Introduction to Proteomics
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Lecture – 12
2-DE: Gel analysis

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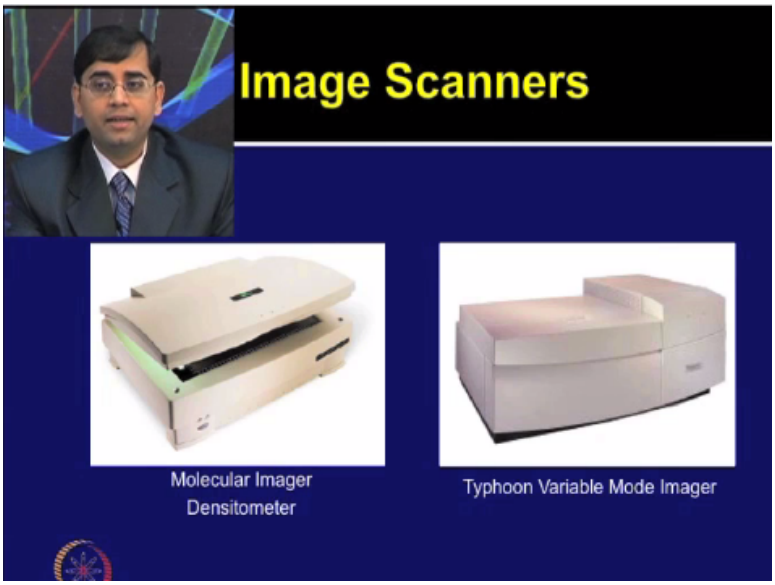

So, image analysis is another one of the very important aspect of the 2 dimensional electrophoresis workflow. There are different types of image scanners available from different vendors such as, one I have shown here molecular imager densitometer, other the typhoon variable mode imager. There are mainly obtaining images scanners available. So, now how to analyse these images, do you want to do the things manually.

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So, can you take your gel patterns and sit 2 of you together and say okay this is my protein in control, this is your protein in treatment, now I am going to look at each spot manually and then going to size the spot based on this comparison. So, that is going to be very, very tedious work and you will not have any information about the whether your spots having any statistical significance or not.

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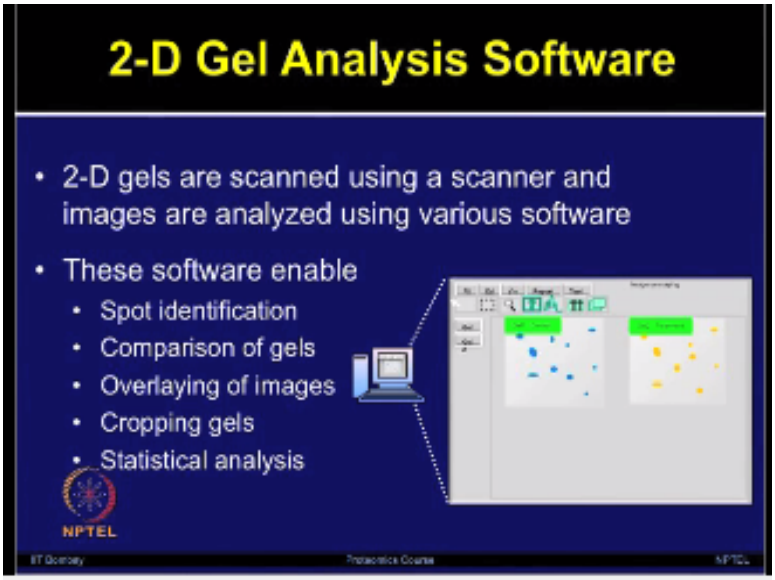

How reports will be those are, so you need to scan it by using good scanners and then finally you need to analyse your image from different software, which are available. So, commercially many software, which are available for doing 2 dimensional gel analysis, I will give you a comparative table at the end but almost all of this software they take the scanned images and analyse your gels by using various steps.

So, all the software enables the spot identification, comparison of the gels, overlaying of the images from your control and a 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 this part of interest, probably you would like to crop those regions.

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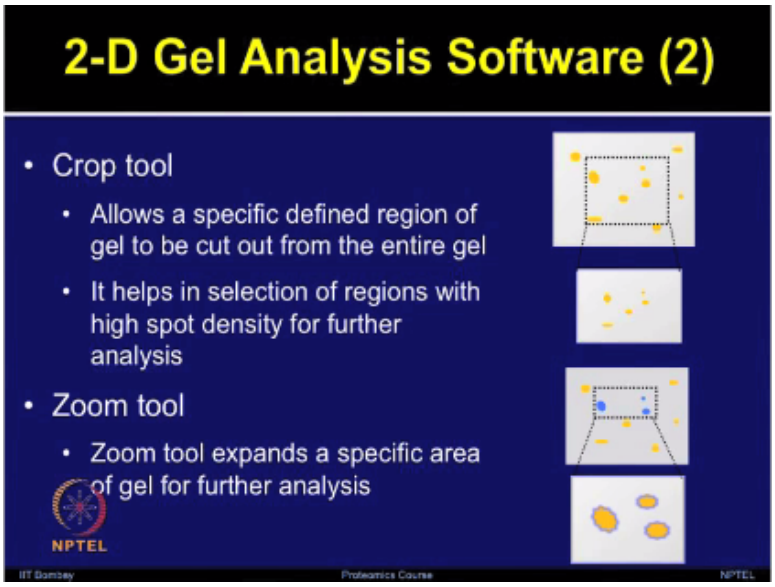

And crop both your control and treatment just uniformly, so this crop tool allows for a very specific defined region of the gel to be cut from the entire gel. It helps to select the region with the high spot density, which can be used for doing further gel analysis. Next, you would like to see your spots 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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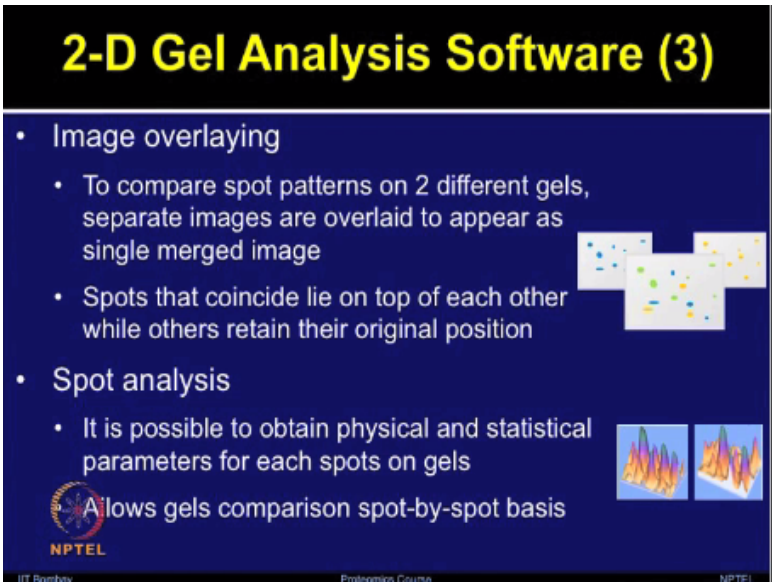

Now, image overlaying is very important aspect because if you are comparing 2 gels on one you have control other you have treatment, you would like to overlay those images together to compare their spot pattern present on 2 different gels because you have acquired 2 separate images now you need to overlay those, so that it can appear as a single merged image. Now spots, which are going to concede on top of each other.

Whereas, 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 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 spot, how they are differing from the control to the treatments.

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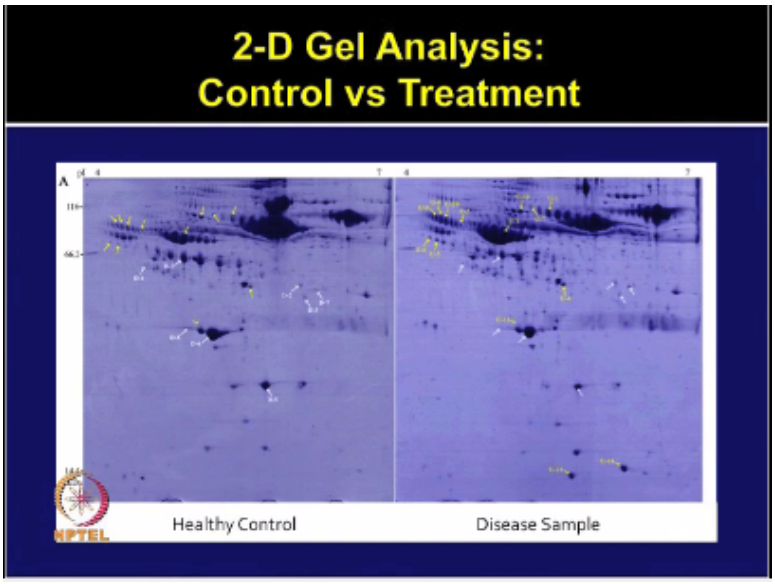

And then you allow the comparison of the gel, is spot by spot basis so often, running a gel or acquiring images and generating lot of data is much straightforward as compared to doing the analysis which is more tedious strip, one has to realistic and go through the gels usually spot by spot to analyse the gels. Now, I am showing you gel pattern of control and its comparison with the treatment gel.

These are taken from one of the healthy control and a diseased 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 a 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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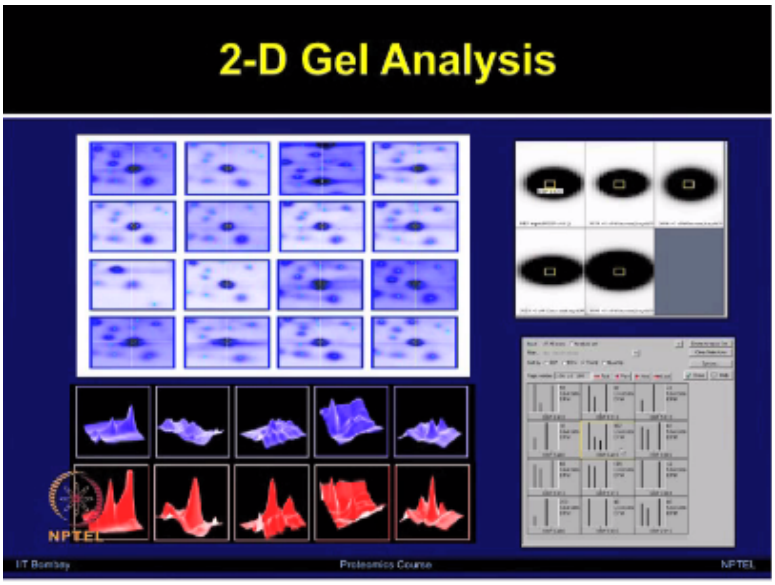

So, all of this analysis can be performed by using different software, as you can see in this image, I am showing you one spot which you would like to compare across 16 gels. Now, you have zoomed into that particular region and further you are looking at the 3 dimensional pattern of each of the spot, how they are differing from the control shown in the black and the blue spots and the treatment which is shown on the black background in 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 it is going a higher amount in the treatment. Now, one can look each of the spot intensity in much detail and then followed by plot different type of parameters for percentage volume or a spot intensity to compare their values and do the statistical comparison.

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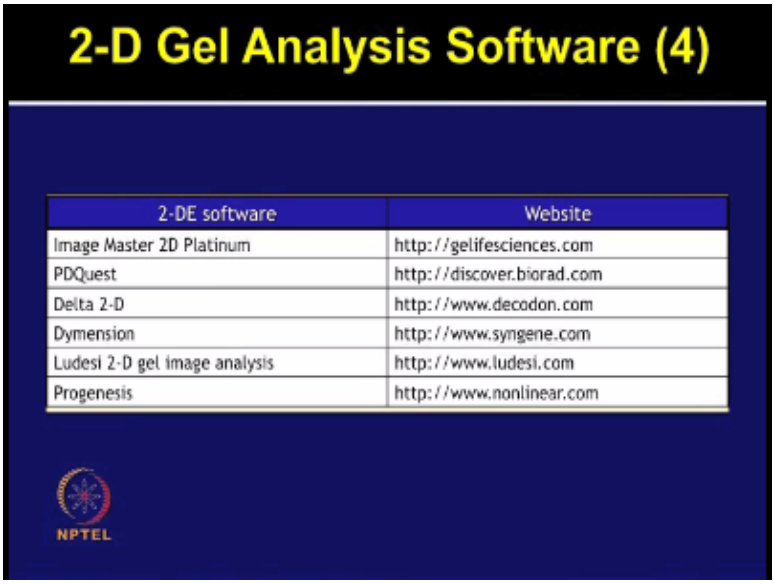

There are various commercial software, which are available for comparing the 2 regions such as image master 2D platinum from GE life sciences, PDQuest from biorad, Delta 2-D from decodon, dymension from syngene, Ludesi 2-D gel image analysis software, Progenesis from nonlinear. These are just a very few number of software, which I have mentioned these are; which are very commonly used.

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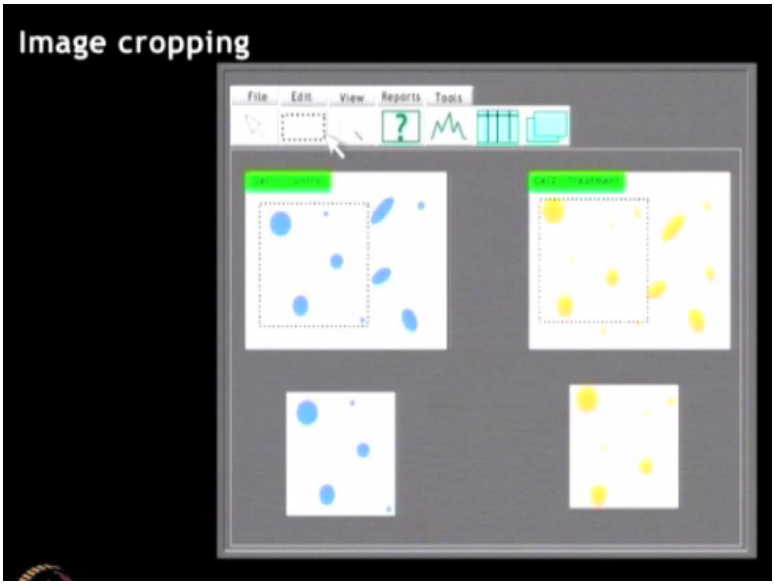

But there are many other good software also available which one can use to analyse regions. Now, let me show you this animation for performing 2 dimensional gel analysis, how to go a step by step to analyse your gels. 2D gel analysis software; in this animation, I will describe you how to analyse the 2D gel images by using a generic software layout. So, first you need to load a gel image.

It is possible to load either a single or multiple gel images simultaneously, this can be done by means of the load option in the file menu, you can save the gel images and then you can crop the area depending upon what area you want to analyse. There are several tools which are available for the analysis of gel. It is possible to crop the gels by selecting a specific region that is to be studied and then selecting the crop gel function.

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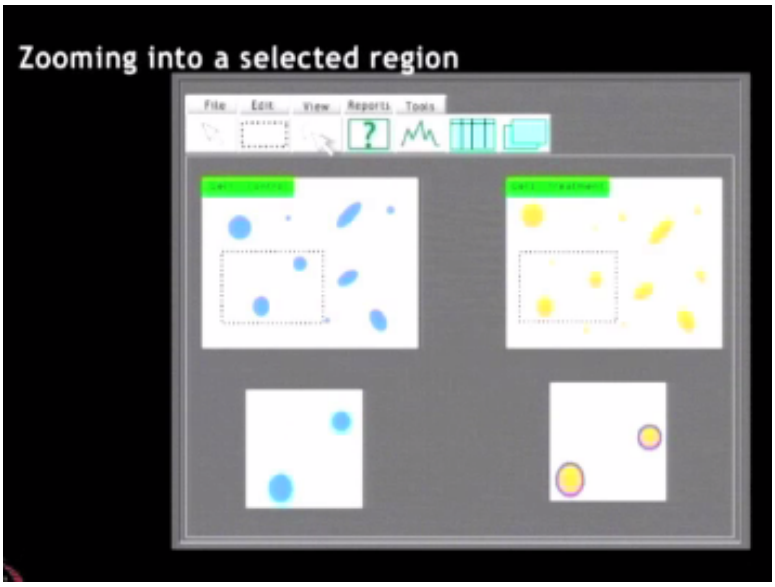

Cropping gel helps in selection of region with high spot density or to reduce the regions which contain high background stains with no spot. Zooming into a selected region, if you want to expand a specific region, you can use zoom tool, a specific selected region of the gel can be zoomed into for viewing the spot more closely and for comparison of spots between the 2 gels. This is particularly useful for gels with large number of spots.

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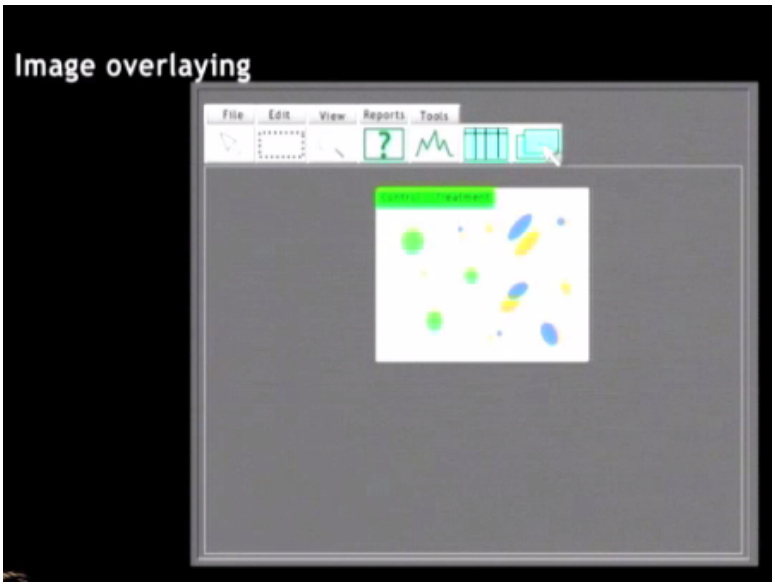

Once you have seen the area you would like to overlay the images; overlaying of images is a particularly useful tool for the comparison of two gels the gels are overlaid such that they appear merged and it spots that concedes will overlap with each other. This is extremely helpful while comparing the 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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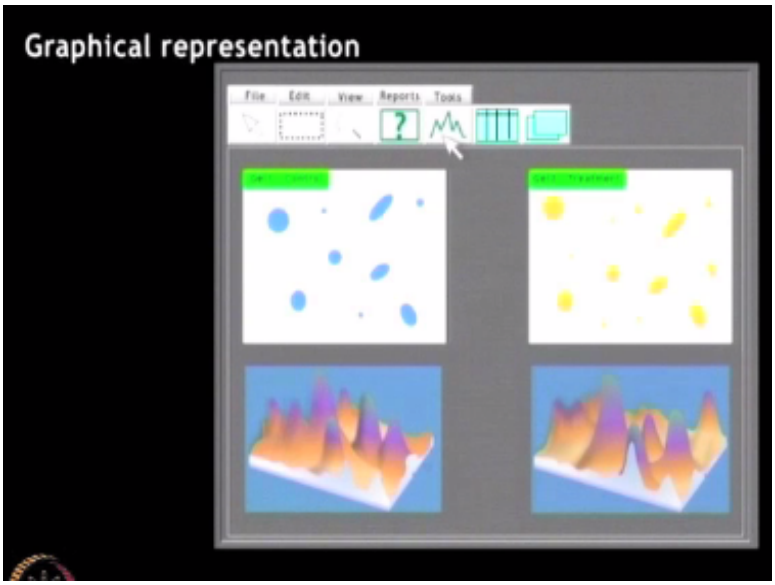

Now, after the analyses, one can look at the graphical representations of these 3 dimensional view of the spots. The spots on the gels can be displayed as 3 dimensional graph either the entire gel can be chosen or a particular region can be selected for this representation. The peaks obtained in the graphical representation are directly related to the spot intensity. Next, we will talk about a spot analysis.

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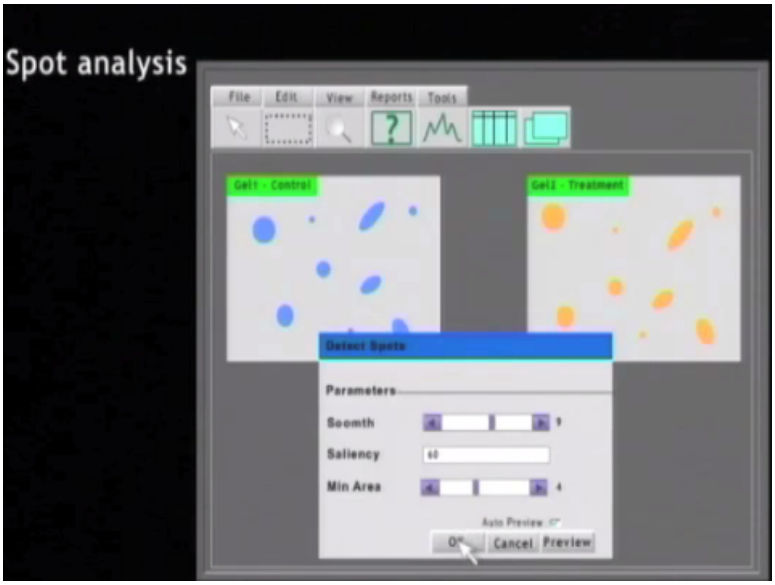

Every spot on the gel can be detected by selecting the detect spot option. Various parameters such as smoothness, saliency and minimum area must be suitably adjusted for maximum clarity.

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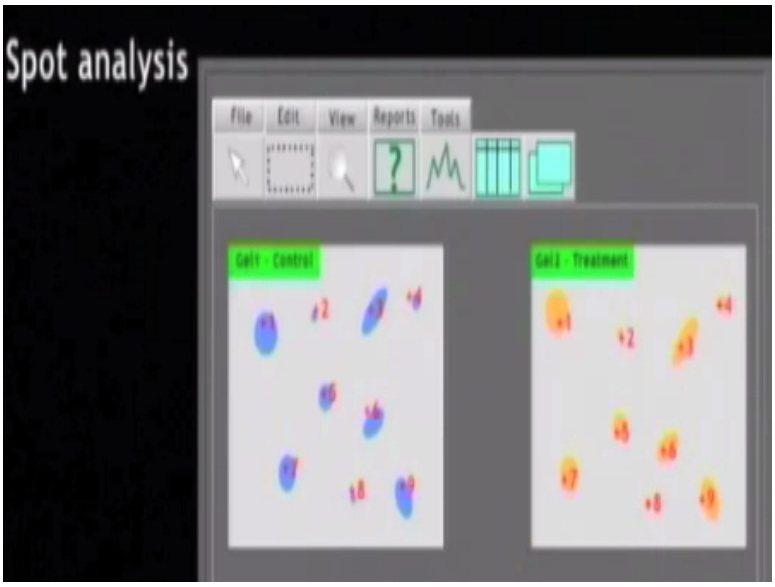

Once this has done, each spot will either be encircled or marked with a cross depends upon the setting along with the spot numbers. Now, describe you the gel matching. The software facilitates interpretation of the gel images by matching 2 different gel images which were obtained in your experiment.

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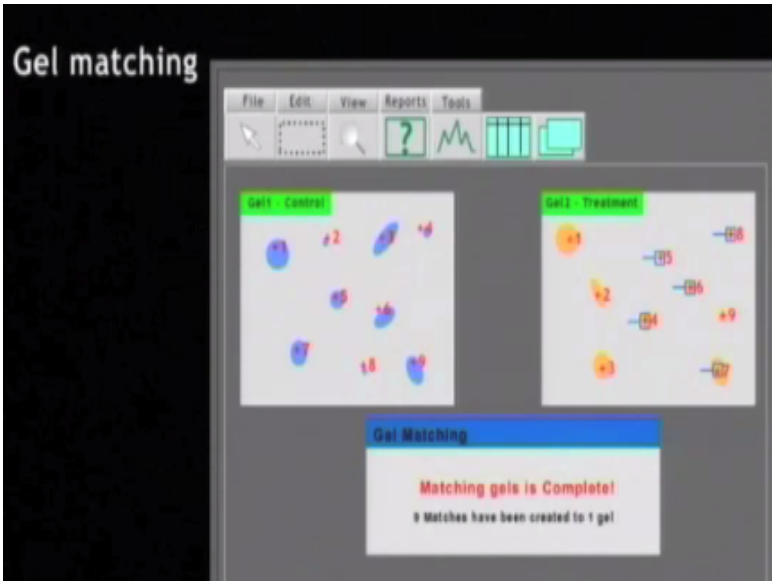

The matching spots are marked and after matching is done, any variation in the spot intensity; spot positions can be indicated by the blue lines as shown in the animation. This provides an understanding about the reproducibility across the gels.

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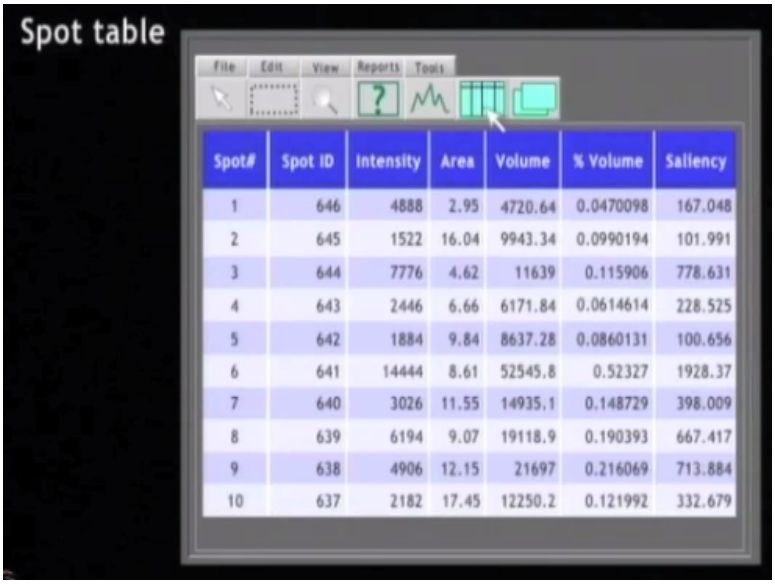

Spot#	Spot ID	Intensity	Area	Volume	% Volume	Saliency
1	646	4888	2.95	4720.64	0.0470098	167.048
2	645	1522	16.04	9943.34	0.0990194	101.991
3	644	7776	4.62	11639	0.115906	778.631
4	643	2446	6.66	6171.84	0.0614614	228.525
5	642	1884	9.84	8637.28	0.0860131	100.656
6	641	14444	8.61	52545.8	0.52327	1928.37
7	640	3026	11.55	14935.1	0.148729	398.009
8	639	6194	9.07	19118.9	0.190393	667.417
9	638	4906	12.15	21697	0.216069	713.884
10	637	2182	17.45	12250.2	0.121992	332.679

Once you have analysed the gel, one can obtain the detailed information for the spots from a spot table; information regarding various physical parameters of each spot can be obtained where this spot table, which provides a spot number in density, area and volume of the spots as well as the saliency of a spots. These parameters help to judge the quality of a gel.

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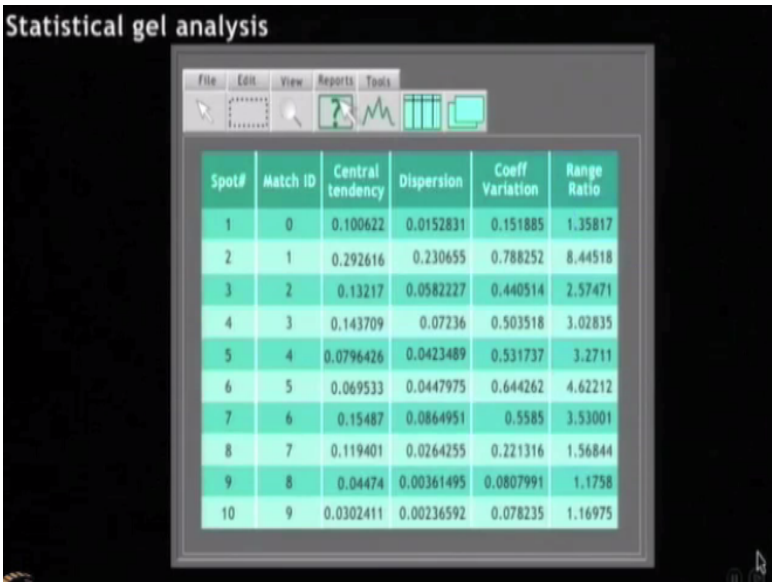

Spot#	Match ID	Central tendency	Dispersion	Coeff Variation	Range Ratio
1	0	0.100622	0.0152831	0.151885	1.35817
2	1	0.292616	0.230655	0.788252	8.44518
3	2	0.13217	0.0582227	0.440514	2.57471
4	3	0.143709	0.07236	0.503518	3.02835
5	4	0.0796426	0.0423489	0.531737	3.2711
6	5	0.069533	0.0447975	0.644262	4.62212
7	6	0.15487	0.0864951	0.5585	3.53001
8	7	0.119401	0.0264255	0.221316	1.56844
9	8	0.04474	0.00361495	0.0807991	1.1758
10	9	0.0302411	0.00236592	0.078235	1.16975

In addition to the physical parameters, various statistical parameters can also be computed for each gel and each spot on the gel such as central tendency mean, median, dispersion, coefficient of variation, a standard deviation or other statistical parameters. The scatter plots and histograms can also be plotted for clear data analysis.

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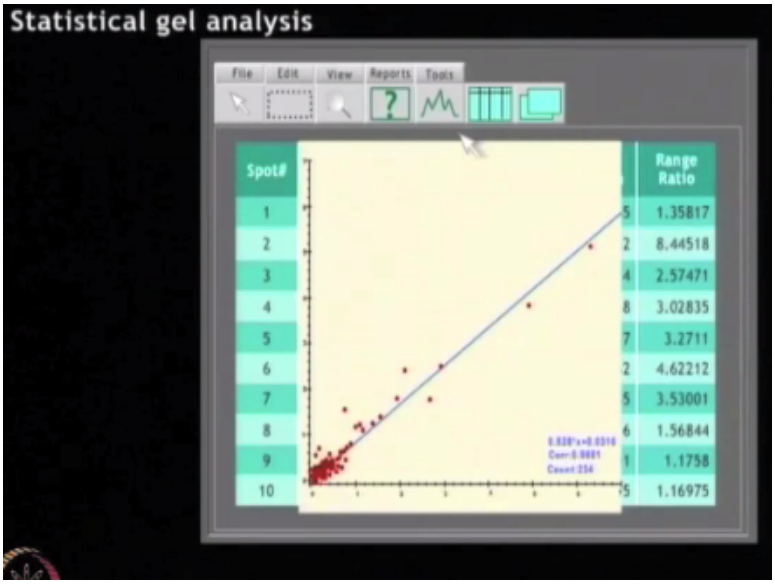

These can provide information regarding inter and intra gel variations.

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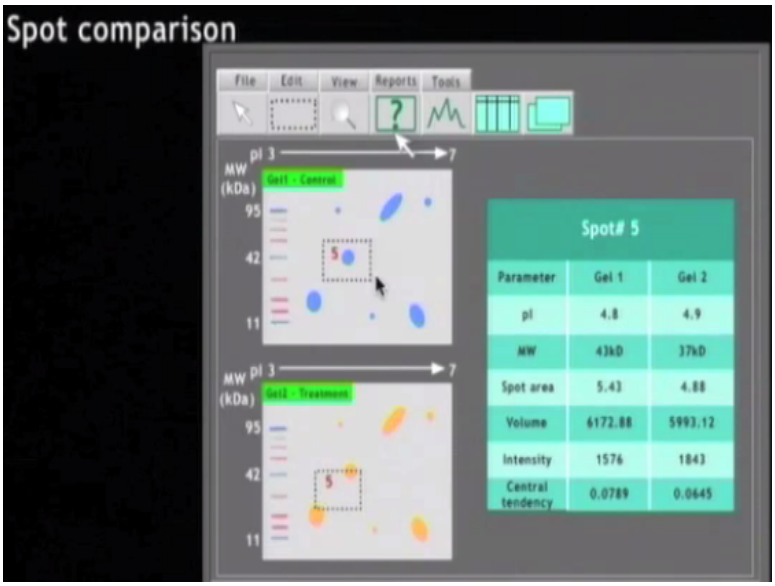

The spot comparison; it is possible to specifically compare a particular selected spot across the gels. When the gel is run with molecular weight markers with molecular weight of unknown proteins can be estimated from this information. For example, as you seen in the animation on the left hand side, you first loaded the molecular weight marker and now from that information, you can compute the information for the unknown protein to calculate its molecular weight and isoelectric point.

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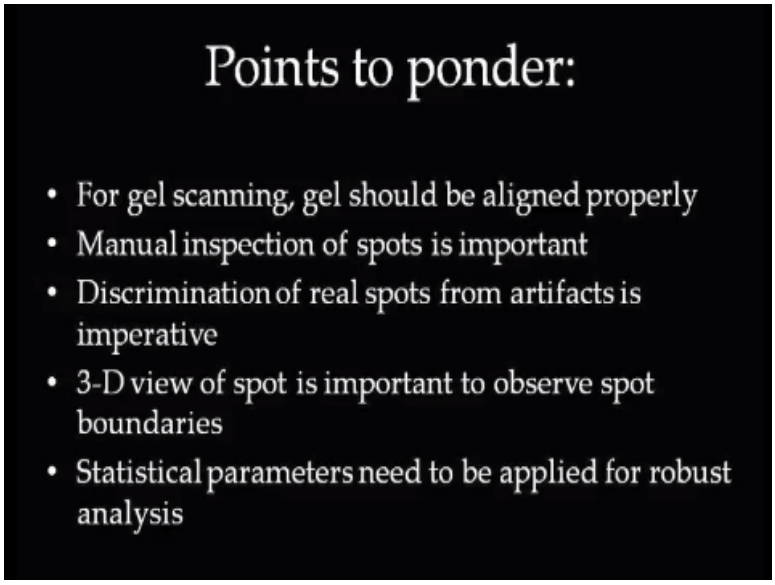

These parameters in addition to the other physical and statistical parameters can be obtained for each spot. Today, I have invited a guest here to discuss the image processing and data analysis by using commercial software, so today we will have Dr. Srinivas from GE Healthcare, who will discuss the; how to analyse gel by using image Platinum software. So, welcome Dr. Srinivas and we would like to initiate discussion with you on image processing.

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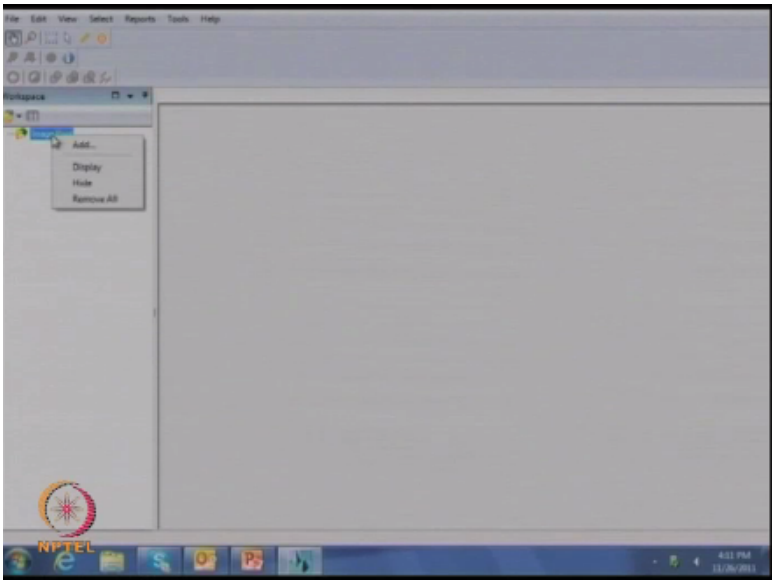

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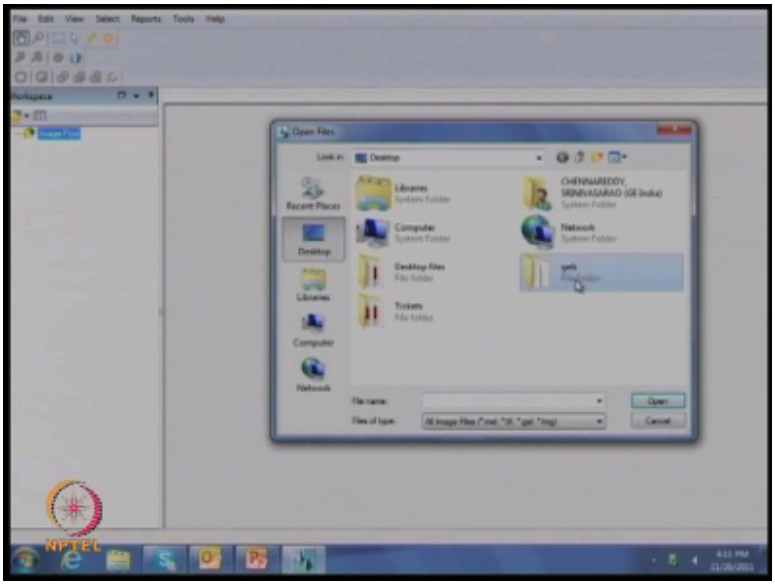

Yeah! This is the software layout, where you can see in your PPTs. This is the original software layout now here; there is there is an option like image loader, add it, wherever you have just stored, you can go to that place.

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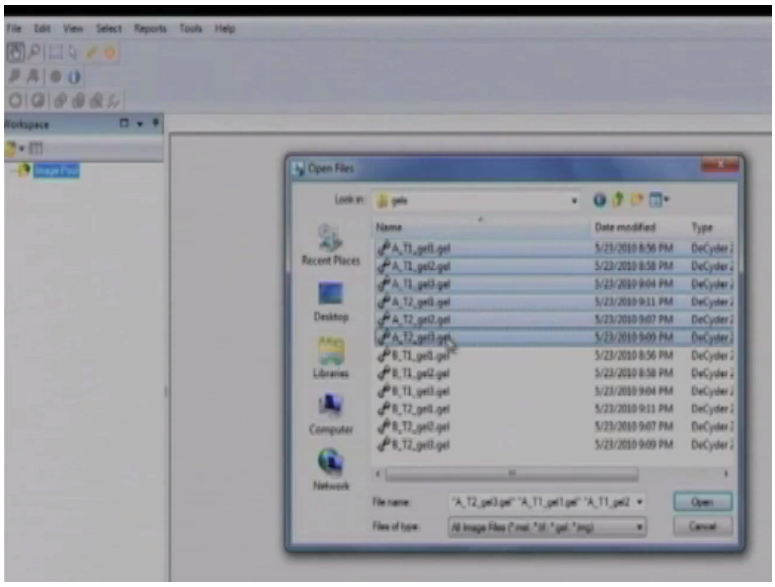

And add the gels like as I have multiple gels here, I am going to add few gels through our software to analyse like gel 1, gel 2, gel 3, and gel 1, gel 2, gel 3.

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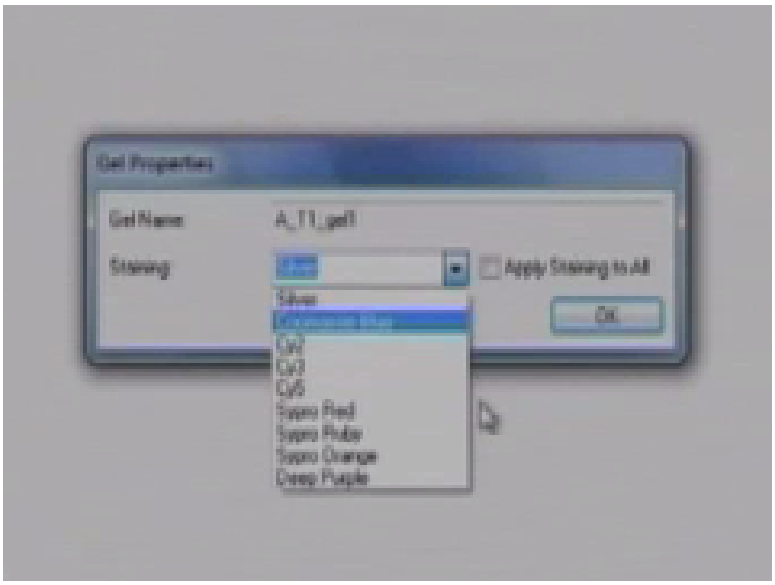

"Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts" So, you are process 6 gels, 6 gels at a time; these are all the 6 gels, basically, 3 or 2 replications like 3 replications for each gel, right. "Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends" Actually, that means one is control and another is the treated in that way, now as which we discussed in our PPTs, this is the thing which is asking for a particular staining.

Here we can give the Commaise or silver or different fluorescent, so as this stained with silver, I am giving this silver and I am applying the same colour for remaining the gels.

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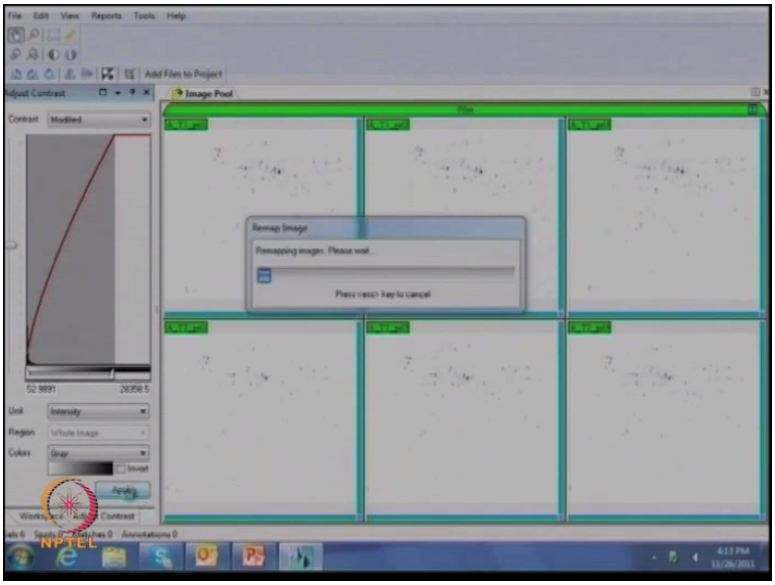

Now, click on okay, now as you can see the 6 gels at a time in this four view, after that now you can see the proper spots in all images; it is a very completely very bright images of which you can see, so now we need to edit the images for visualization. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts” It will be change the contrast; contrast and brightness; different features, “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends”.

So, this is the button where you can adjust contrast and brightness, so as I can show here if you; now we can see any differences now after changing also but one can have to press on apply.

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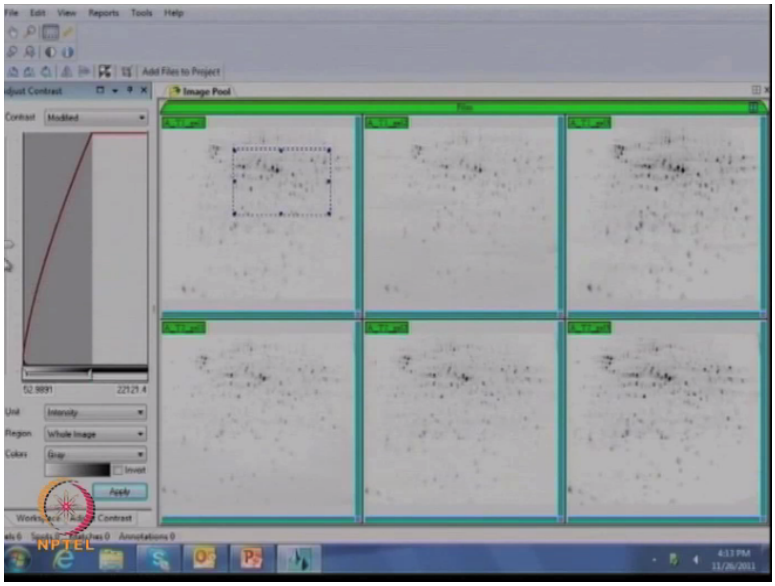

Another feature is available here, “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts” so, now more spots available here; yes, more sharp, “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends” otherwise there is an option that, you can select this particular

area okay, then change the parameters now. Now, we can see real time changes in that particular spots.

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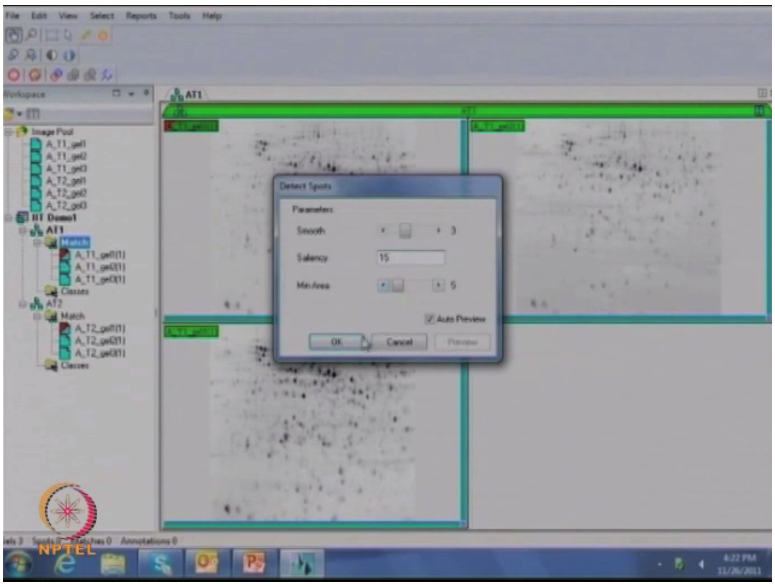

Now, display them; now you have all of your gels here, once you have the gels, we can directly detect the spots now, okay from this button you can detect them. Now, the 3 parameters; smoothness, saliency and minimum area. The smoothness as we can see if you are increasing the smoothness, it is going to under splitting and if you are decreasing this smoothness, it is going to over split, so it is an inversely proportional from this splitting, so it is average value from to 1 to 5 actually.

So, but one can set as a 2 to 3, this value is sufficient for the start actually, and saliency, again this is also again one can set up 150 but to start very good value is almost 15 to 30, okay I am just giving 15 for this initially, then minimum area 5 is quite good enough. If you think in your spot there are more spectacles like there are much Artifacts are there, you may increase this also.

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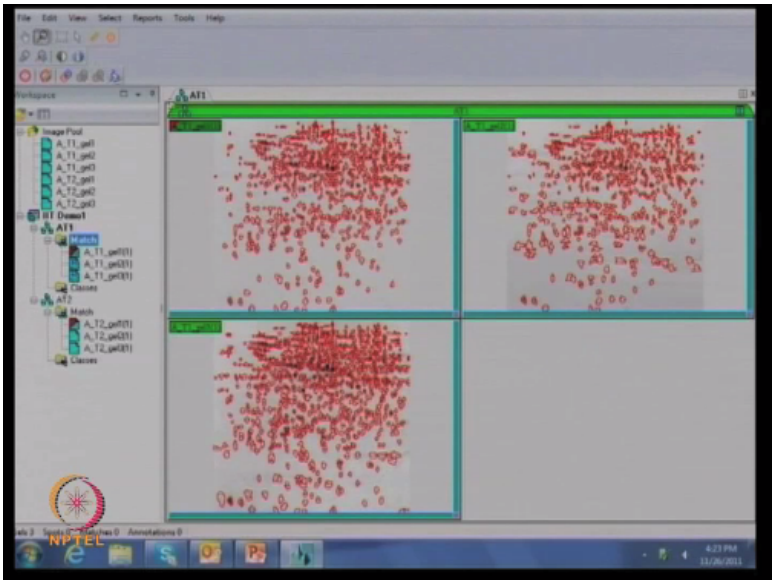

In that case, you can be removed very easily more artifacts, otherwise 5 is okay fine value; “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts” all the 3 parameters now, one can actually accept those; be exactly and then it will be spot, exactly that was the software detecting the spots. Now, it is detected all spots in this gel, if you are satisfied with this particular detection, then it is fine otherwise, we should zoom in my region and see like; yes, exactly I am going to do the same.

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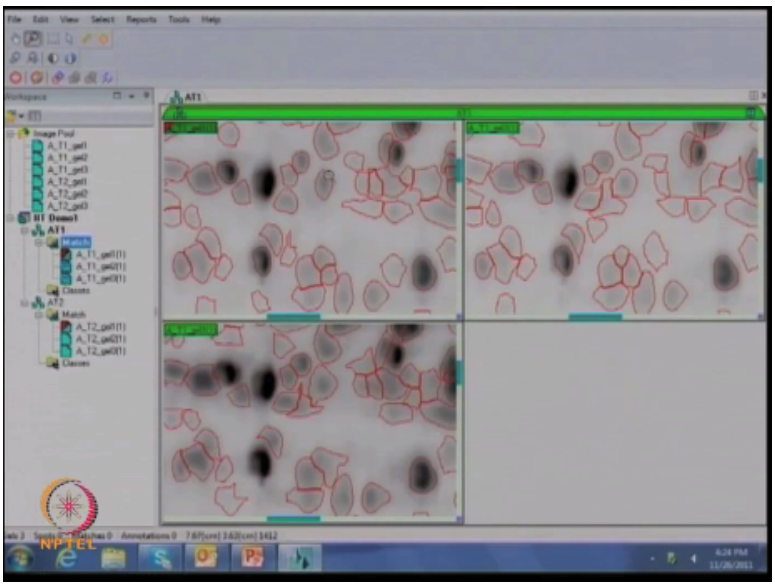

And now you can zoom this particular region, see how this spots are boundary as well as how the detection is there. Can you apply the same zoom in parameter for all the 3 gels? Yes, definitely first come back to original, now select one region and then apply that for all this; no need, you just select like the same region it is going to; got it, right. I think it is very useful to see this way because exactly; I think the parameters are very well defined; exactly.

Because it has properly defined the boundaries; properly define the boundaries and let us have a spot where one can know; quickly know, exactly if you want to edit these particular spots, right where as you can see there I think you cannot see there is a protein but it is showing as a protein, one can delete this kind of spots also like, one should have to go to edit this part. There is an option like edit enable, go there and just delete the spot, wherever the spot you would like to delete.

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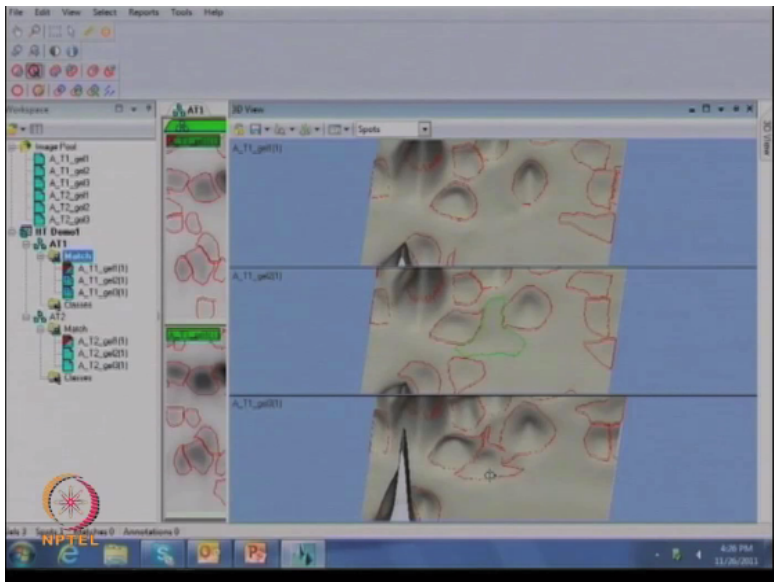

This particular spot I am not interested in this then I can delete it. But, before we take a delete, can we just have a look on the 3D profile; Yes, definitely to ensure that we are not actually deleting a real spot. Definitely; definitely; reports, 3D view, so the spot, which you are interested in discussing that is the green boundary but it is only present in this particular spot but it is not there and but we can see some portion here actually that particular spot see; but that is why it is detected there also, right.

Sometime it was like treatment may have that is spot appearing due to the application of protein or it is totally shed down, exactly because but it is not the case exactly here because these are all the three replication of the regular same protein, so they should be the same but this somewhat it is not there so we can delete these two spots here exactly, or in this case I guess it is doubtful right, so yeah it is doubtful or let it be we can then finally, we can see the statistical data whether it is there in both just as well as treatment also.

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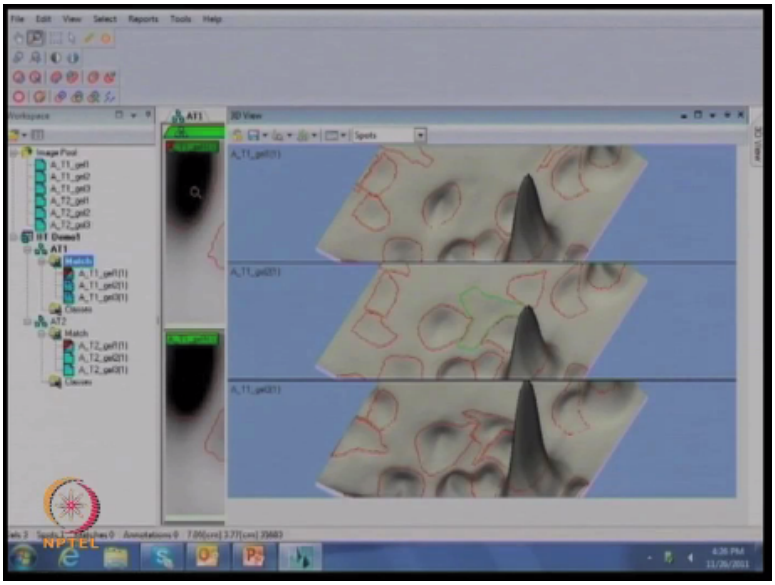

So, that one can go with the statistical parameters, right this will be helpful there, so no need of deleting also in this case. Now, let us look at in detail about one of the real spots. Otherwise, I can visualize this thing; I can more zoom so that one can see more; yes, now this is the intense spots, which you are visualising, another boundaries are marked from the 75% from the top, yes.

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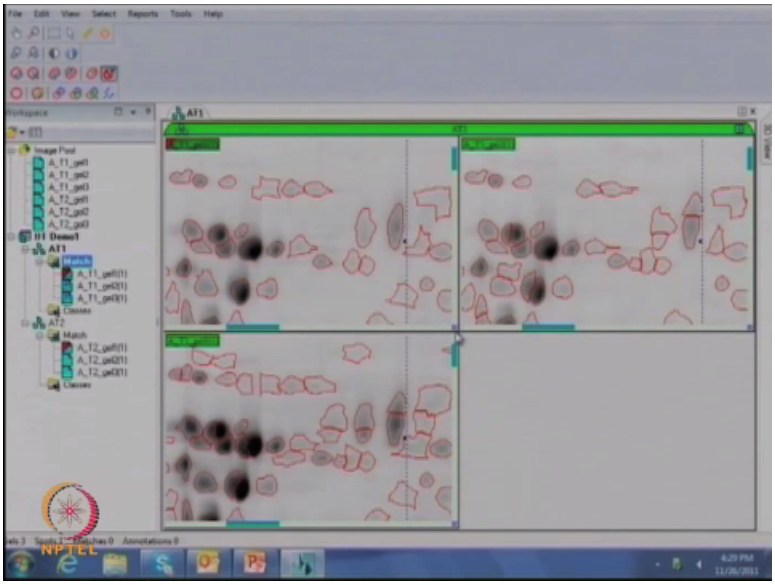

Now, let us say; we can actually zoom out and go back to the whole gel, so let us say if in a region we have missed out some spot like, software has missed out, yes then there is an option to add the spot, exactly, you see this is the adding like; let me zoom a; let me select a particular area, interested in this particular area I am selected and let me zoom that now, you can see this particular area in all gels okay, right.

I think it has defined the spot boundary is quite well overall; yes, so that is why it is better to align more on the automated; yes, exactly, that is what it is; exactly, this is what; if you want to include some spot wherever if you are interested, you can definitely add like this kind of extra portion we can as easily we can deal it actually like you can reduce this particular portion; seconds better ways to remove the spot and then draw this spot.

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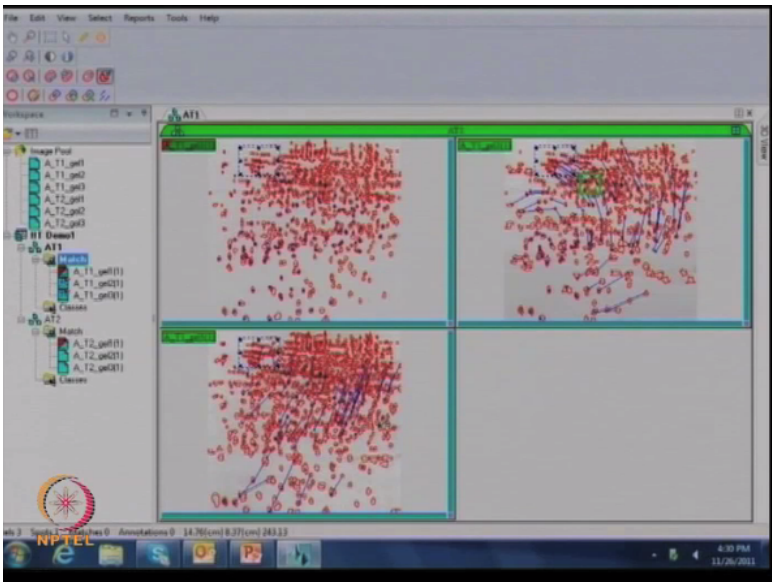

Yeah! that is also better way but both ways you can do actually, yes we can delete it or we can redraw this both as well as you can do. Now, we find now, all spot boundaries are okay and everything is fine now, we can match these 3 gels how our reproducibility of our 3 gels; how reproducible of our 3 gels; okay this is what which we can do here, so come to your original state without zooming and match them.

This is the match option, okay you can match them, so now you are applying the same analysis parameters on both control and treatment; yes, exactly, now we are matching only control gels only; only one group, how our reproducibility is there within one group, this is what we have to check initially okay. "Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends" So, if you have supposed maybe instead of 3 replications, if you have maybe 5 replications, maybe 6 replications out of 5, 6 replication, one can remove that particular gel and they can take remaining 4, 5 gels.

"Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts" So, if there is some time during various experimental run, if there is 1 or 2 gels, which are very bad; very bad which are going to affect the overall reproducibility; exactly so by looking at this type of parameters here, one

can decide okay out of 6 gels maybe one of that is very bad and it affect our statistical parameter exactly; so, may be one should remove that, exactly and then select those one which are having the very good number of matching, yes that should be done. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends”

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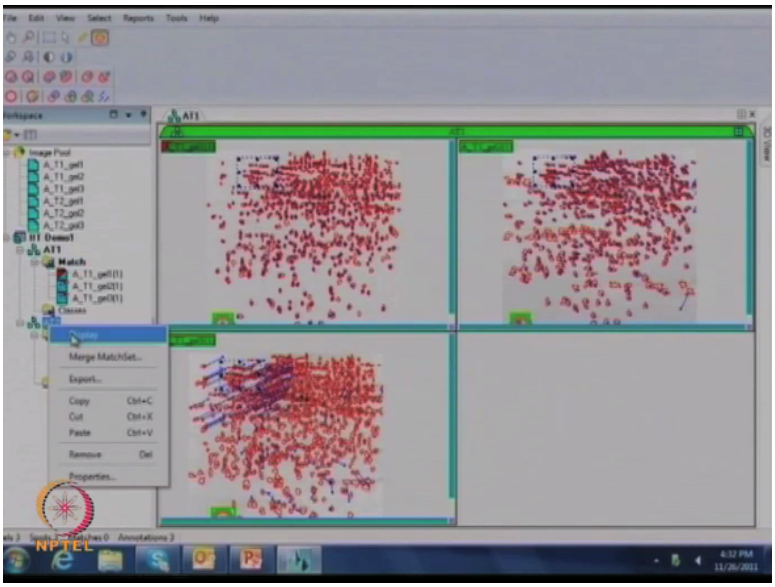

And now, we can see in these 2 gels, there are few vectors which you can see, this we can able to remove by adding the landmarks out of it okay. Now, this is the landmark option okay you have to add your landmark initially in the reference gel only, like if suppose you can think this is a landmark sort 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.

“Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts” Now vectors are; I think improving, yes; based on the land mark position, again match them, so from 146, it has increased to 746, exactly by increasing the; by adding a landmark and now vectors are matching well, yes see; but you can see if there are few vectors but still that; but these are all moving at the same side, so that means there is not a like irregular, so I think one need to go through each region very carefully.

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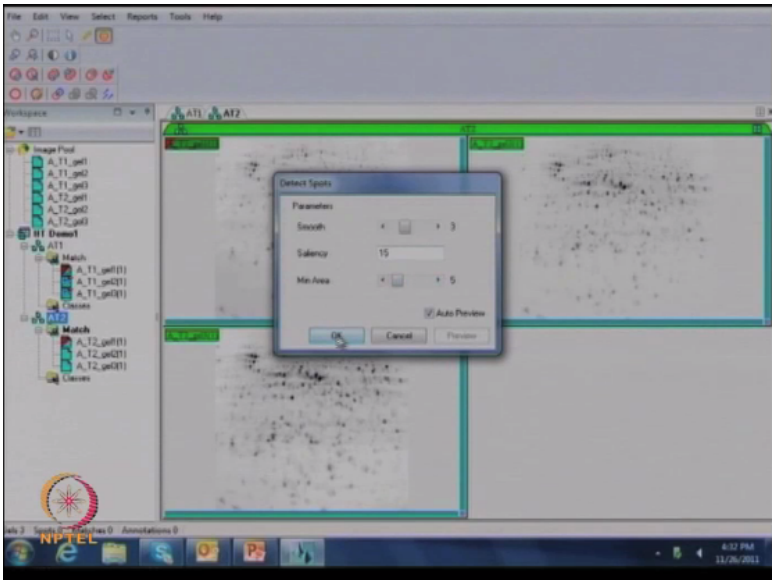

So, if you can add some more; or one more landmark here, it can be removed very easily, this is what we can do, now we can save this particular thing and apply the same parameters to our treatment gel also, that is important because we do not want to make any change in controls and treatments, so you can see no need to remember also the same parameters, if you can detect your spots the automatically gives the same parameter earlier which you have used, okay.

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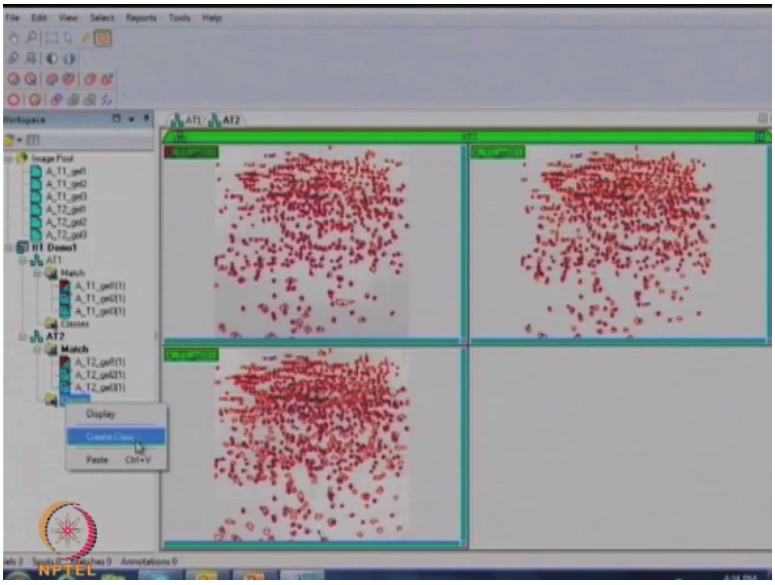

Now, so the same smoothness is 3 and saliency 15, minimum area is 5, so no need to change anything then click on okay, there is a long process of analysing it or rather I think sort of quite quick. It is very quiet quick then it is completely finished, now the same way we can go through each and individual gel by zooming and 3D view and you can select which are all this spots and which are all the not spots then you can directly match them. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends”

Now, I am doing the same matching 12,074 matches have been done, so these gels are more reproducible that means less compared to other set, yeah! see there is no even vectors also without giving landmark also there is no vector, so this is very fine gel, so now, what are they rep; what is the replication which we did was confirmed, okay; these replications are okay. Now, one can analyse in the class analysis in between these 2 classes like inter classes and intra classes sort of things; "Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts" so between control and treatment group, yeah.

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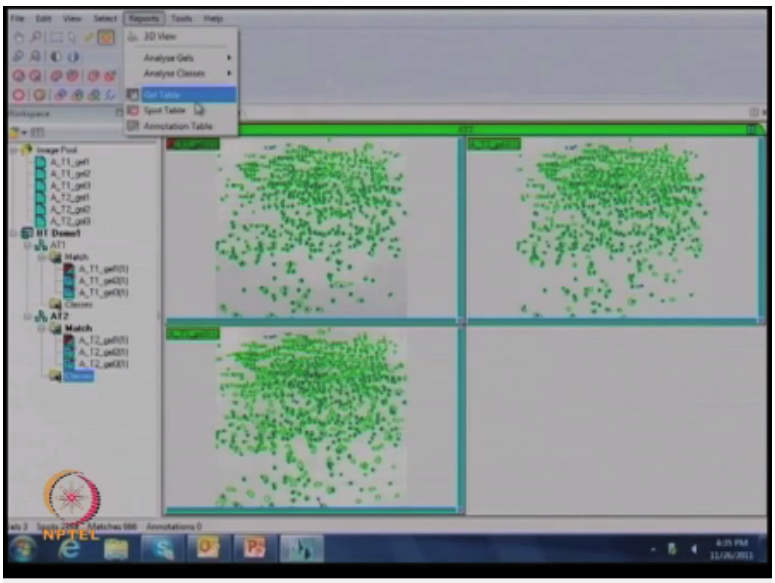

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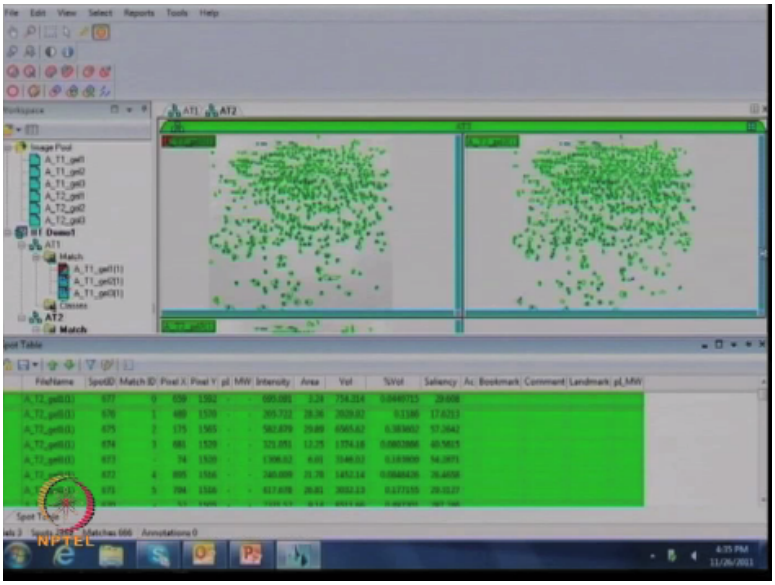

One can actually now do the statistical analysis, so now I am creating the classes here otherwise before that I would like to go to one report point, what is the gel table okay, that is; these are all

gels, which are present here; shift a; you can select all the spots go to spot table see now; what parameters are displayed here in the spot table? This is the filename and this spot ID, it has given this particular spot ID for all proteins and this is the pixels.

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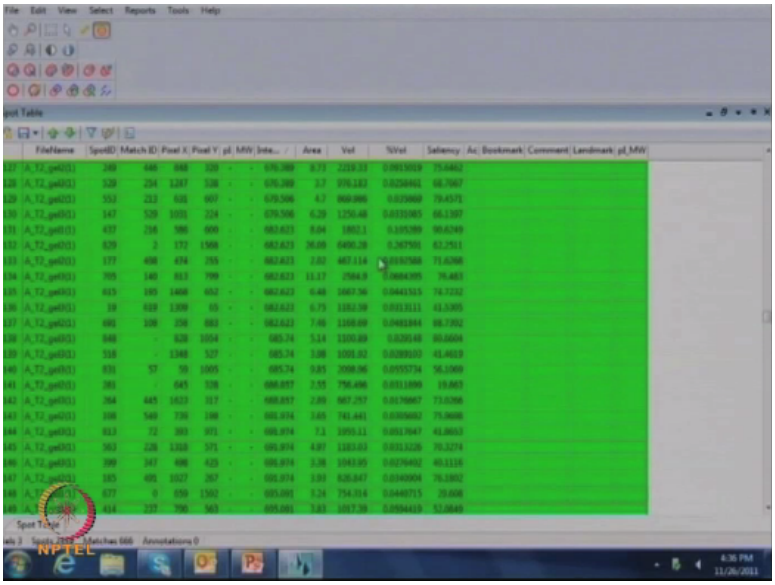

Let me; this is the match ID that means out of 6 gels, this match head it is giving; one second, this is the file name, thus this is the gel number, then spot ID which is given a complete number for all spots, then match ID, then this is the pixel size and x axis and pixel at y axis then if you can; we can able to give the PA also and you can calibrate the PA as well as molecular weight also then intensity of particular spot and area of that particular spots and volume of that particular spot and percentage volume of that particular spot and salience of that spot.

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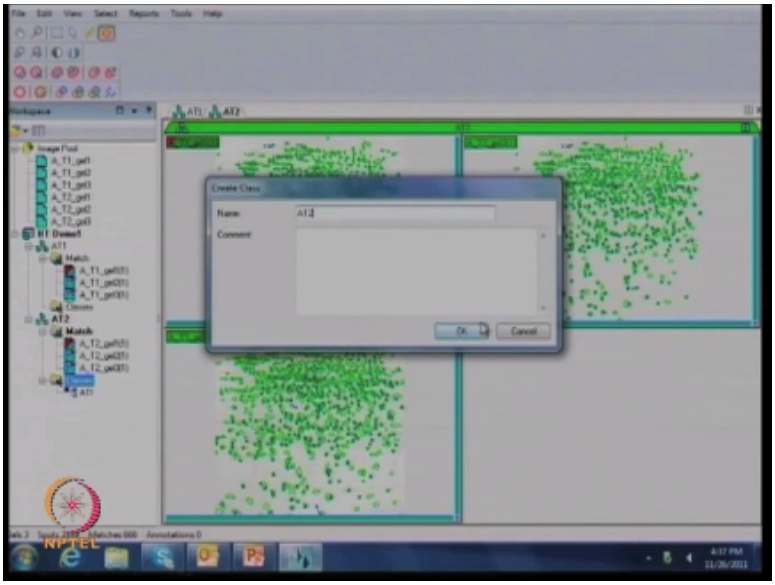

These are all the things which you can see; so all these parameters for all the gels be obtained from the software; these are all the parameters which you can see in the software okay. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends” So, one if you are satisfied with all these values then you can go ahead your next level of analysis that is class analysis okay. Now, I am going to create a class here, create a class, this is 81, another is 82, which is control and treated.

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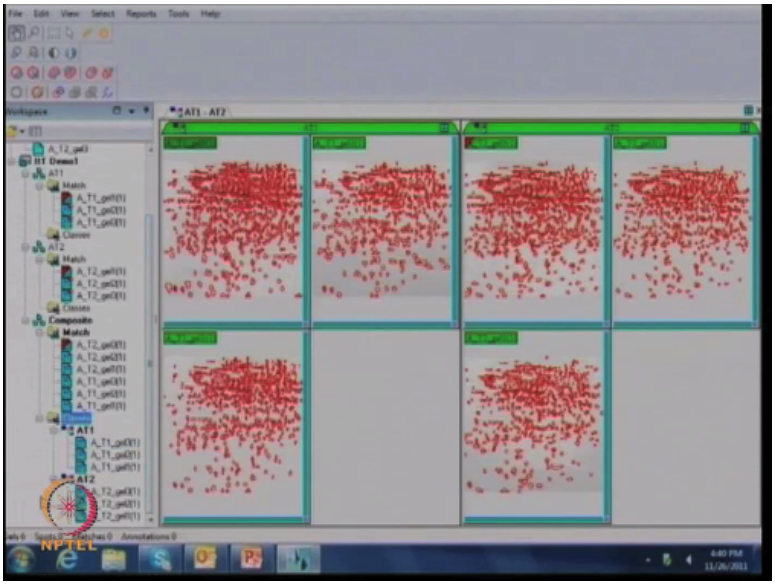

Now, again we can shift these gels into 81 to 81, another match set which I am creating, so where we can easily shift; move easily before going to class, so that; “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts” so you are going all the 6 gels; all the 6 gels into composite max set, so all 6 gels are here and now you can create again the same way classes, so by adding different folders I think one can actually avoid any sort of carry or mistakes with previous one; yes, exactly.

So, that all the steps are stored; yes all the steps are stored, now again, I am shifting all 82 gels into 82 folder, this is the class folder again, 81 folder, this is for class analysis, now we can see both gels together now, 3 gels from the control and remaining 3 gels cells from the treated okay, right, this is what which you can see. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends”.

Now, it is behaving; it is taking values as a single gel of 81 and single gel of the 82, now it is going to average of these 3 gels and now it is another averaging this remaining 3 gels.

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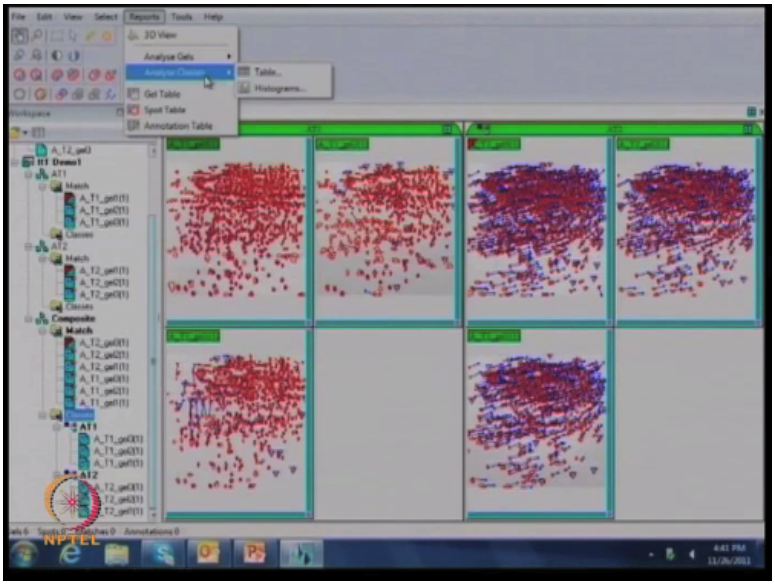

So, a single value you can get from the control, another single value can get from the treated. Once you did the same thing then quickly match them again, one can remove this; all these vectors by adding the again or particular landmark, so same process what we did earlier.

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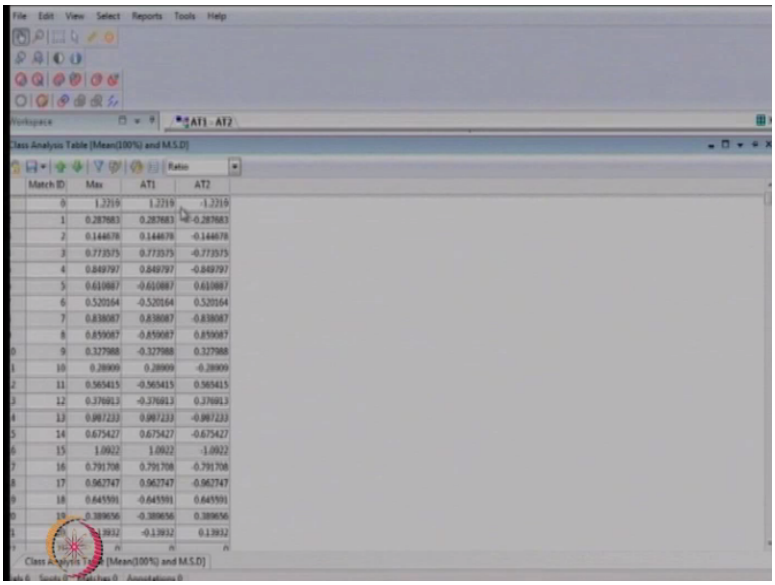

Now, go to reports where you can see the analysis classes go to table, now you have the match ID that match ID is the particular spot number as well as the maximum area of this accomplice maximum; whatever the maximum area as well as the; this is the 81 value and 82 value okay, "Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation starts" so for the control and treatment yeah, both groups; we had 6 gels, right yes, so what are the values in each of the 6 gels for each spot; for each spot exactly.

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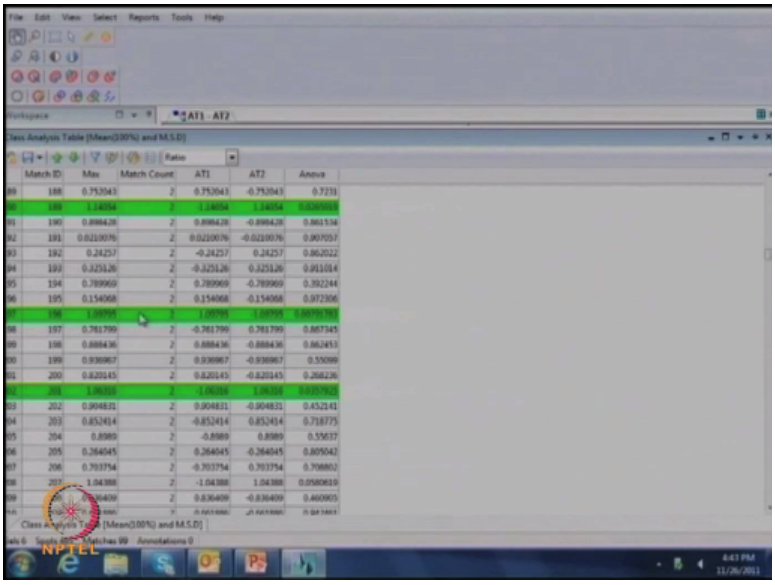

Oh, this is the averaged value again, average of all the 3 gels from each; ratio value if you can see, this is the ratio value which is giving the up regulation or down regulation okay and I think we need to add yeah now, this is the statistical parameter of various software gives the option for; exactly so this is the ANOVA value which we can see. “Dr. Sanjeeva Srivastava – Dr. Srinivasarao conversation ends”.

On the basis of this ANOVA value we can easily select our interest of protein as well as you have the fold regulation also this is one fold up regulator and this is the 1.2 fold down regulator. The same we can see the whole values now, no need to go each an individual spot, you can filter them easily, now what we are going to do is first at least ANOVA values which we are going to take; the maximum we are going to take is maybe 0.05 as the statistically significant value, so those many spots it can be highlighted, right.

So, this is the spot which is already undergoing this particular threshold and you can see all these spots which you have these particular; this particular ANOVA value, those are all highlighting here. Now, it can be very easy; one can easily go through that and this particular table you can easily export to excel also from there you can have your complete data also; this is what the complete analysis software gives you the output.

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

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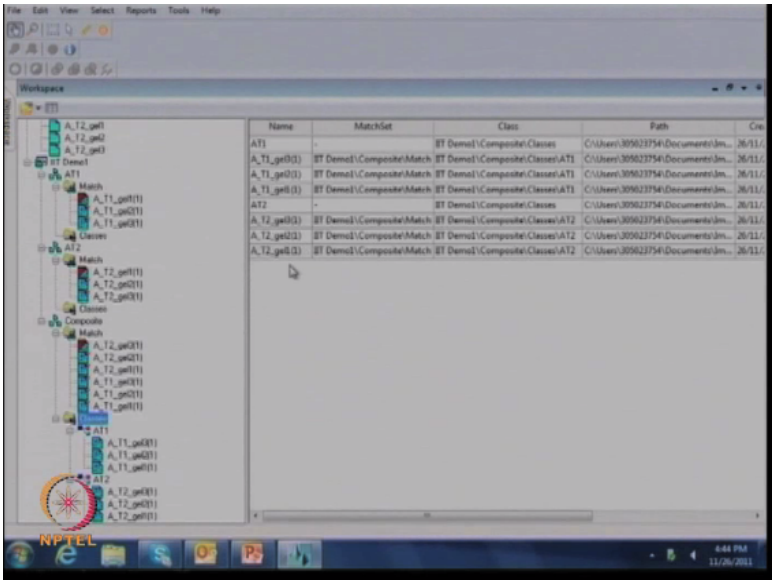

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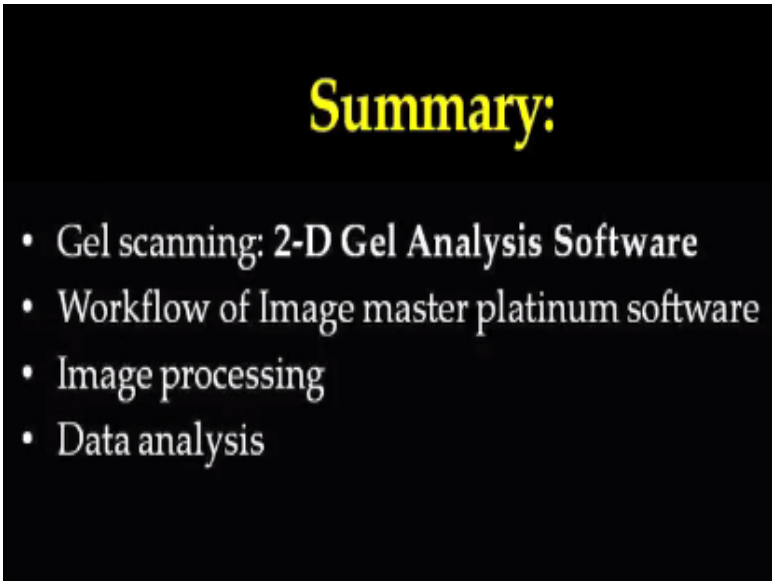

And then one can actually still go back to those spots which software say this is significant and look at manually each spot to verify, that is the real spot. So, thank you very much Srinivas for very useful discussion and demonstration on the software for image processing on 2 dimensional gels and will continue our lecture flow for further 2 dimensional difference in electrophoresis in the next class.