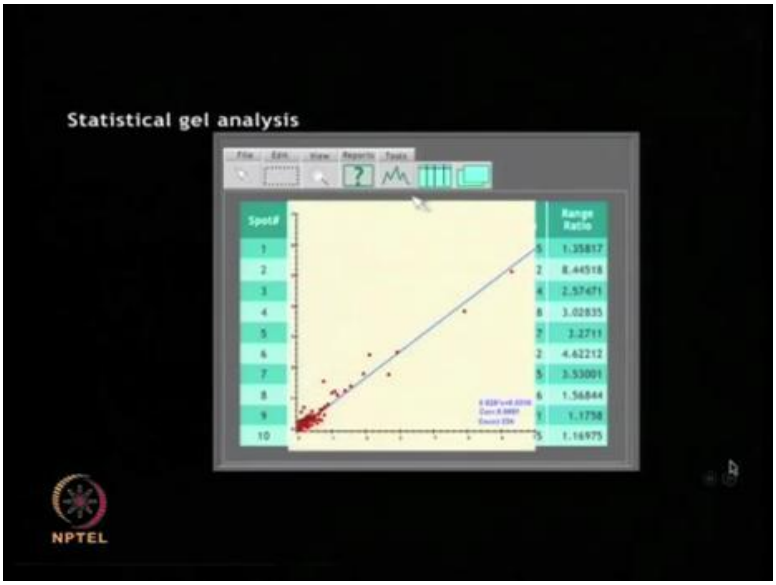

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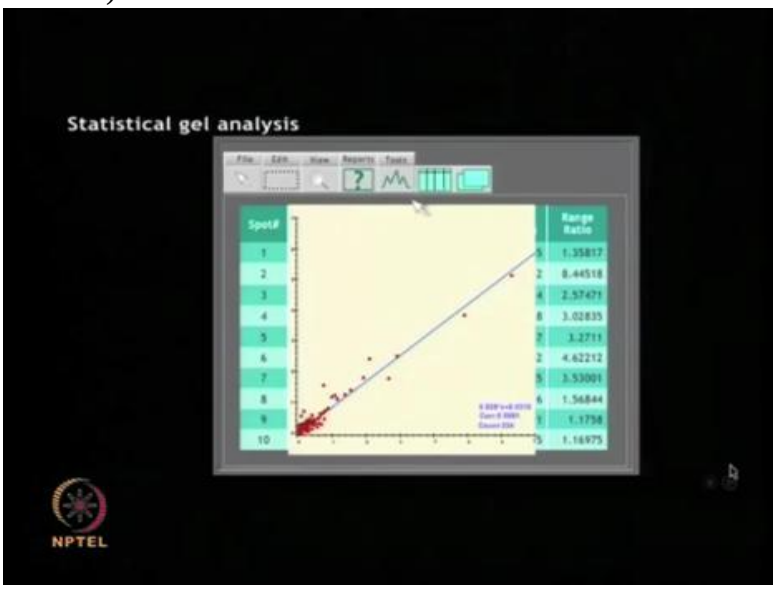

The scatter plots and histograms can also be plotted for clear data analysis. These can provide information regarding inter and intra gel variations

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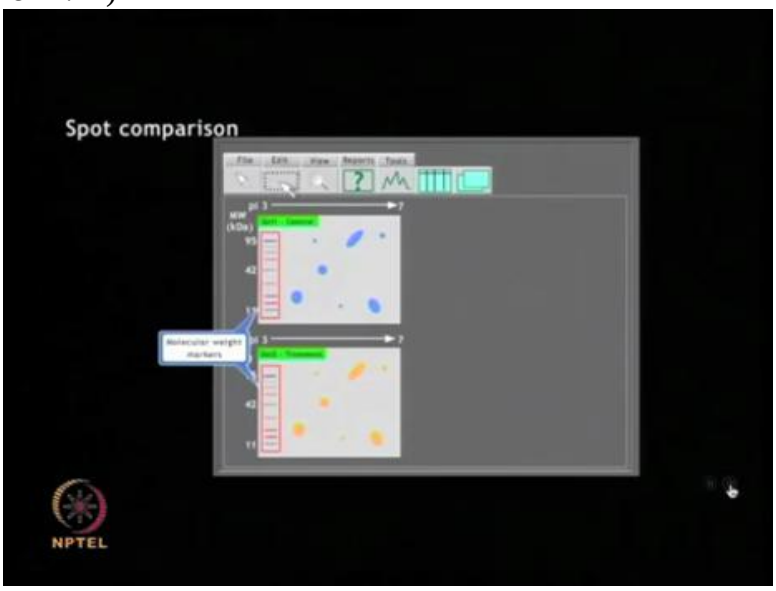

Spot Comparison, it is possible to specifically compare a particularly selected spot across the gel.

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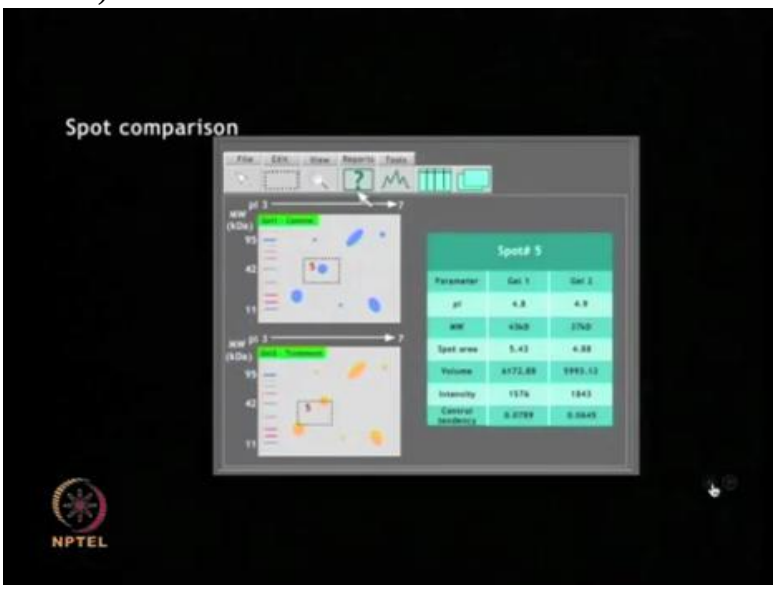

When the gel is run with molecular weight markers with molecular weights of unknown proteins can be estimated from this information.

For example, as you see in this animation, on the left hand side, you first loaded the molecular weight marker and from that information, you can compute the information of the unknown protein to calculate its molecular weight and isoelectric point.

These parameters in addition to other physical and statistical parameters can be obtained for each spot.

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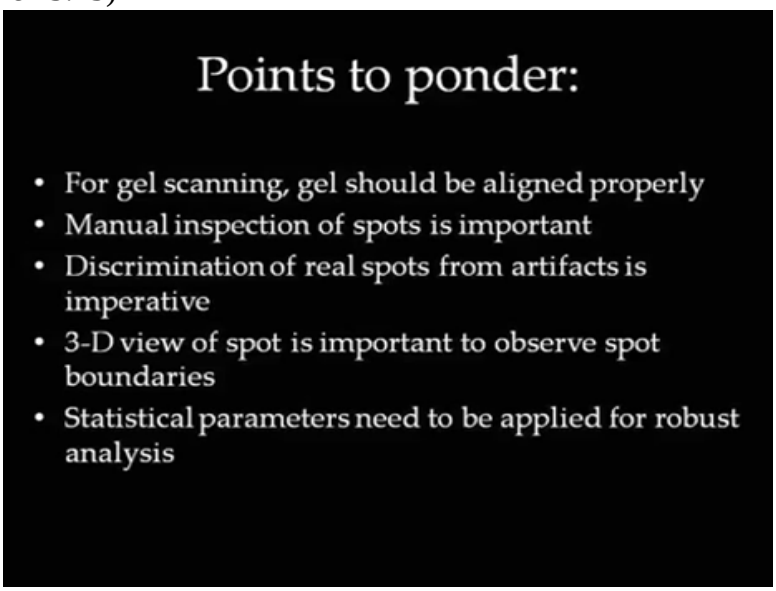

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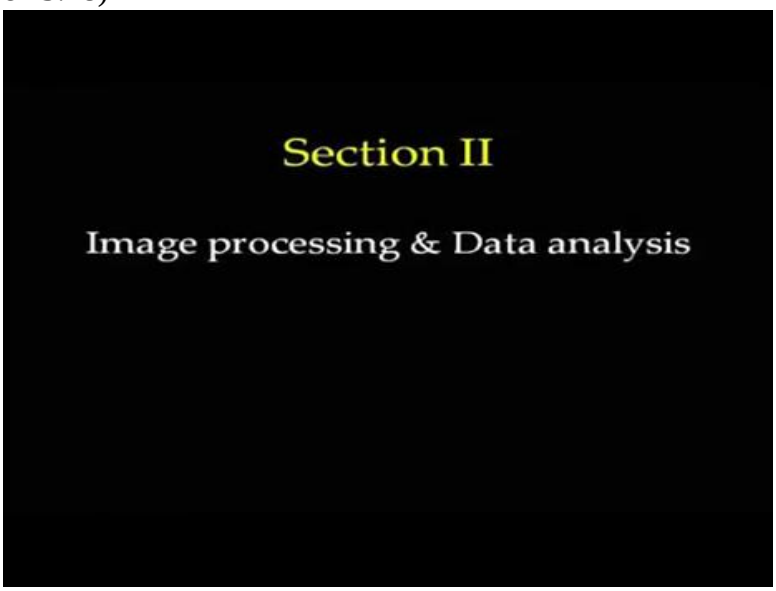

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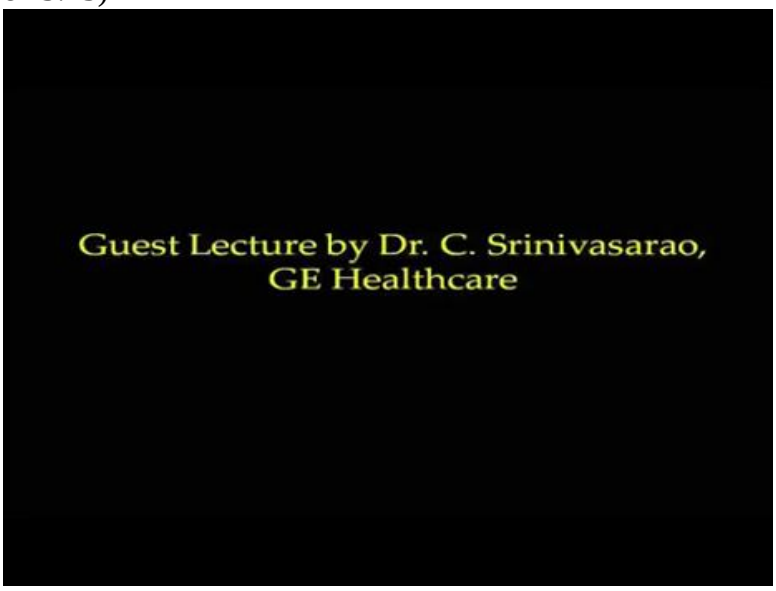

Today I have invited a guest here to discuss the image processing and data analysis by using commercial software. So today we will have Doctor Srinivas from GE Healthcare who will discuss how to analyze gel by using Image Platinum software.

Professor- Expert conversation starts

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Professor: So welcome Doctor Srinivas and we would like to initiate discussion with you on image processing.

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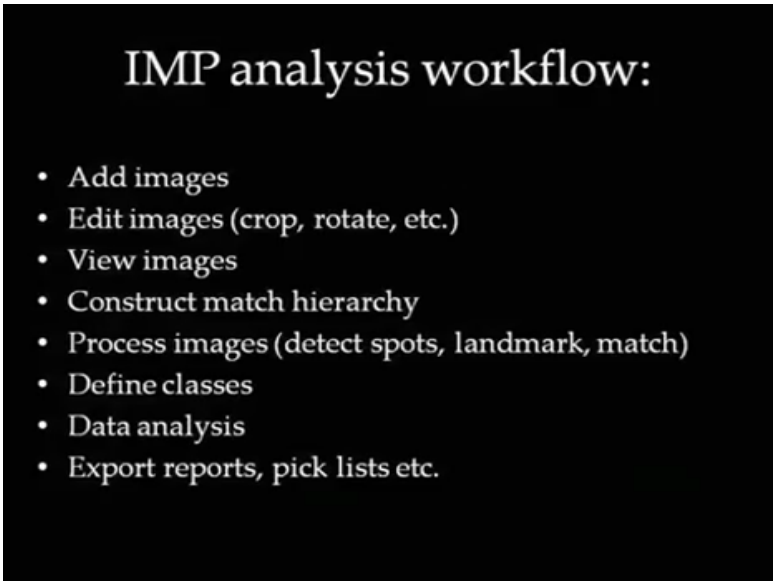

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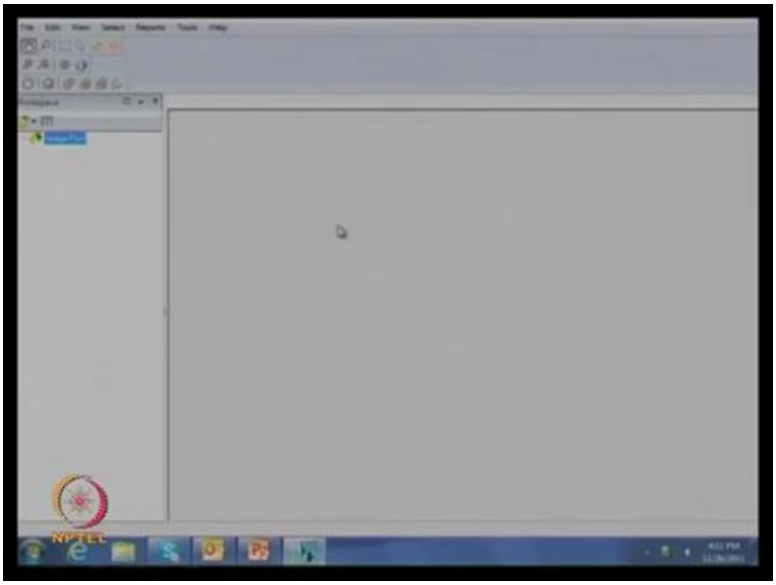

Expert: Yeah, this is the software layout where you can see in your PPTs; this is the original software layout.

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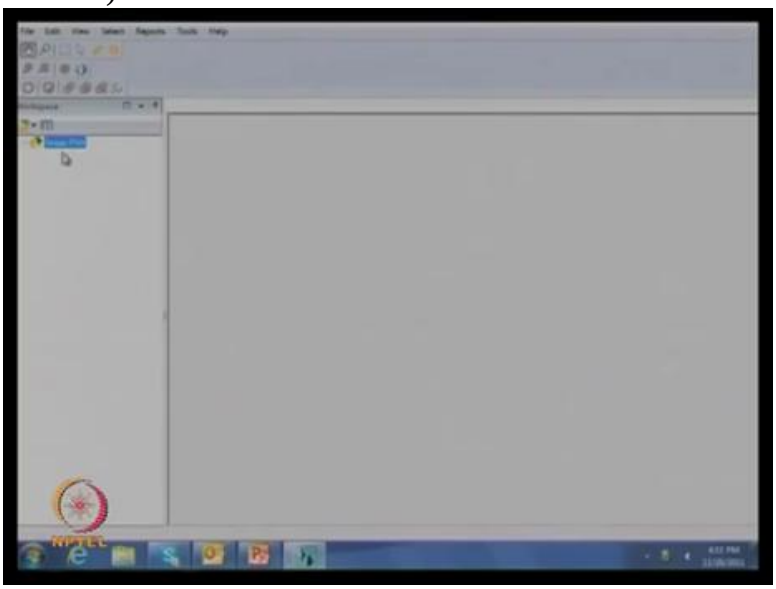

Expert: Now here there is option like Image Loader. Add it,

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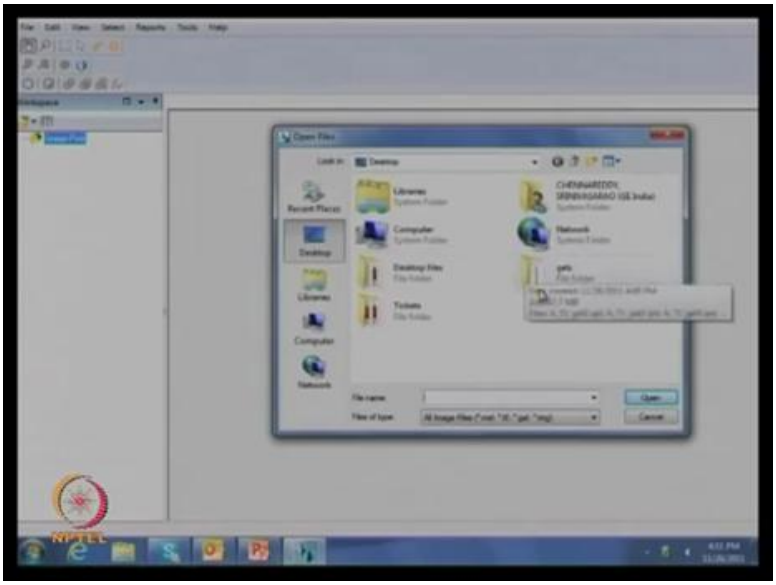

Expert: … wherever your gels stored, you can go to that place and add the gels. Like as I have made gels here,

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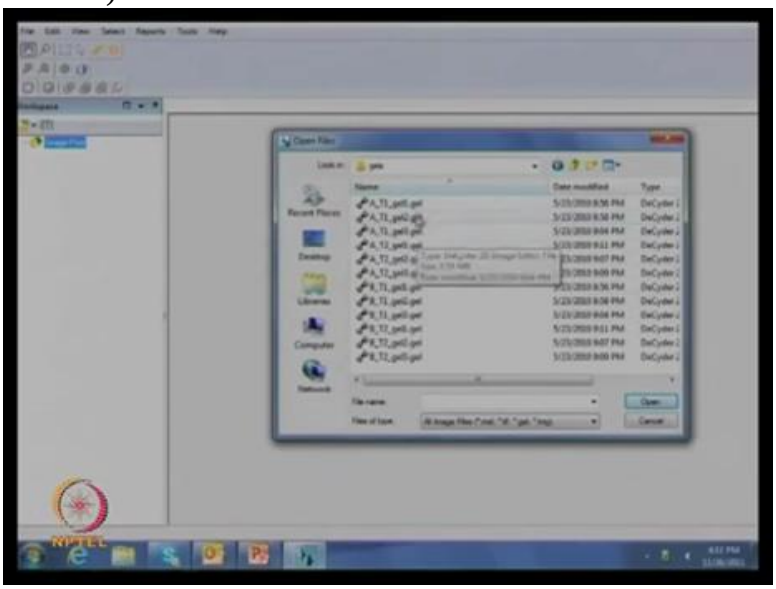

Expert: I am going to add few gels through our software to analyze. Gel 1, Gel 2, Gel 3; Gel 1, Gel 2, Gel 3.

Professor: So you are processing 6 gels at a time.

Expert: 6 gels at a time. These are all the 6 gels.

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Expert: Basically 3 of 2 replications like 3 replications for each gel.

Professor: Right

Expert: That means one is the control and another is the treated, in that way.

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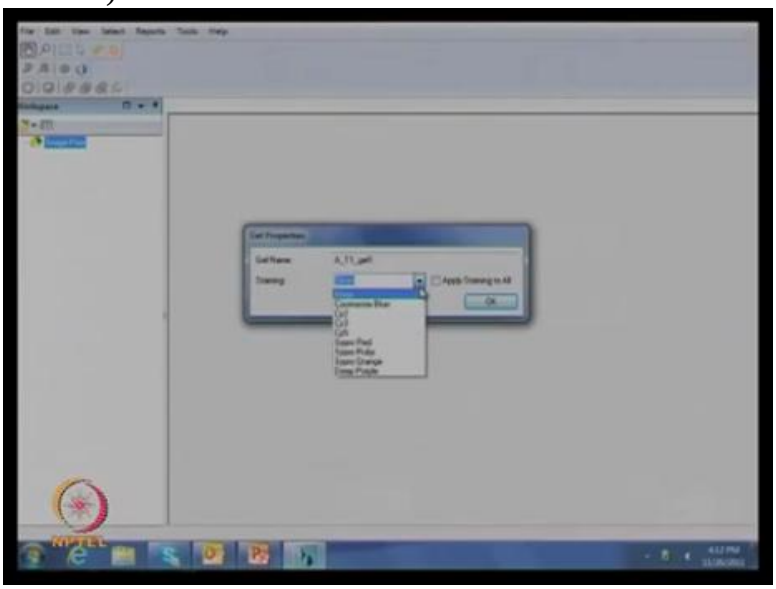
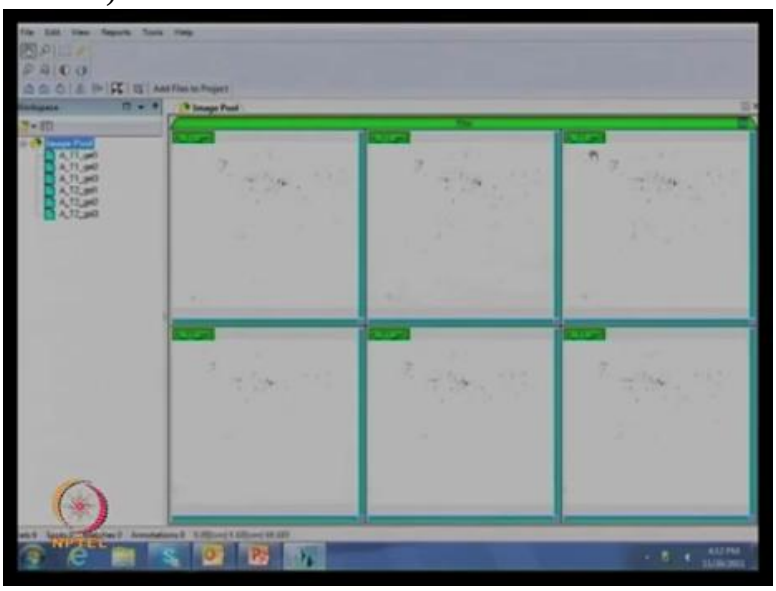


Expert: Now as we discussed in our PPTs, this is the thing that is asking for particular staining.

Expert: Here we can give Coomassie or silver or different fluorescent. So, as this stained with silver, we are giving the silver and applying the same color for remaining the gels Now click on Ok.

(Refer Slide Time 17:15)



Expert: Now as you can see the 6 gels at a time in this overview after that. Now you can't see the proper spots in all images. Completely very bright images which you can see, so now we need to edit the images for visualization

Professor: We need to change the contrast...

Expert: Contrast and brightness...

Professor: Different...

Expert: Different features.

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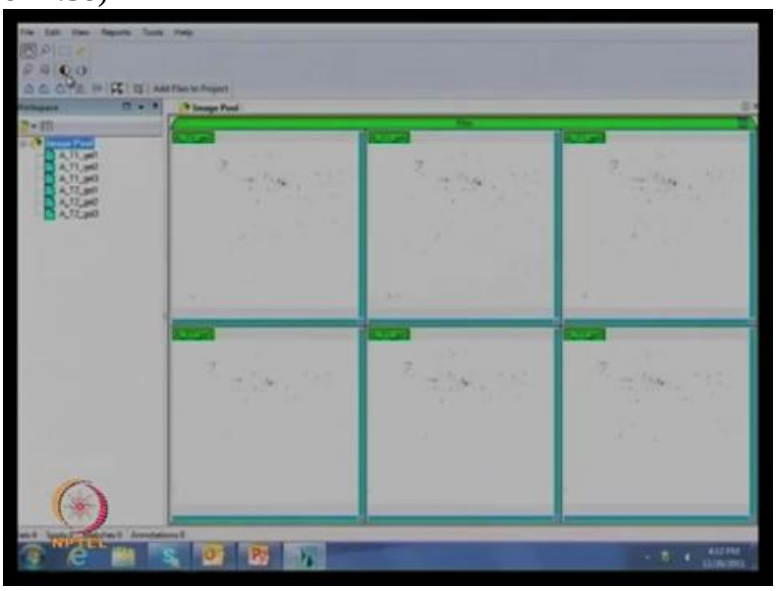

Expert: So this the button where you can adjust contrast and brightness. So as I can show here....

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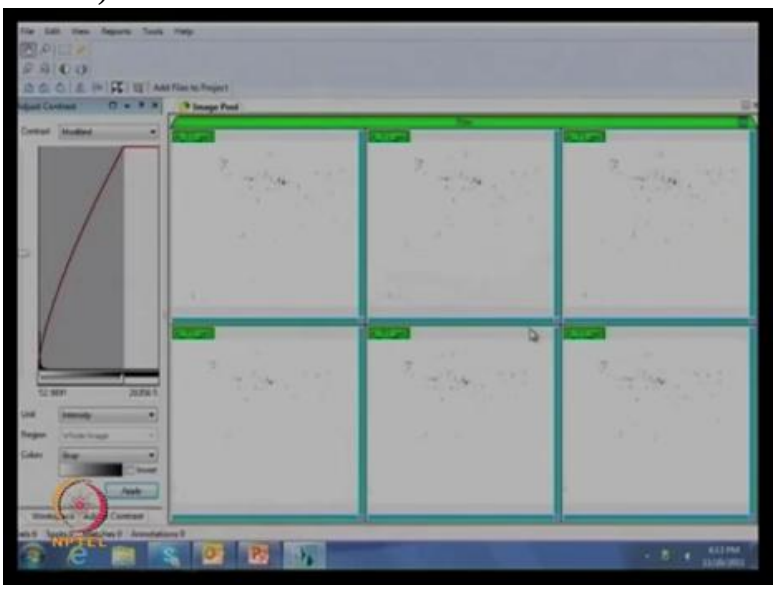

Expert: now we can't see any differences now after changing also but one can have to

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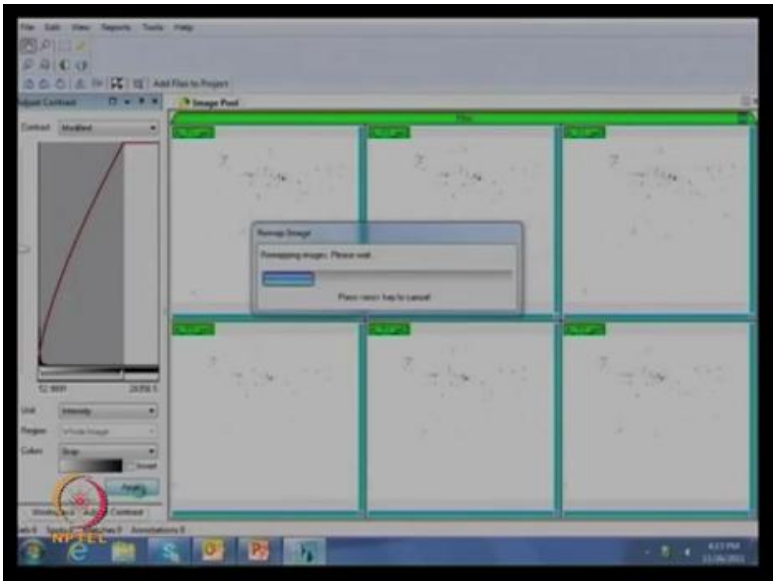
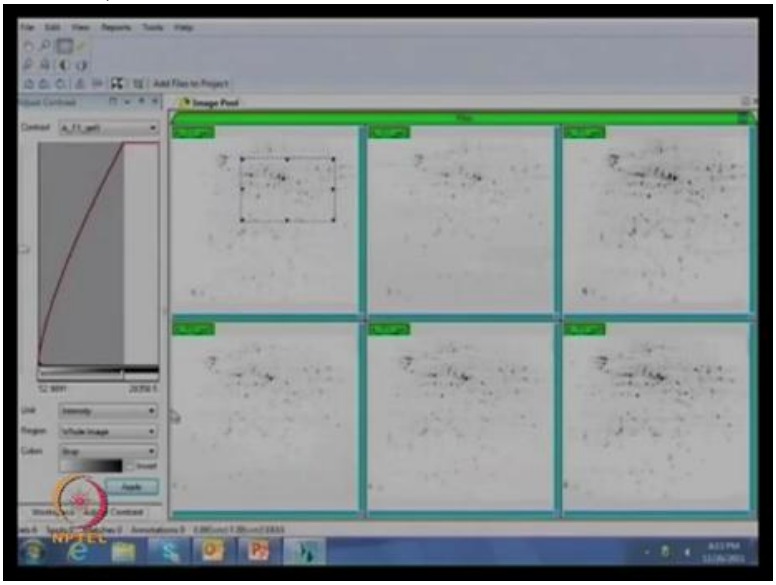


Expert: press on Apply. Another feature is available here...

Professor: So more subtle...

Expert: And more spots are; yes more spots; otherwise there is an option.

(Refer Slide Time 18:09)



Expert: It can select a particular area, Ok

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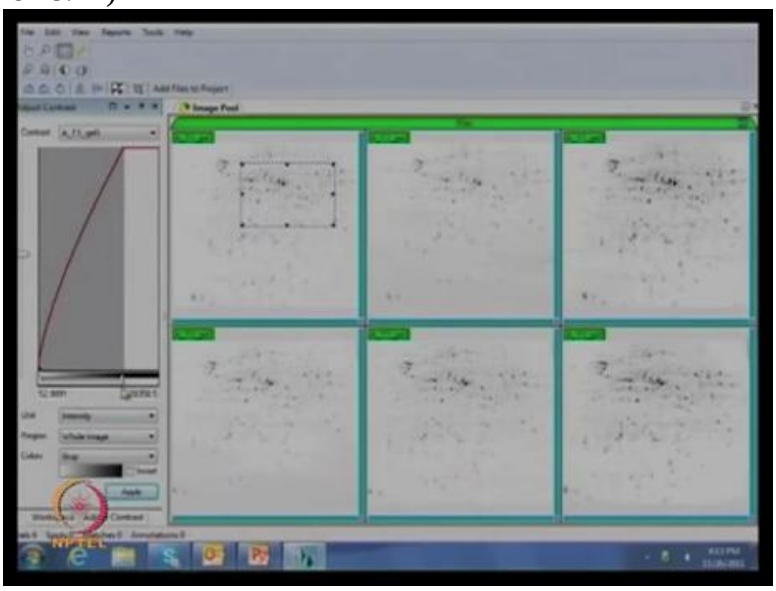
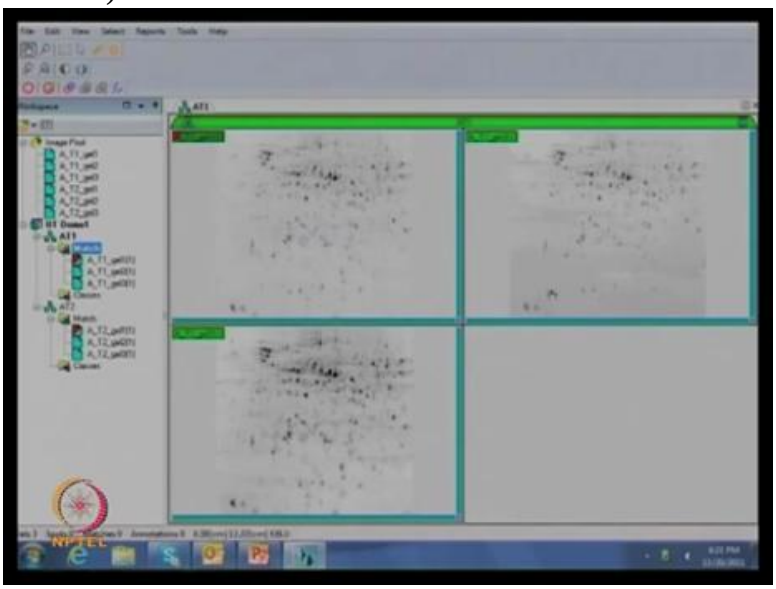


Expert: and then change the parameters now. Now we can see real-time changes in that particular spots. Now display them

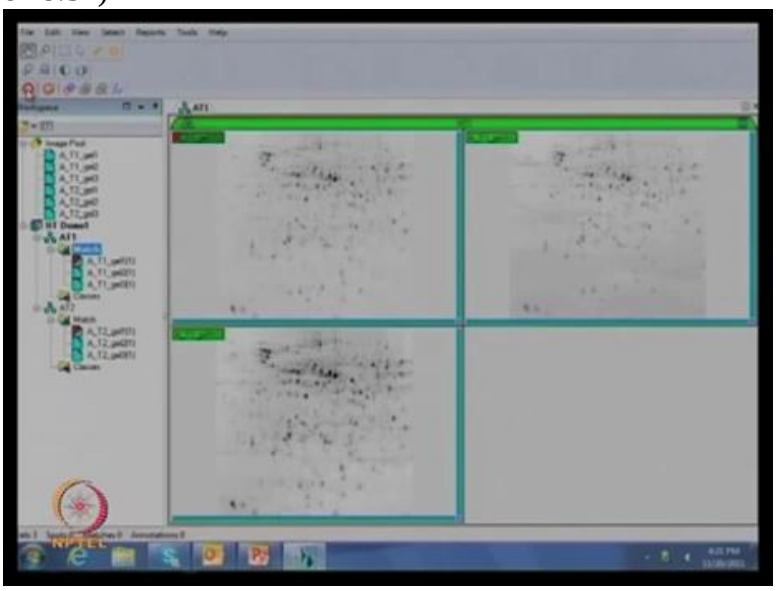
(Refer Slide Time 18:22)



.Expert: Now you have all of your gels here, Ok. Once we have the gels we can directly detect the spots now.

Professor: Ok

(Refer Slide Time 18:31)



Expert: From this button…

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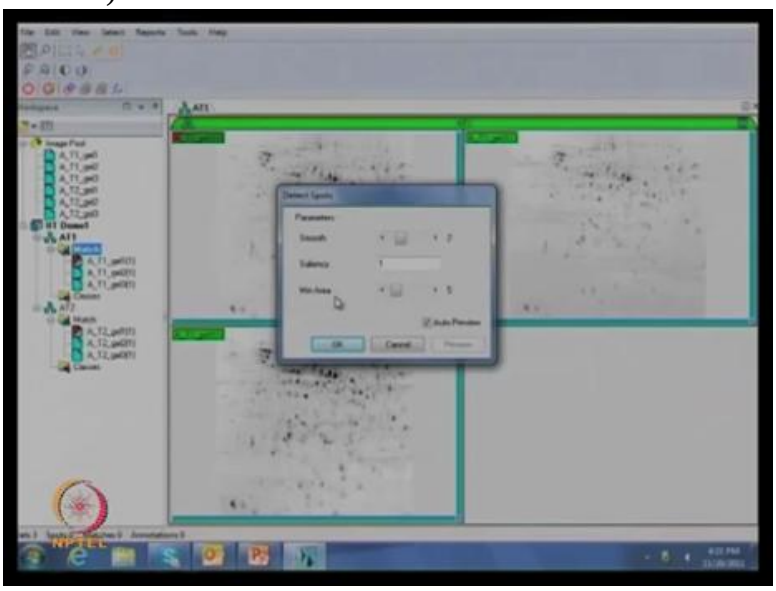

Expert: …you can detect. Now 3 parameters; smoothness, saliency and minimum area; the smoothness as we can see

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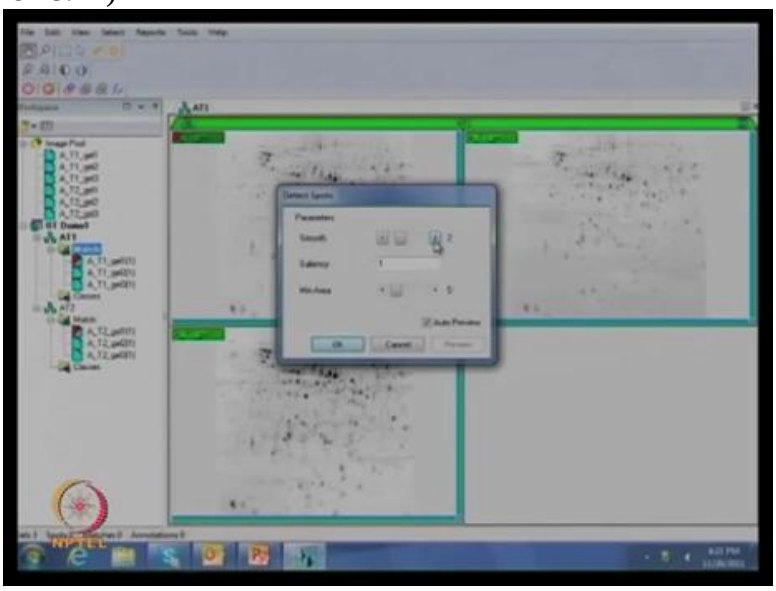
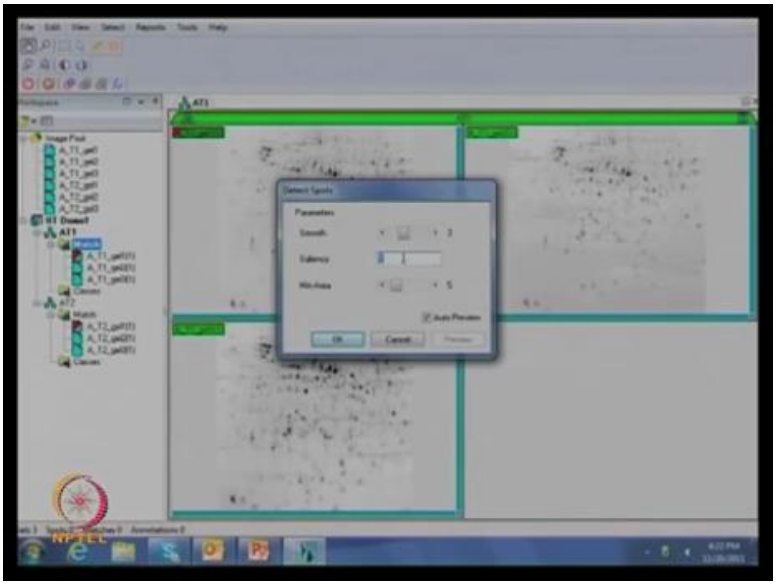


Expert: if we are increasing the smoothness, it is going to over...it is going to under splitting and if you are decreasing the smoothness, it is going to over split. So it is inversely proportional from this splitting. So its average value from 1 to 5 actually so but one can set as 2 to 3, it is...this value is sufficient for...

Professor: To start.

Expert: Start actually,

(Refer Slide Time 19:13)

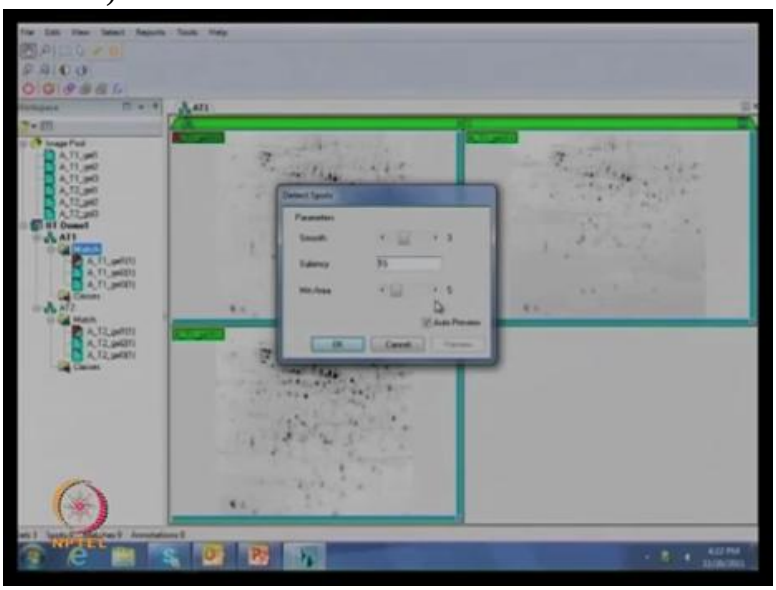


Expert: and saliency, again this is also again...one can set up to 100, 150 but to start, very good value is almost from 15 to 30.

Professor: Ok

Expert: Ok, I am just giving 15 for this initially,

(Refer Slide Time 19:25)



Expert: then minimum area 5 is quite good enough. If you think in your spots there are more speckles, there are much...

Professor: Artifacts...

Expert: Much artifacts are there, you may increase this also. In that case it can very easily remove artifact. So in this...otherwise 5 is Ok, fine value.

Professor: So all the 3 parameters now, one can actually accept those

Expert: Exactly

Professor: And then detect the spot

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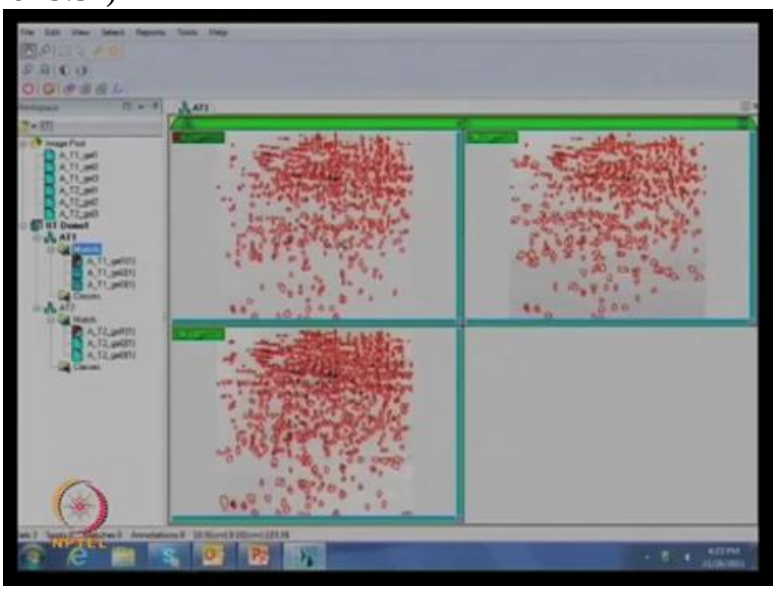

Expert: Exactly. That is, now the software is detecting the spots. Now it has detected all spots in this gel. If you are satisfied with this particular detection, then it is fine, otherwise...

Professor: May be you should zoom in regenerating like…

Expert: Exactly, I am going to do the same,

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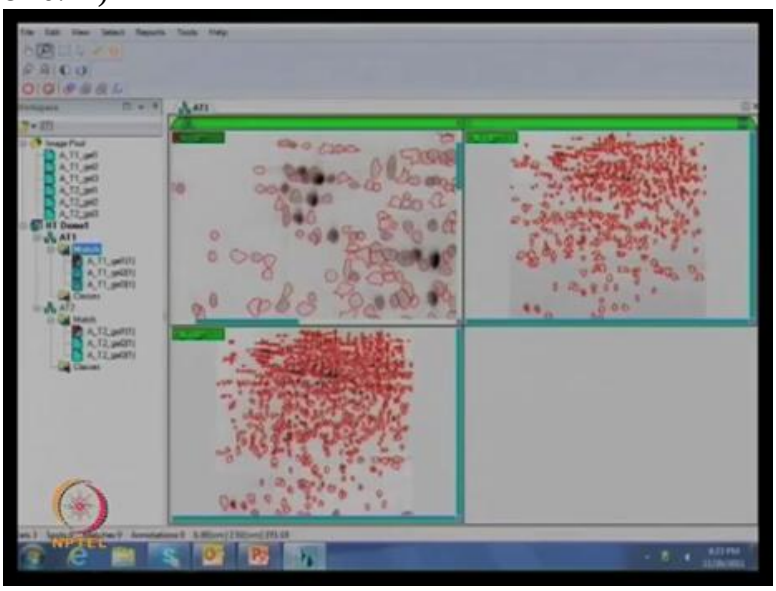

Expert: …and now we can zoom this particular region

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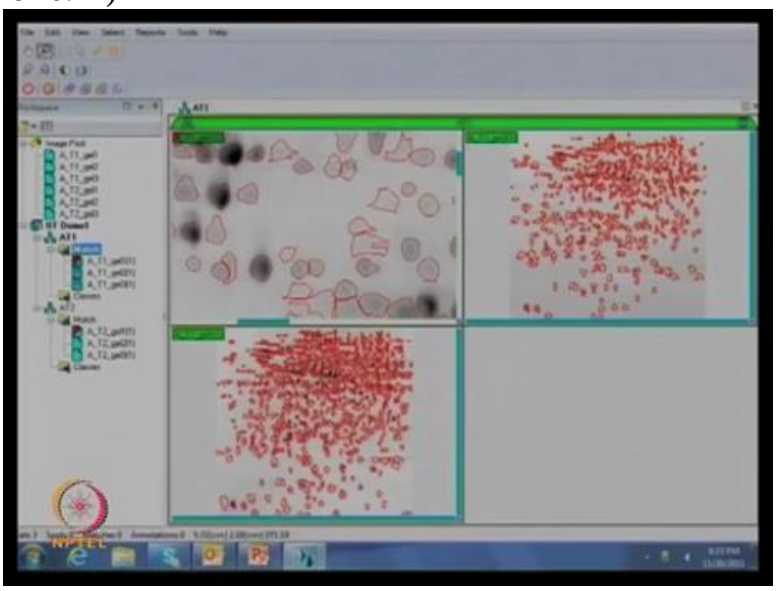

Expert: see how these spots are boundary as well as how the detection is there...

Professor: Can you apply the same zoom in parameter for all the regions?

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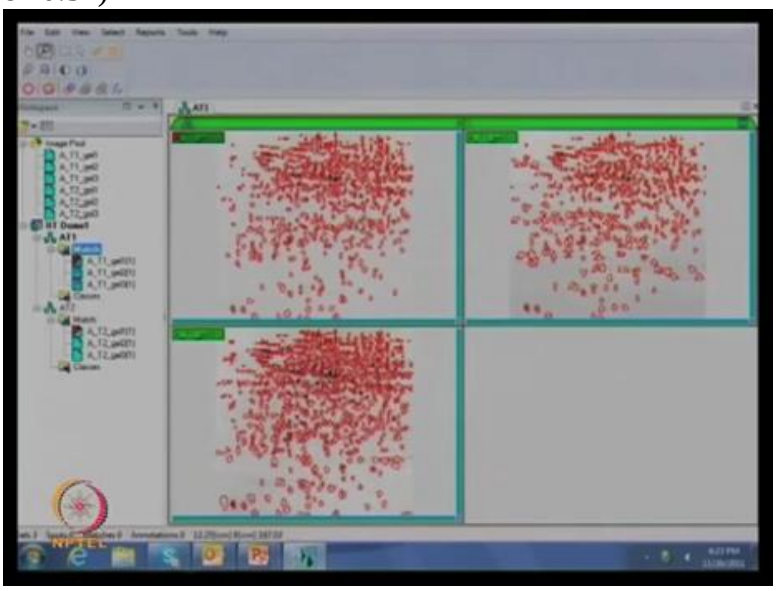
Expert: Yes definitely. First come back to original

(Refer Slide Time 20:34)



Expert: … and again

Professor: Let’s select one region and then apply that for all....

Expert: No, you just select like...

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Expert: the same region…

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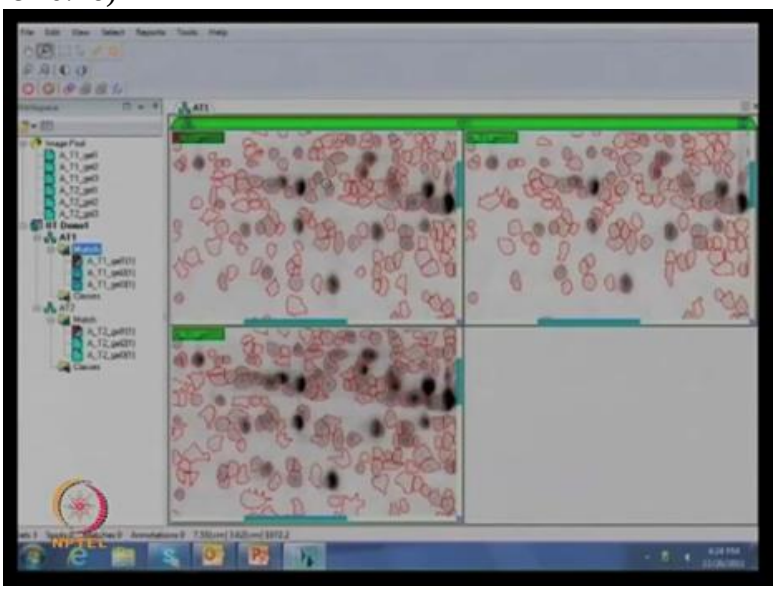

Expert: it is going to...

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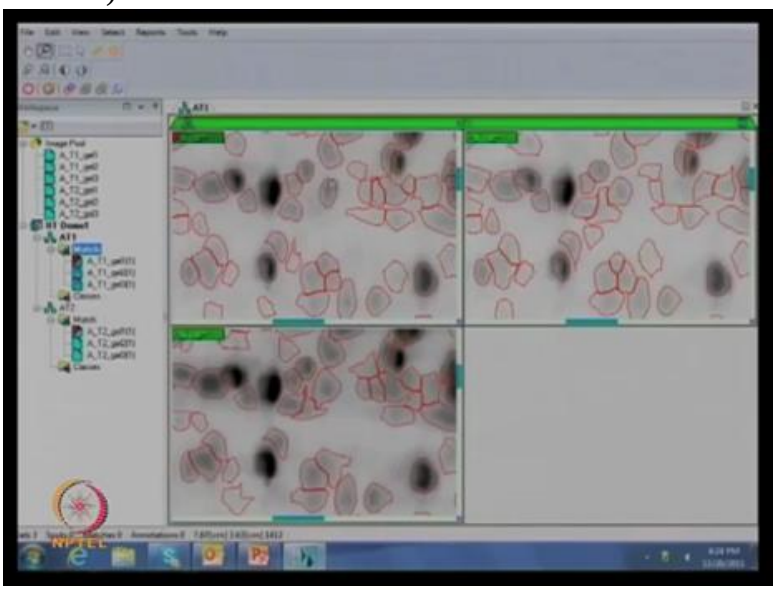


Expert: got it?

Professor: Right, I think, it is very useful to see this way.....

Expert: Exactly

Professor: Because now I think the parameters are now well-defined

Expert: Exactly

Professor: Because it has properly defined the boundaries,

Expert: Properly define the boundaries.

Professor: And let’s see spots where one can actually, no, tweak around.

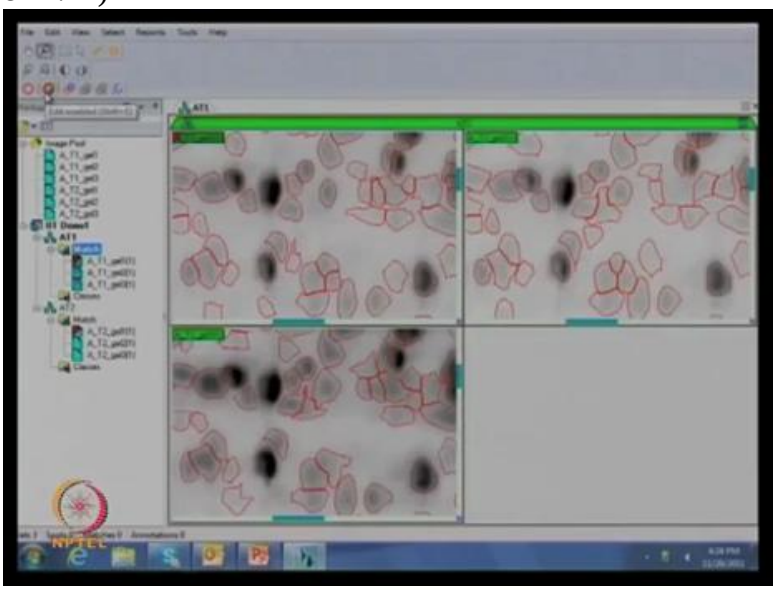
Expert: Exactly, if you want to edit this particular spot...

Professor: Right

Expert: Whereas, there you cannot see there is a protein but it is showing as a protein. One can delete such kinds of spots also.

Professor: Right

(Refer Slide Time 21:21)



Expert: One should have to go to edit the spots. There is an option like edit Enable.

Professor: Right

Expert: Go there and yes, delete this spot, wherever the spot you would like to delete.

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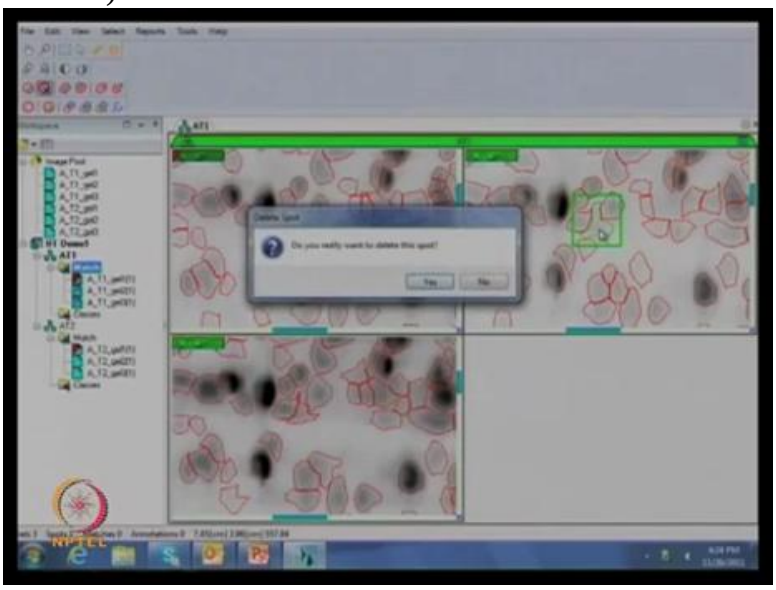


Expert: This particular spot I am not interested in this then I can delete it.

Professor: But before we take the decision, can we just have a look on the 3D profile...

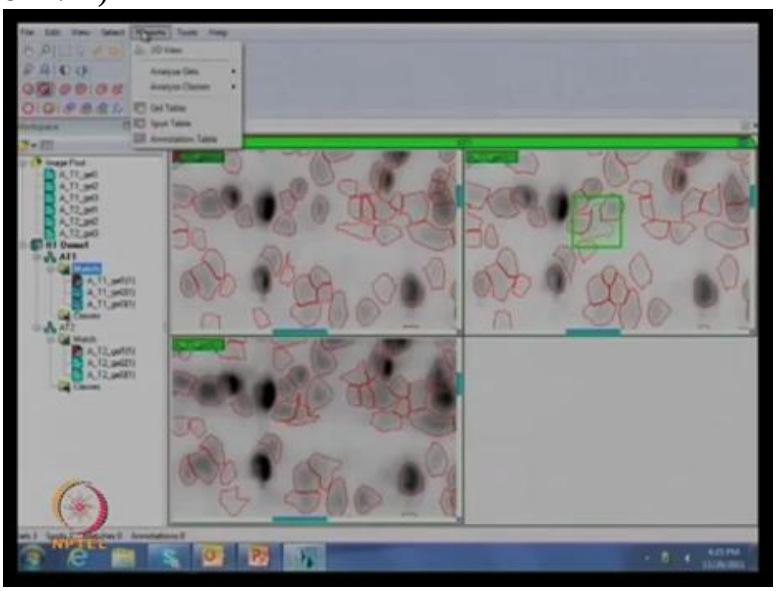
Expert: Yes, definitely

Professor: To ensure that we are not actually deleting...

Expert: Yes, definitely

Professor: The real spot

(Refer Slide Time 21:44)



Expert: Definitely, definitely. Reports...3D View

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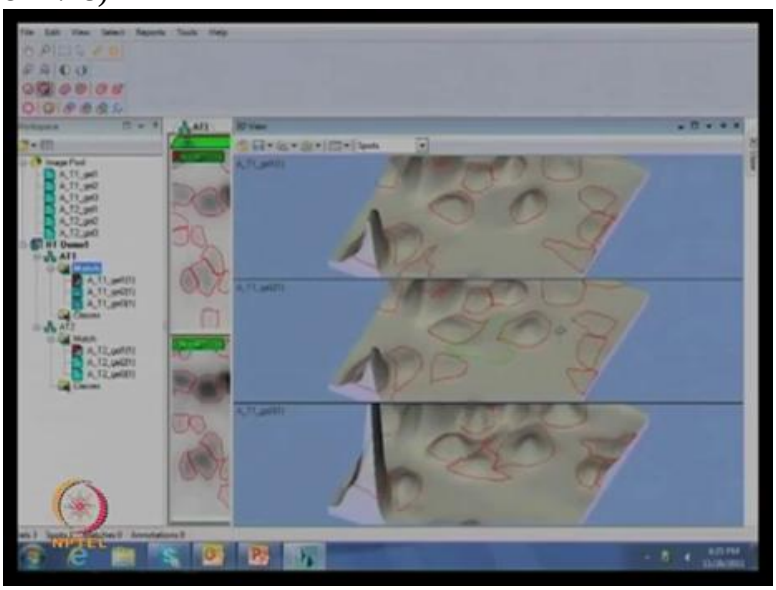

Professor: So the spot which you are interested in discussing, that is highlighted in green boundary...

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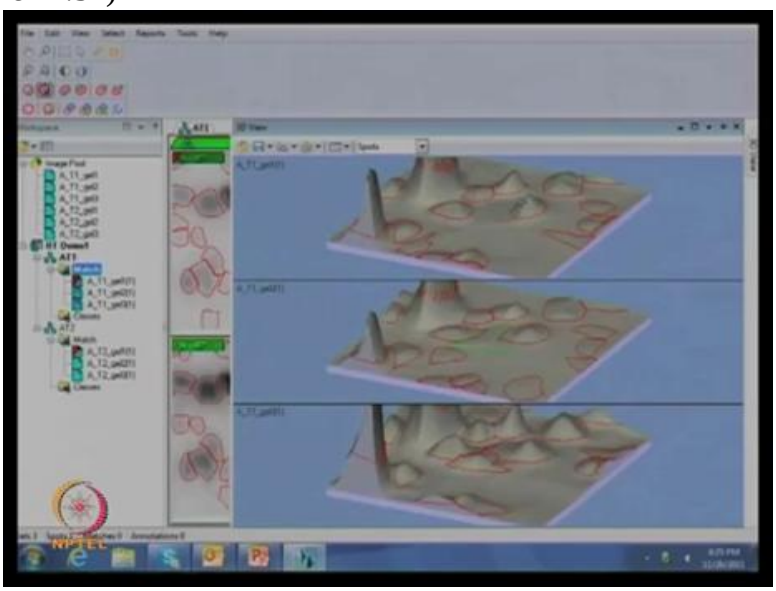

Expert: Yeah, green boundary. It is only present in this particular spot but it is not there...But we can see some portion here, that particular spot; that is why there also

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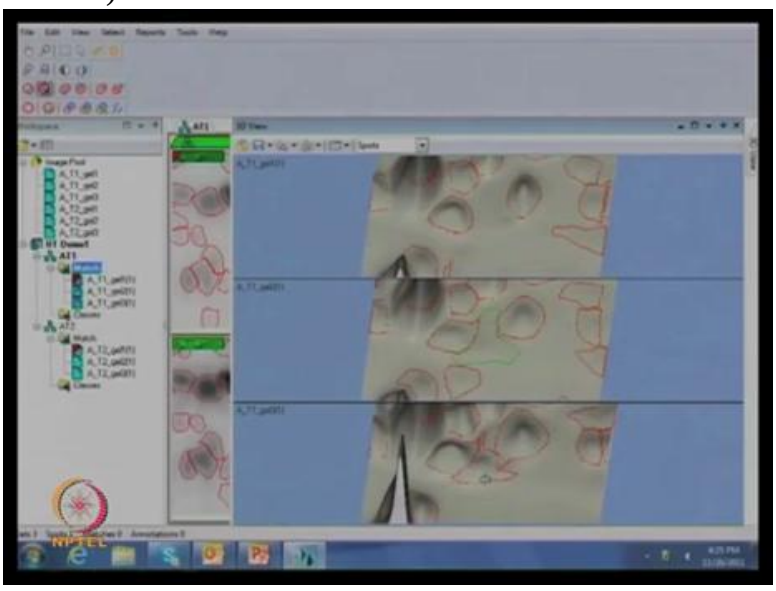


Professor: Right. I think sometimes it is possible like....

Expert: Yeah

Professor: Your treatment… has that spot appearing...

Expert: Yeah

Professor: Due to the application of the protein.....

Expert: Yeah, exactly.

Professor: Or it is totally shut down...

Expert: But it is not the case exactly here because these are all the 3 replications of the same protein

Professor: OK, this should the same

Expert: This should be the same, but somewhat is not there so we can delete these two spots, exactly

Professor: Or in this case I guess it is doubtful, right, so....?

Expert: Yeah, it is doubtful or let it be, then finally we can see the statistical data where it is there in both gels and as well as treatment also

Professor: Right

Expert: So that one can go with the statistical parameters.

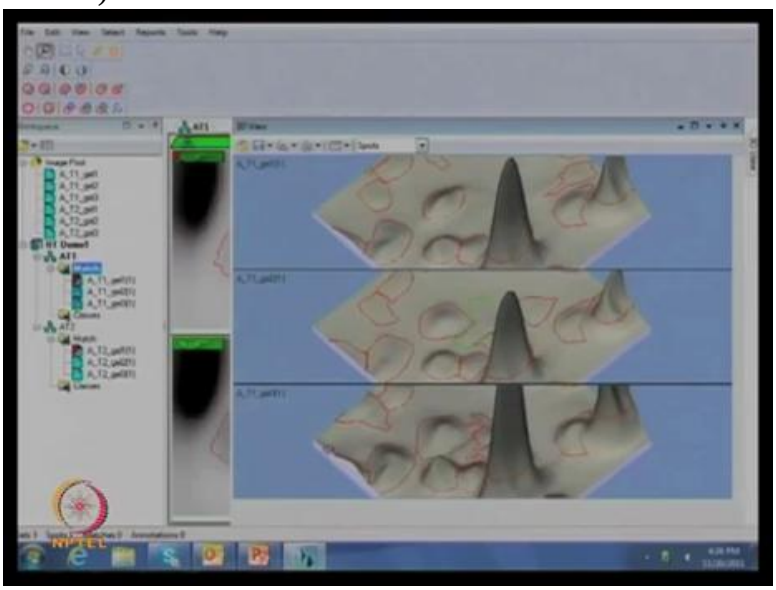
Professor: Right

Expert: This will be helpful there. So need of deleting all....

Professor: Now let's look at some detail about one of the real spots

Expert: Otherwise I can visualize it, I can more zoom so that one can be see more,

(Refer Slide Time 23:02)



Professor: So now this the intensity spots

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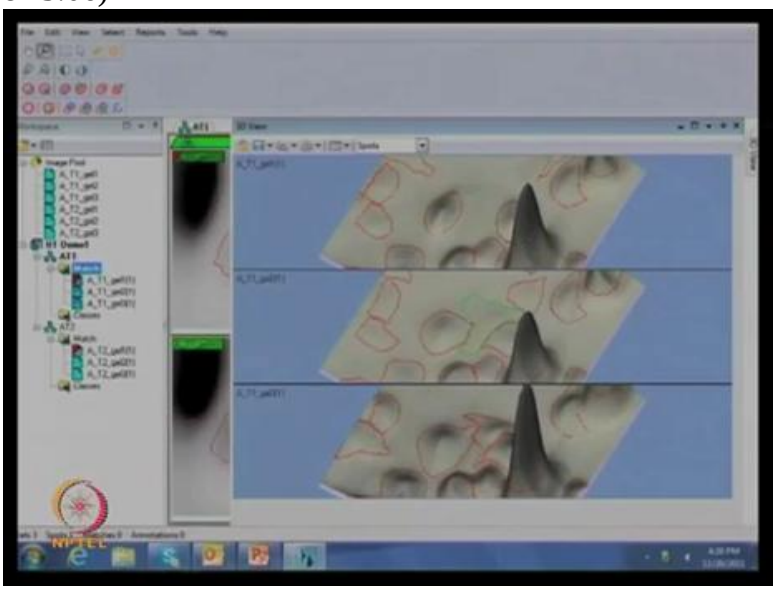


Expert: Yes, this is the

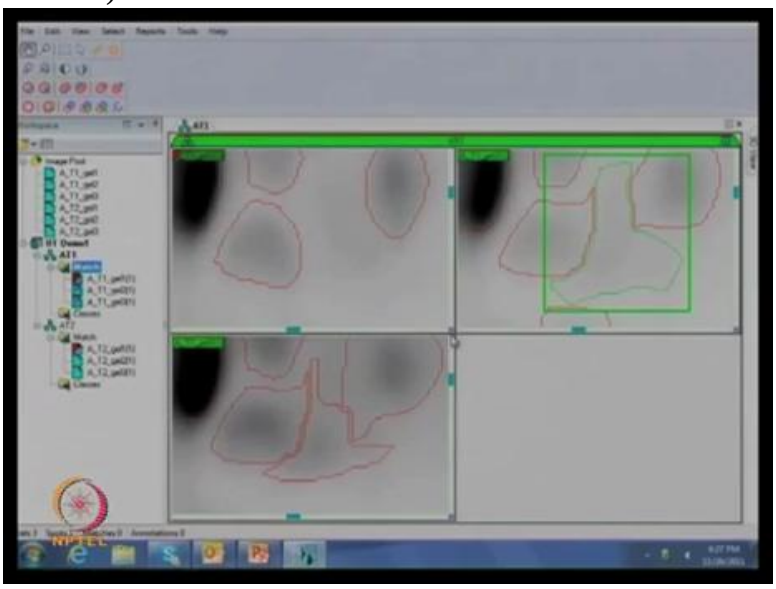
Professor: Now this is the intense spots

Expert: Intensity spots which you are visualizing,

Professor: … another boundaries are marked in these 75% from the top

Expert: Yes

(Refer Slide Time 23:22)



Professor: Now let's say we can actually zoom out and go back to the old gel. So let's say, in a region we have missed out some spots

Expert: Yes

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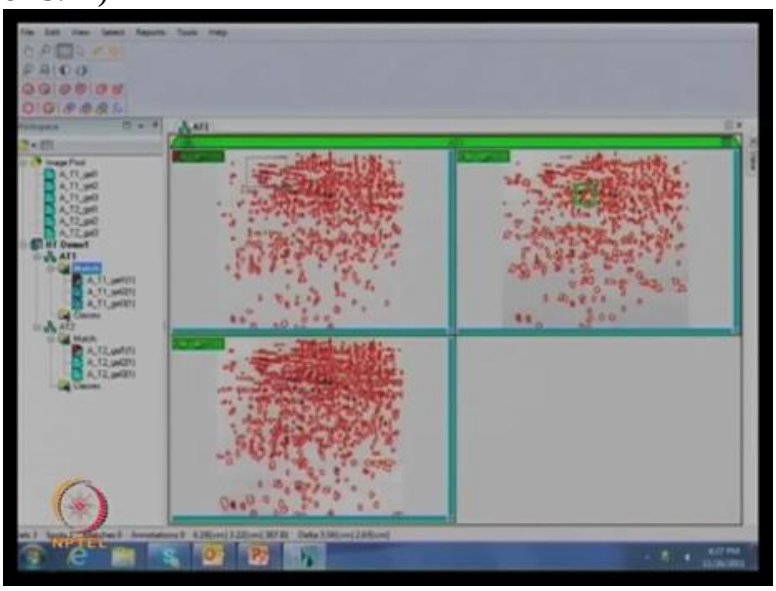

Professor: Then there is an option to add this spot as well.

Expert: Exactly, exactly. This is the adding, like let me zoom; let me select a particular area.

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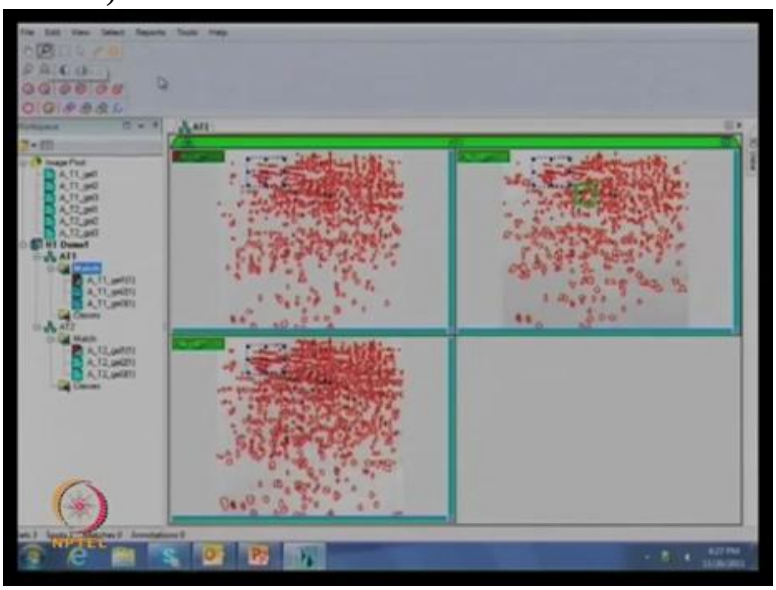

Expert: I am interested in this particular area. I have selected everywhere and let me zoom that.

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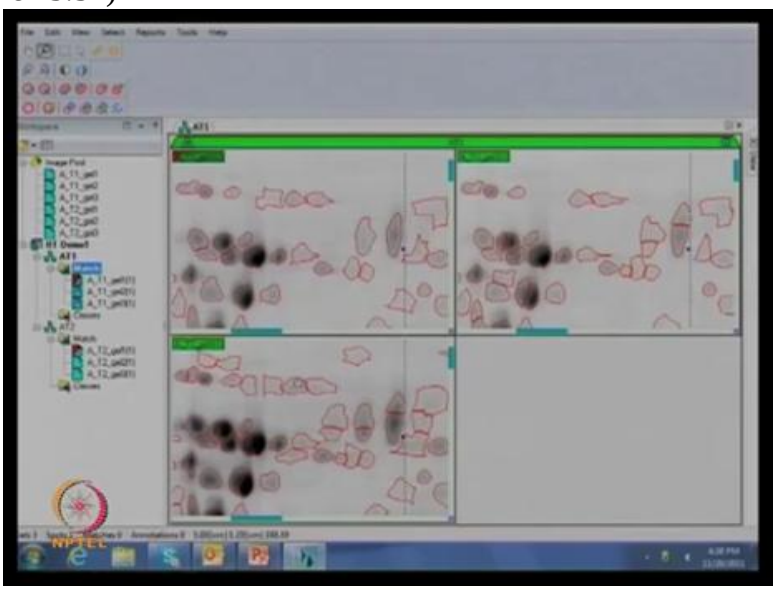

Expert: Now you can see this particular area in all gels. Ok

Professor: Right, I think it has defined the spot boundaries quite well overall...

Expert: Yes

Professor: That is why it is better to rely more on automated....

Expert: Yes, exactly that is why.

Professor: Automated detection

Expert: Exactly. This is what...if you want to include some spots wherever you are interested, you can definitely add. Like this kind of extra portion we can easily delete actually. Like you can reduce this particular portion...

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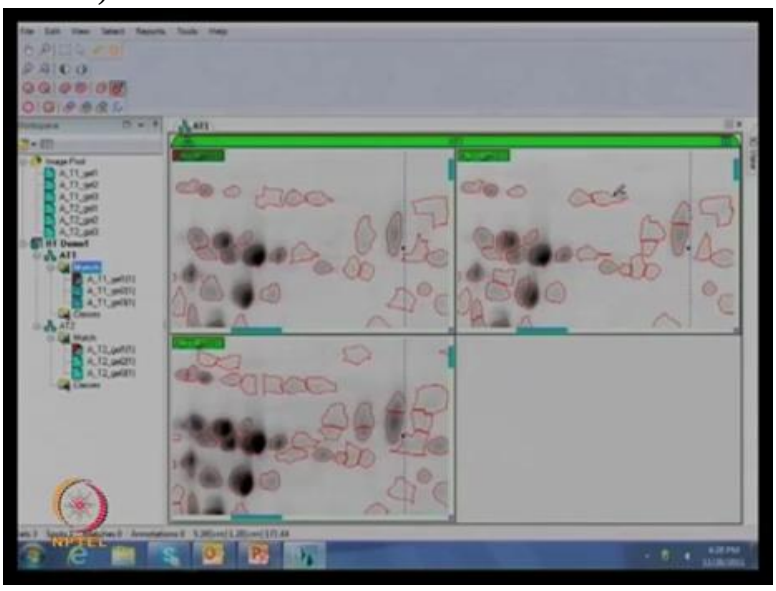

Professor: Second better way is to remove the spot and draw the spot again....

Expert: Yeah, that is also better....both ways you can do actually. Yes, you can delete it, or you can redraw that is, both ways you can do. Now we find all spot boundaries are Ok and everything is fine, now we can match these 3 gels. How reproducibility of all 3 gels, how reproducible are all 3 gels? OK, this is what which we can do here,

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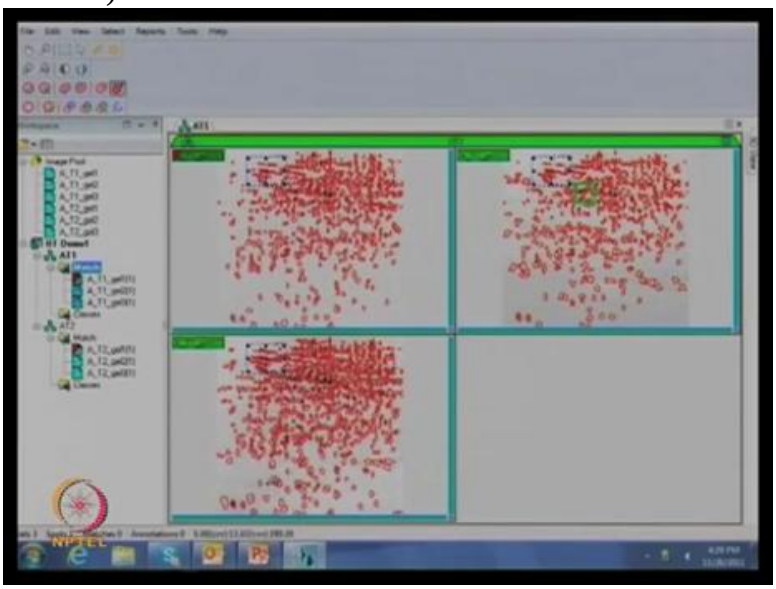

Expert: so come to your original state without zooming and match them.

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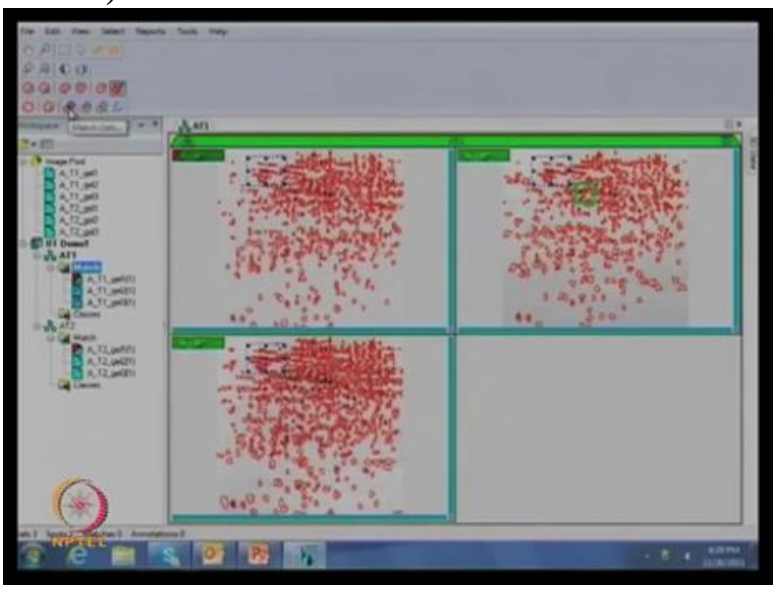

Expert: This is the match option

Professor: Ok

Expert: You can match them

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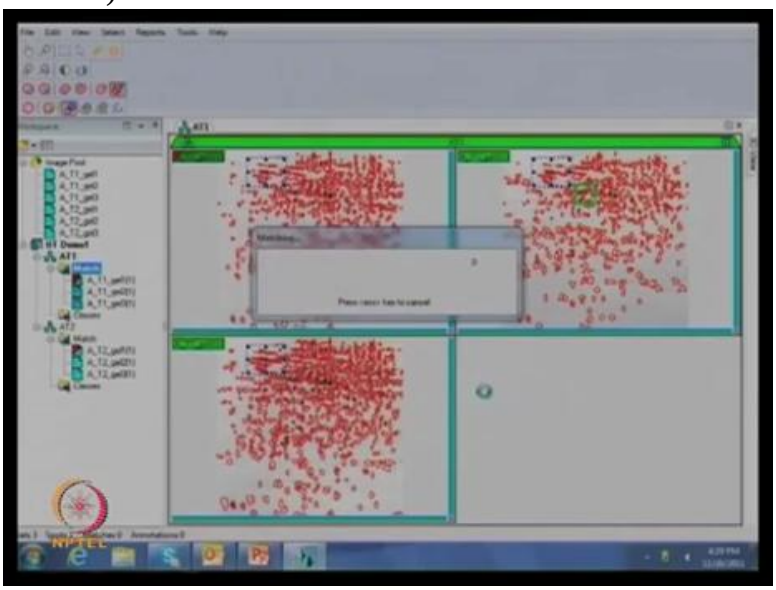


Professor: So now, you are applying the same analysis parameters on both control and treatment gels...

Expert: Yes, exactly

Professor: And matching

(Refer Slide Time 25:14)

Expert: Yes, exactly...Now we are matching only control gels.

Professor: Only one group?

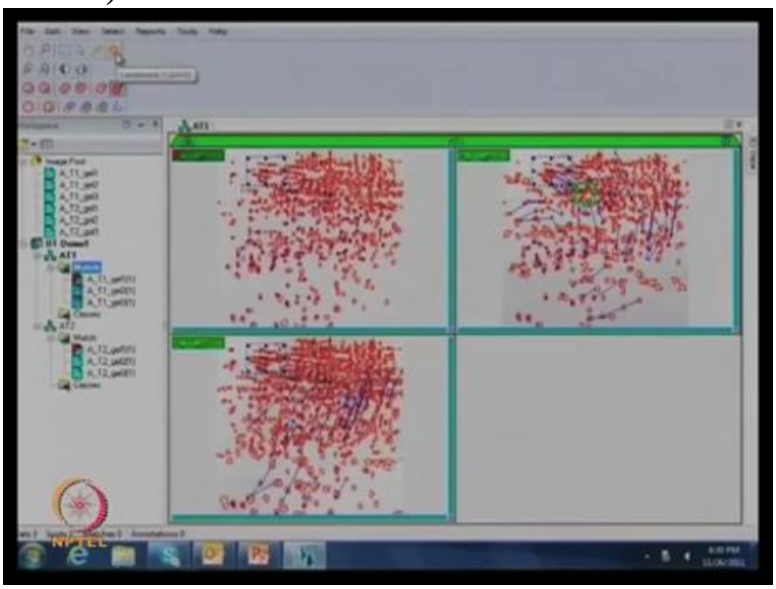
Expert: Only one group, how reproducibility is there....?

Professor: Within...

Expert: Within one group, this is what we have check initially.

Professor: Ok

(Refer Slide Time 26:13)



Expert: So if you have, suppose may be instead of 3 replications, if you have may be 5 replications, may be 6 replications, out of 5-6 replications, one can remove that particular gel and they can take remaining 4 or 5 gels.

Professor: So if there is some time during various experimental run, if there are 1 or 2 gels which is very bad...

Expert: Very bad

Professor: Which is going to affect the overall reproducibility...?

Expert: Exactly

Professor: So by looking at this type of parameters...

Expert: Yes

Professor: One can decide, Ok out of 6 gels one of that is very bad...

Expert: Yes

Professor: and is going to affect our statistical parameter...

Expert: Exactly

Professor: So may be one should remove that.

Expert: Exactly

Professor: Then select those only which are having very good number of matching.

Expert: Yes, that should be done

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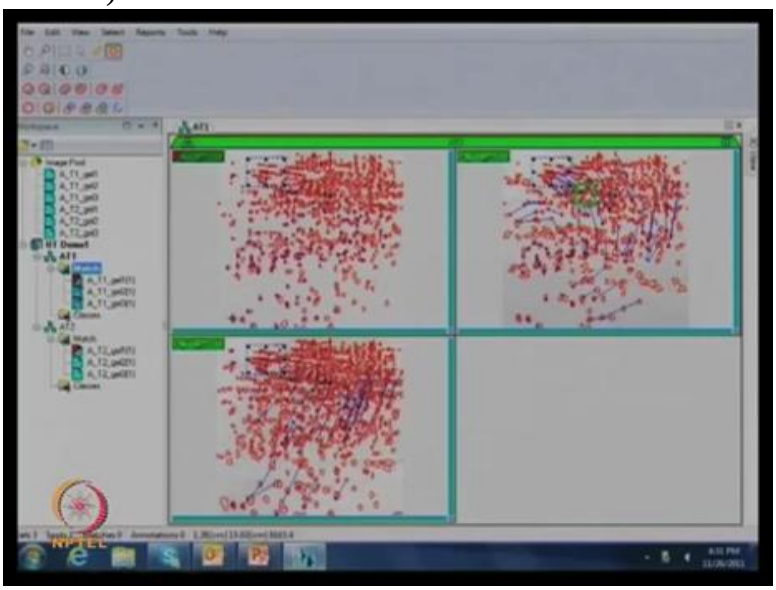

. Now we can see, in these two gels there are few vectors which you can see. This we can able to remove by adding...

Professor: Yes

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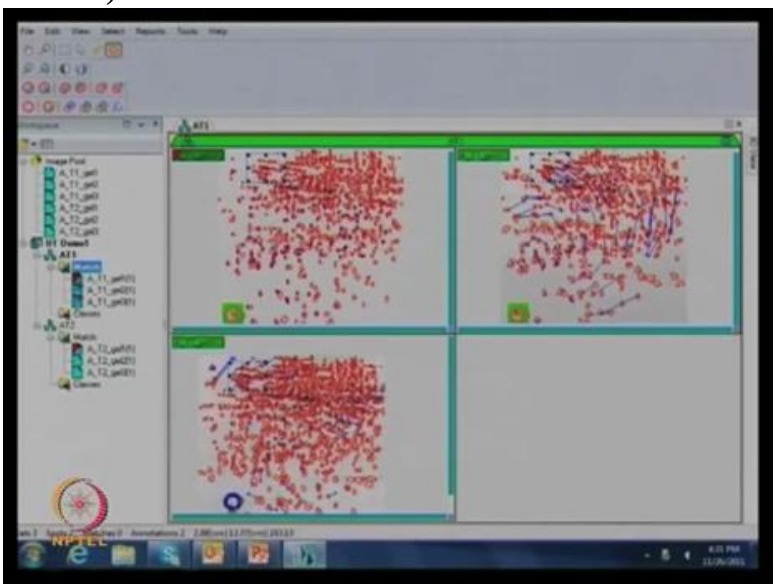


Expert: Now we can see, in these two gels there are few vectors which you can see. This we can able to remove by adding...

Professor: Yes

Expert: the landmark sort of thing, Ok. Now this is the landmark option, Ok. You have to add your landmark initially in the reference gel only, like suppose you can think this is a landmark sort of thing, I am just going to add a landmark here, so I have to adjust this landmark to the same position in remaining all gels..

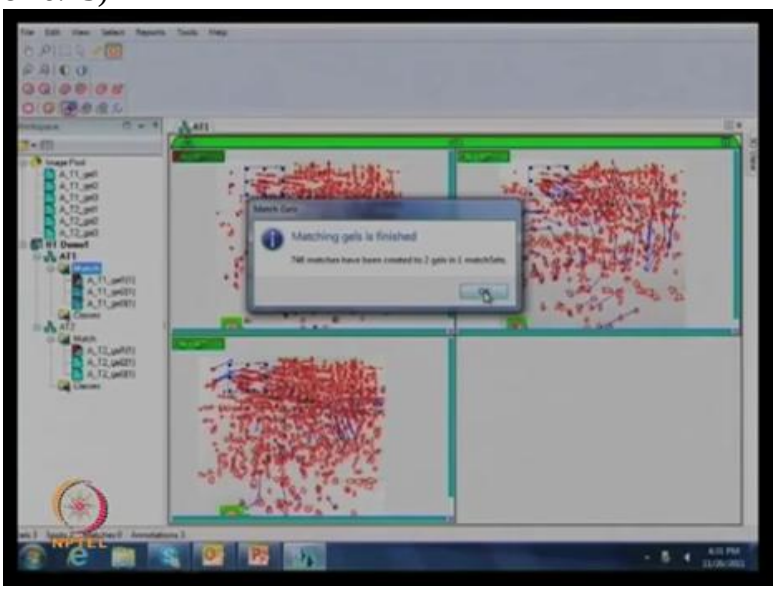
Professor: So vectors are, I think, improving...

Expert: Yes

Professor: Based on the landmark position.

Expert: Again match them

(Refer Slide Time 26:43)



Professor: So from 146 it has increased to 746.

Expert: Yes

Professor: By increasing the...

Expert: Exactly...

Professor: By adding a landmark

Expert: Landmark

Professor: And vectors are matching well.

Expert: See

Professor: Right

Expert: But you can see there are a few vectors

Professor: Vectors are still there.

Expert: Still there but these are all moving at the same side, so that means it is not like, irregular.

Professor: Also I think one needs to go through each region...

Expert: Go through each region, yeah very carefully. So you if you can add one more landmark here, it can be removed very easily. This is what we can do. Ok, now we can save this particular thing

(Refer Slide Time 27:21)

Expert: … and apply the same parameters to our treatment gel also.

Professor: That is important because...

Expert: Yes

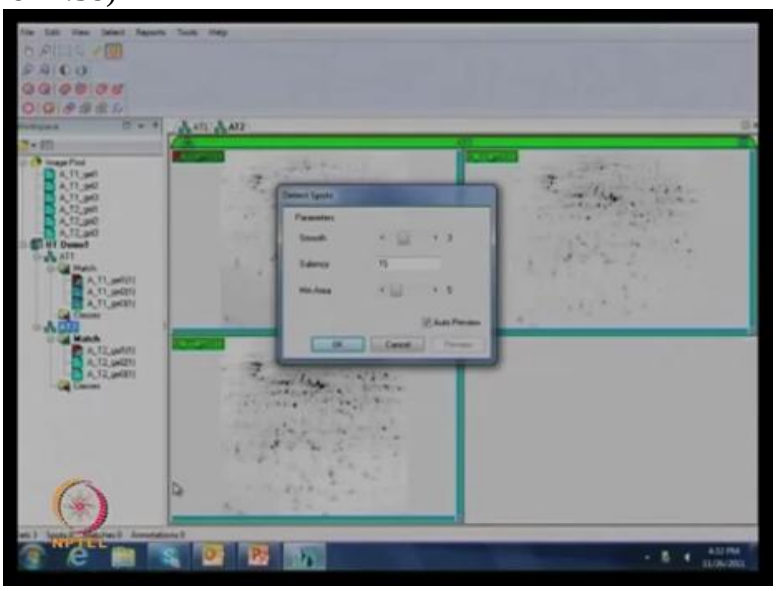
Professor: We don't want to make any change...

Expert: Any change...

Professor: …In controls and...

Expert: ...treatment. So you can see, no need to remember also the same parameters;

(Refer Slide Time 27:38)

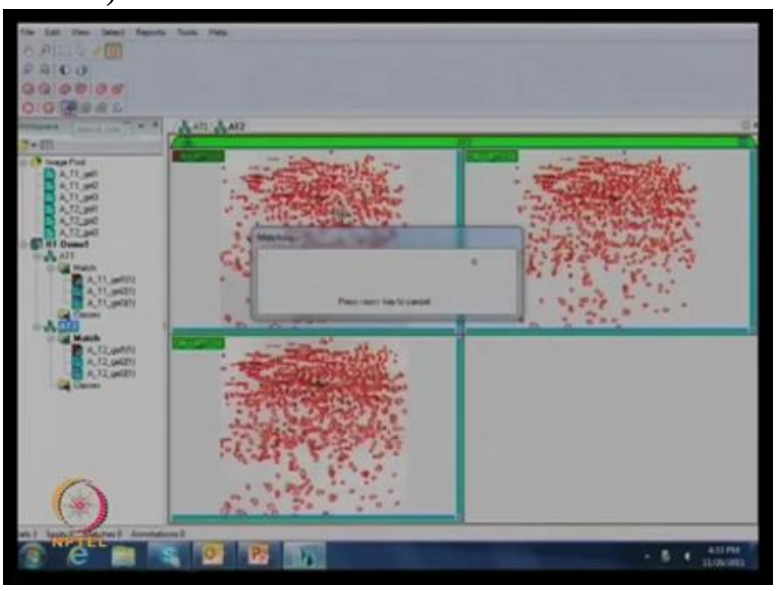


Expert: …if you can detect your spots, they automatically give the same parameters which you have used. Ok, same smoothness is 3 and saliency 15 and minimal area is 5. So no need to change anything then Click on Ok.

Professor: There is a long process of analyzing it; or rather it is quite quick?

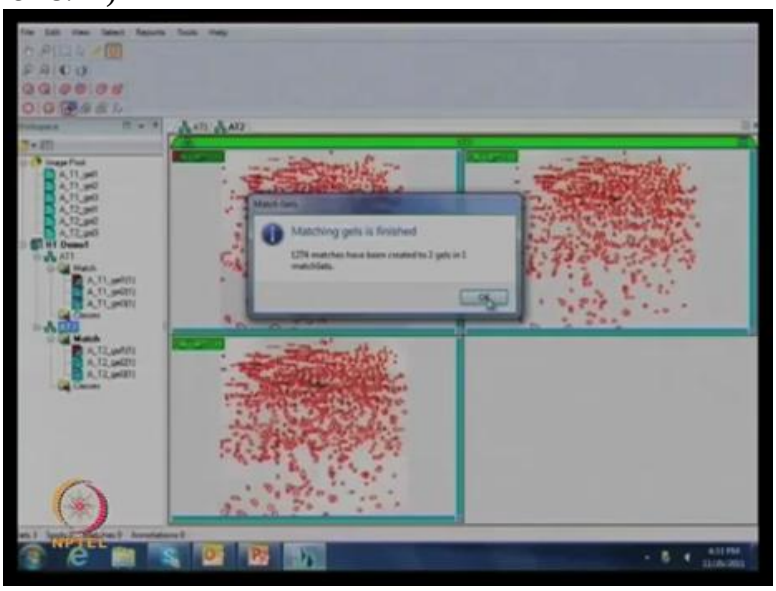
Expert: It is quite quick, than...

(Refer Slide Time 28:07)



Expert: it is completely finished. Now the same way we can go through each and individual gel by zooming and 3D view and you can select which are all the spots and which are all the not spots. Then you can directly match them. Now I am doing the same matching,

(Refer Slide Time 28:12)



Expert: 1274 matches have been done. So these gels are more reproducible as compared to...

Professor: Other source.

Expert: Yeah, see...even vectors also, without giving landmark also there is no vector

Professor: Right

Expert: So this is very fine gel so now, what are the... the replication which we did was confirmed. These replications are Ok. Now, one can analyze in the Class Analysis, in between these two classes, like inter classes and intra classes.

Professor: Between control and treatment group?

Expert: Yeah

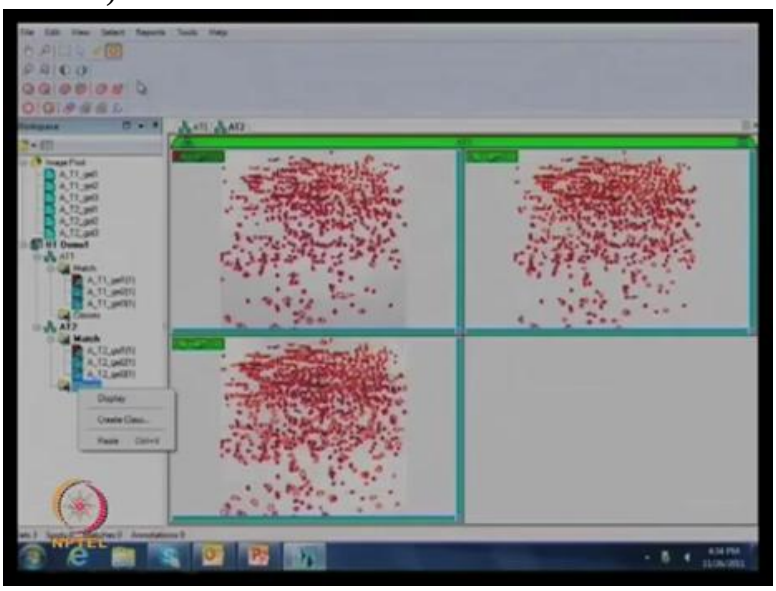
Professor: One can actually now do the statistical analyses.

Expert: Statistical analysis.

(Refer Slide Time 28:53)

Expert: So now I am creating the classes here...otherwise...

(Refer Slide Time 28:55)

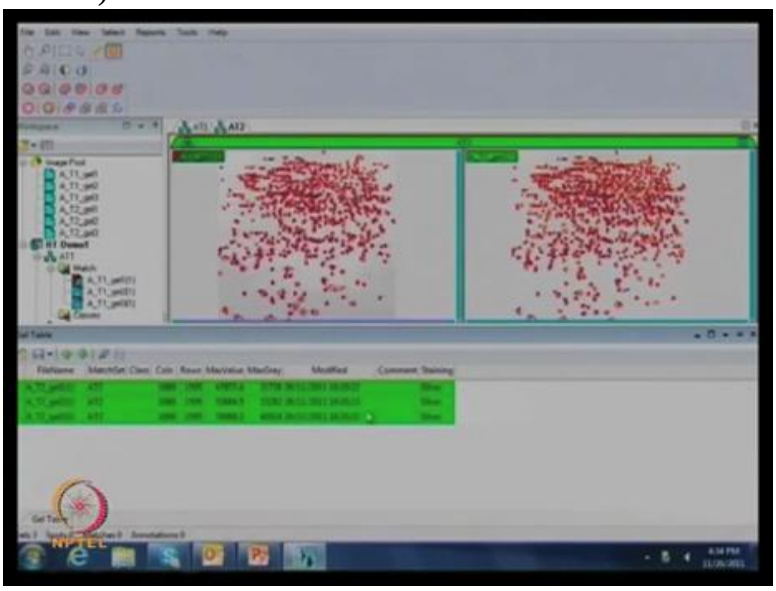


Expert: before that I would like to go to one report point,

(Refer Slide Time 29:00)

Expert: what is a gel table...Ok?

(Refer Slide Time 29:05)



Expert: These are all the gels which are present here.

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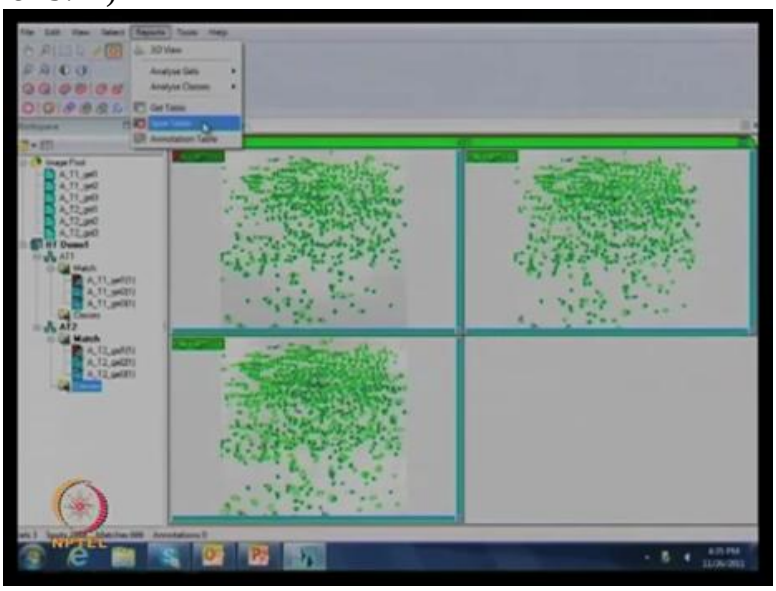
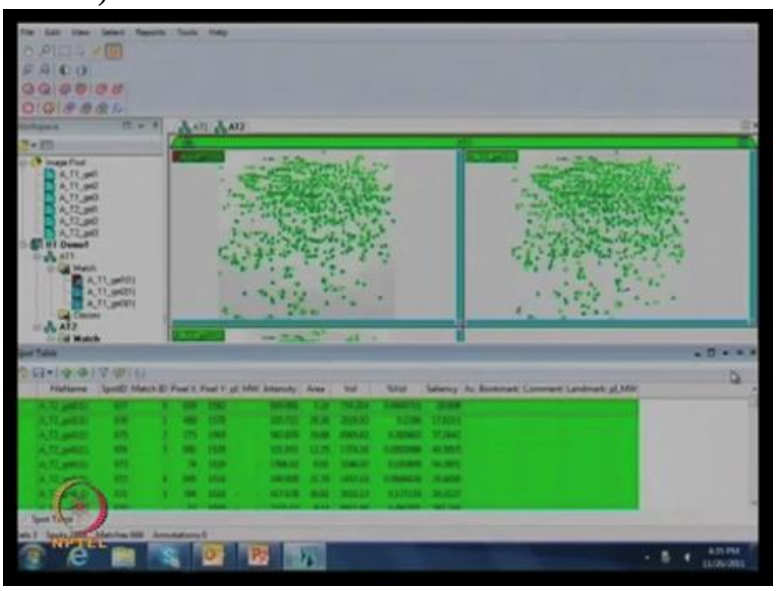


Expert: Shift-A, you can select all the spots, go to spot table, see now...

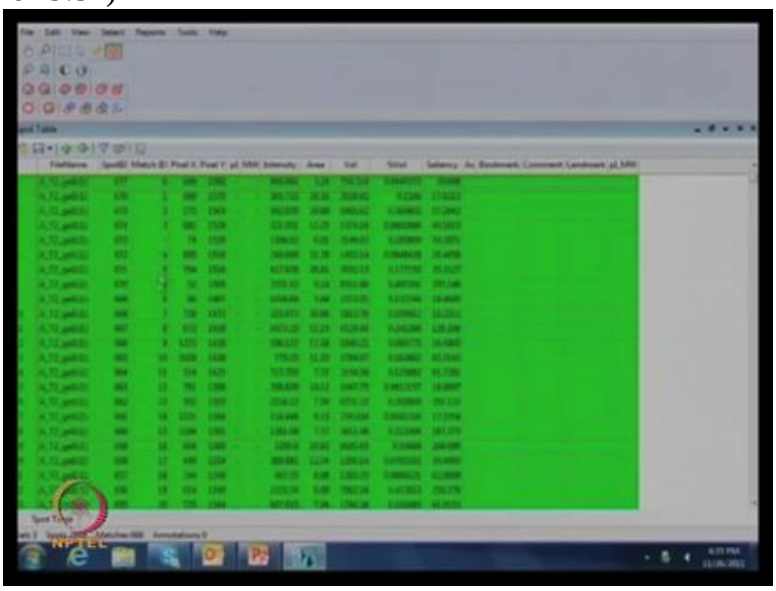
(Refer Slide Time 29:31)



Professor: So what parameters are described here on the spot table?

Expert: This is the file name and the spot ID. It has given a particular spot ID for all proteins and this is the pixels… that is the...let me...

(Refer Slide Time 29:54)



Expert: this is the Match ID that means out of 6 slides, Match ID is giving....

Professor: Just give me quickly like what are the....

Expert: This is the file name, this is the gel number, then Spot ID which is given complete number for all spots, then Match ID, that is the pixel size at X-axis and pixel at Y-axis, then you can be able to give the pI also and you can calculate the pI as well as the molecular weight also, then intensity of that particular spot and area of that particular spot and volume of that particular spot and percentage volume of that particular spot and saliency of that spot. These are all these things which you can see here...

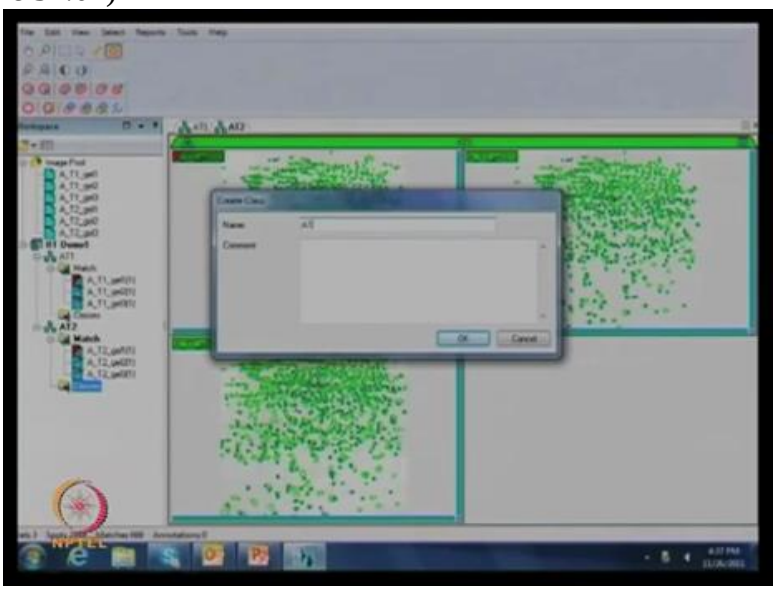
Professor: All these parameters for all the gels...

Expert: All the gels...

Professor: Can be obtained

Expert: Obtained from this software. These are all the parameters which you can see in this software. Ok, so one, if you are satisfied with all these values, you can go ahead, your next level of analysis that is Class Analysis,

(Refer Slide Time 31:02)



Expert: Ok. Now I am going to create a class here. Create a class

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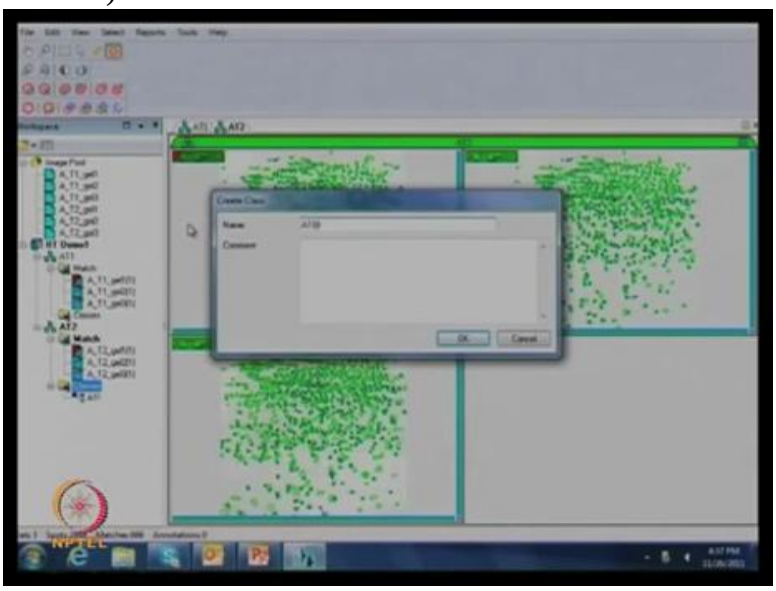

Expert: This is AT1,

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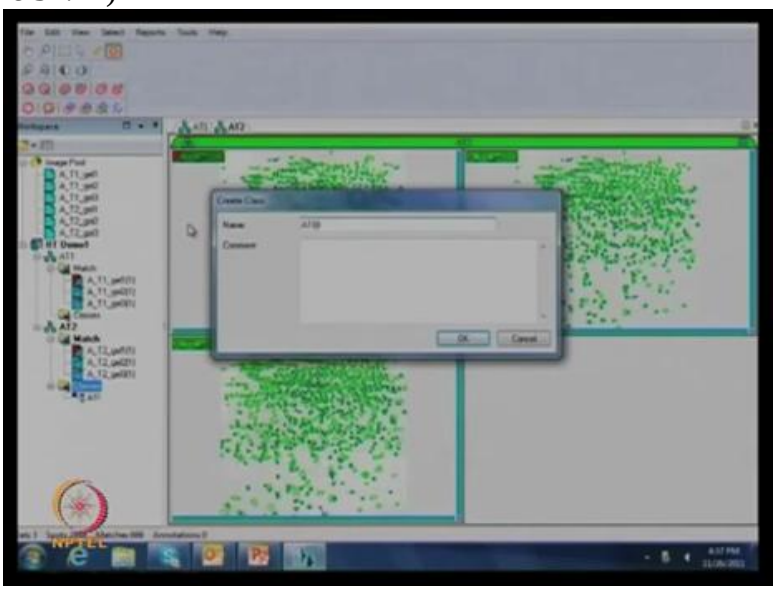

Expert: another is the AT2 which is control and treated. Now I can shift these gels from AT1 to AT1; another match set which I am creating where we can easily, move easily before going to class, so that...

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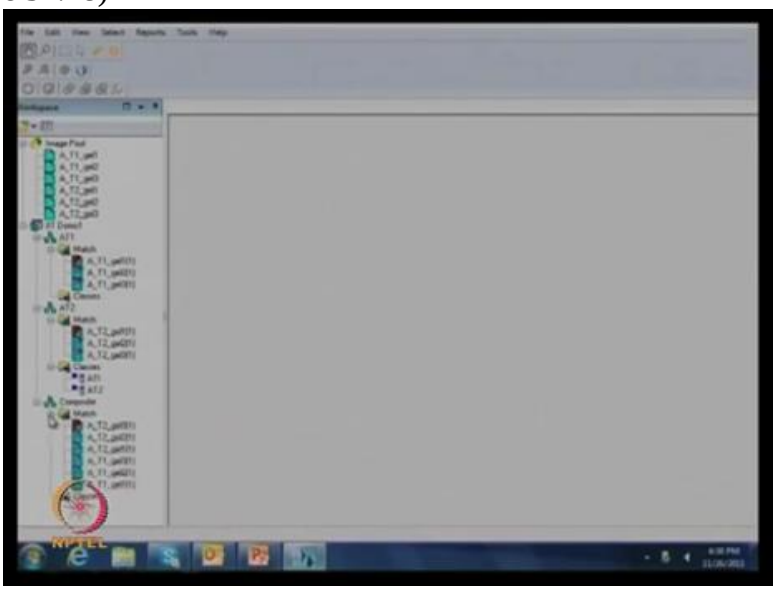

Professor: So you are moving all the 6 gels from...

Expert: All the 6 gels in 2 composite Match set, so all 6 gels are here

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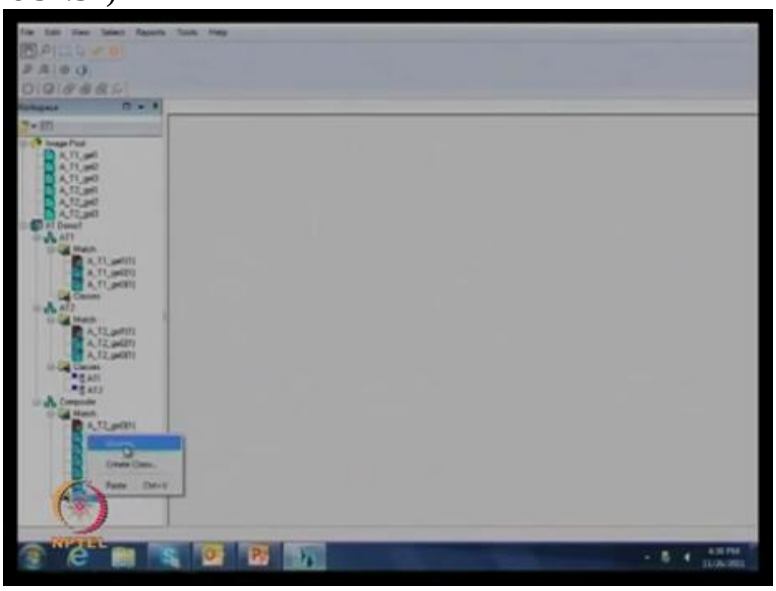

Expert: and now, we can create again the same way, classes...

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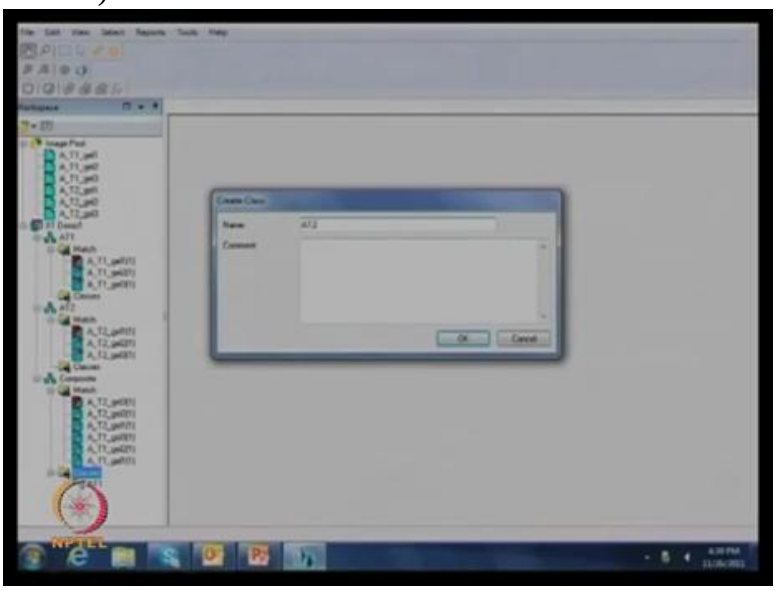

Professor: So by adding different folders, I think one can actually avoid any carryover mistakes previously...

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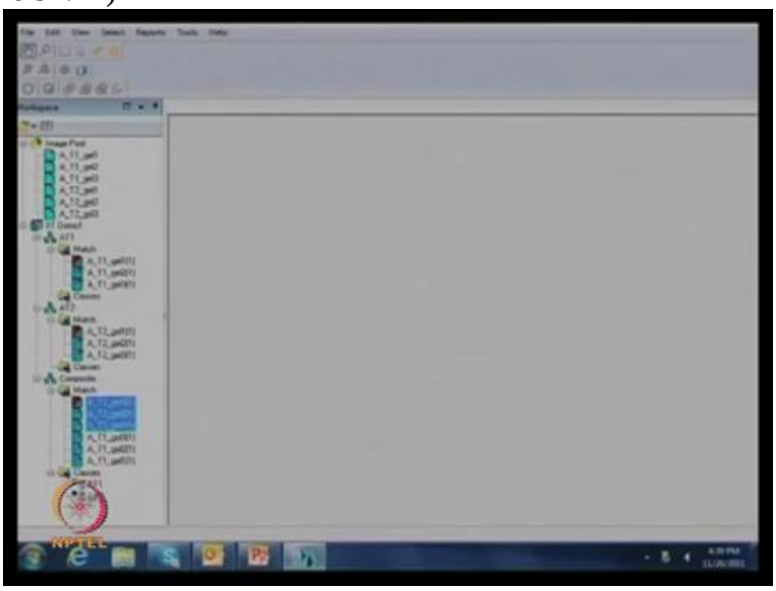


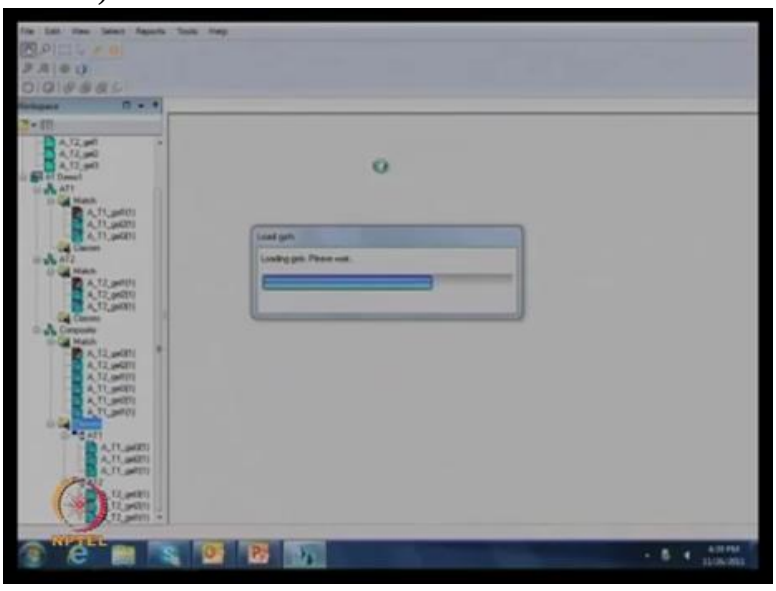
Expert: Yes, exactly

Professor: All the steps are stored.

Expert: Yes, all the steps are stored. Now again I am shifting all AT2 gels in AT2 folder. This is a class folder again, AT1 folder.

Professor: This is for Class Analysis.

(Refer Slide Time 32:29)



Expert: This is for Class Analysis. Now we can see both gels together

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. Now 3 gels from the control and remaining 3 gels from the treated, Ok?

Professor: Right.

Expert: This is what which we can see. Now it is behaving...it is taking values as a single gel of AT1 and single gel of AT2. Now it is going to average of these 3 gels and now it is another averaging these remaining 3 gels. So a single value you can get from the control, another single value you can get from the treated. Once you did the same thing, and then quickly match them again. One can remove all these vectors by adding the...again the particular landmark, so...

Professor: Same process...

Expert: Same process what we did earlier; now we go to Reports where we can see the Analysis Classes, go to Table

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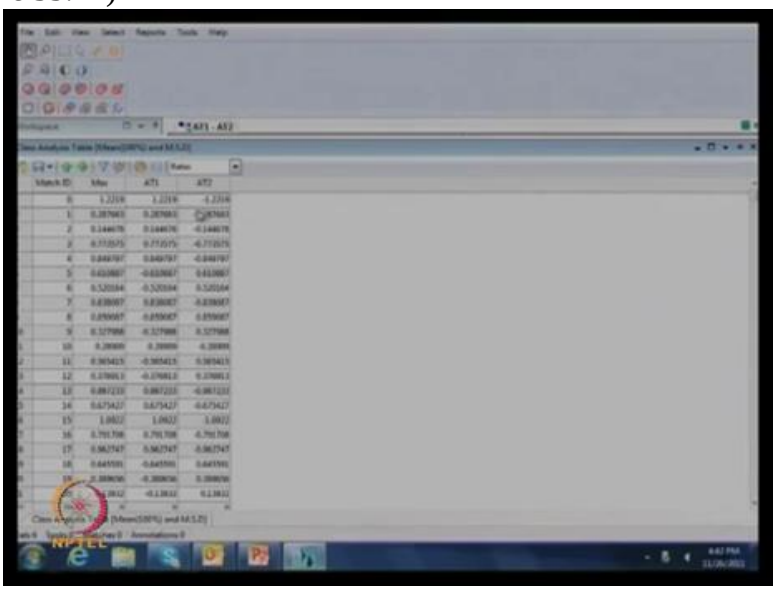

Expert: now you have the Match ID. Match ID is the particular Spot number as well as the maximum area of the....whatever the maximum area as well as the...this is the AT1 value and AT2 value...

Professor: So, for the control and the treatment...

Expert: Yeah...

Professor: Both groups...we had 6 gels right?

Expert: Yes

Professor: So what are the values in each 6 gels, for each spot?

Expert: For each spot, exactly....this is the averaged value again...

Professor: Average of all the 3

Expert: All the 3 gels...

Professor: From each...

Expert: Yeah. Ratio value, you can see, this is the ratio value which is giving the up-regulation or down-regulation,

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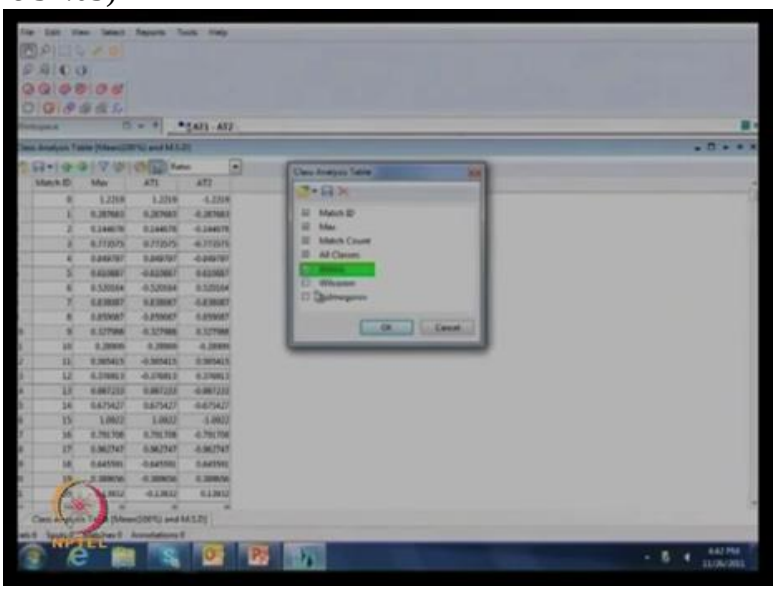

Expert: Ok and I think we need to add...

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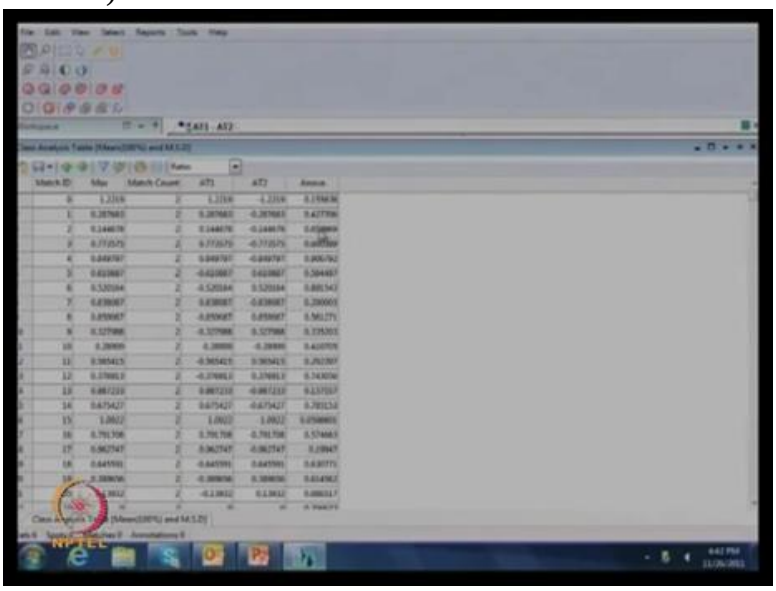

Expert: yeah, this is the statistical parameter

Professor: Various software gives you option for the table to...

Expert: Yes, exactly, so this is the ANOVA value which we can see; on the basis of this ANOVA value, we can easily select our interest of protein as well as you have the full regulation also...this is one fold up-regulator and this is the 1.2 fold down-regulator. The same way, you can see the whole values. now, no need to go each and individual spot. You can filter them easily.

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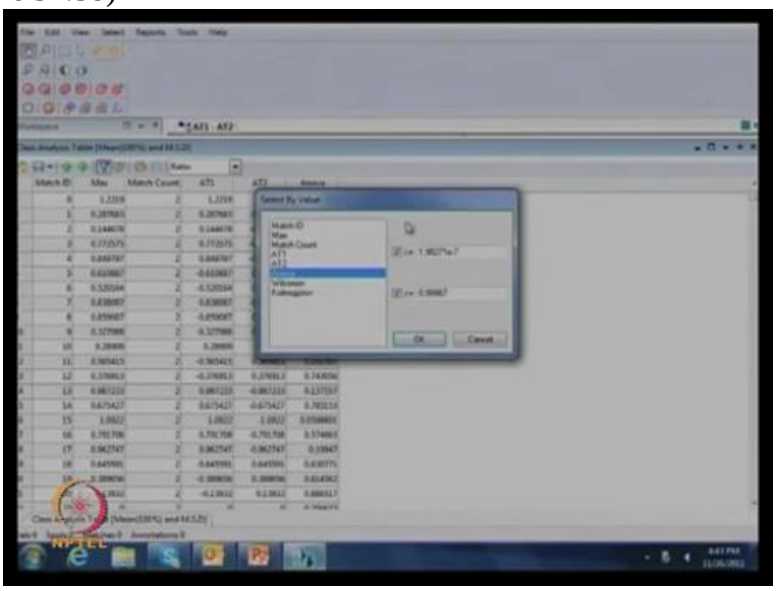
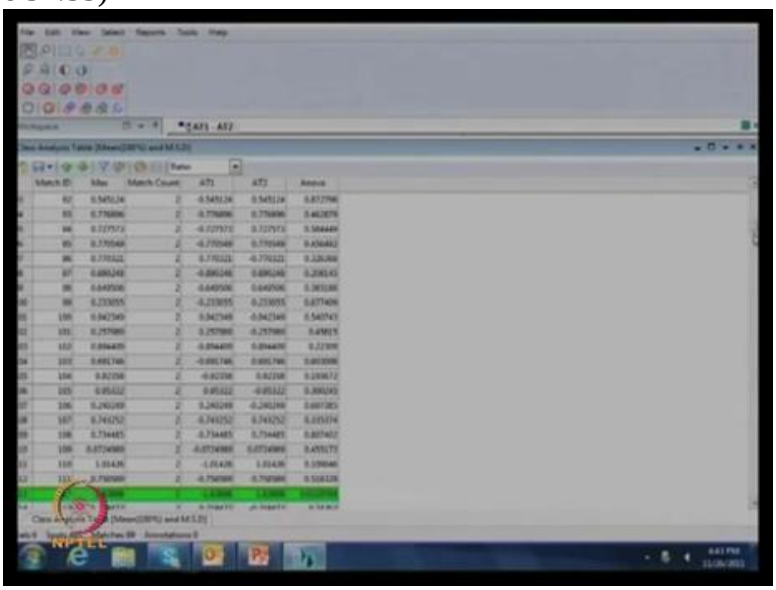


Expert: Now what we are going to do, is first at least ANOVA values we are going to take, the maximum we are going to take, as 0.05 as the statistical significant value, so those many spots can be highlighted.

Professor: right.

(Refer Slide Time 34:53)



Expert: So this is the spot which is already undergoing the particular ANOVA value

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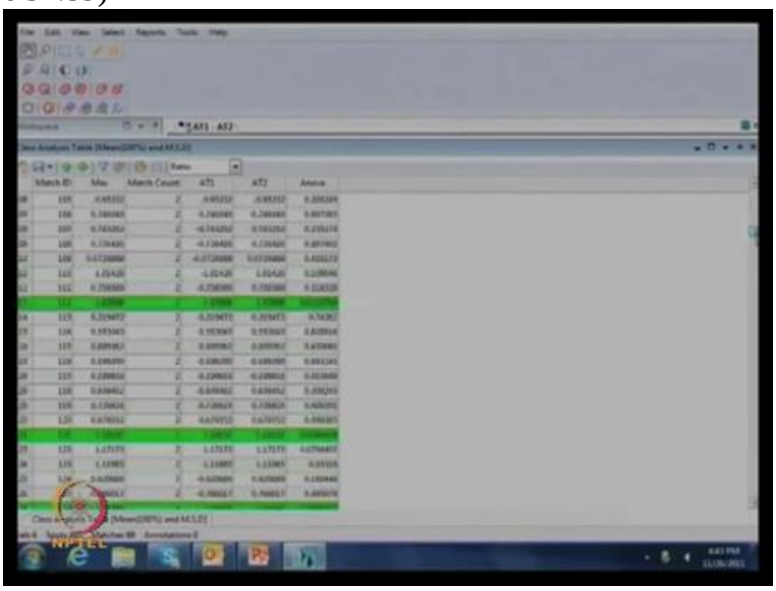

Professor: This is the threshold value

Expert: Threshold and see, all the spots which you want, which you have this particular ANOVA value, those are all highlighted here. Now, one can very easily go through ...and this particular table you can easily export to Excel also and from there you can have complete data also. This is what the complete analysis data gives you the output.

(Refer Slide Time 35:26)

Professor: So, I guess it was very useful to see, like different steps what are required for performance analysis, and also the software gives you lots of options for doing different fold chains and statistical analysis of how significant data is.

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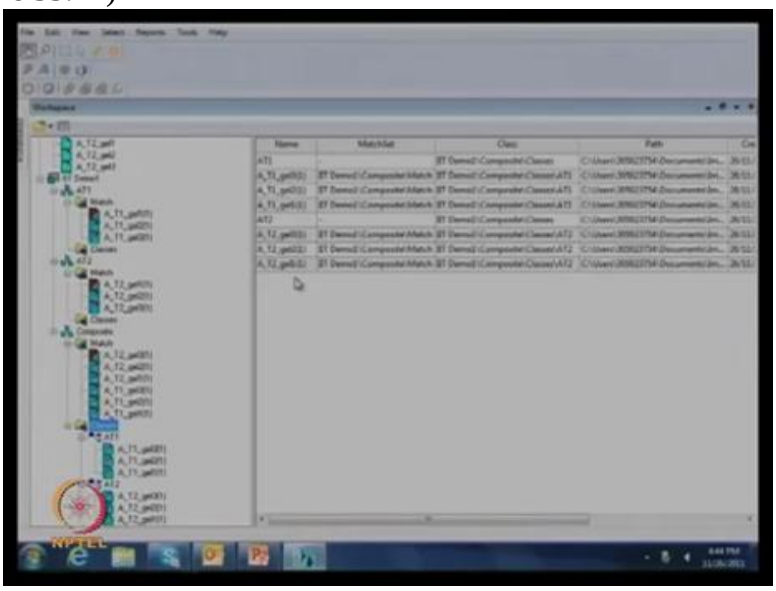

Professor: And then one can actually still go back to those spots which software says as significant and look at manually each spot to verify.

Expert: Yes

Professor: It is real spot.

(Refer Slide Time 35:50)

Professor: So thank you very much Srinivas for useful discussion and demonstration of the software for image processing on two dimensional gels. and...

Expert: Thank you

Professor- Expert conversation ends

We will continue our lecture flow for further two-dimensional difference electrophoresis in the next class.

(Refer Slide Time 36:07)