Proteomics: Principles And Techniques
Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture No. # 15
Two - dimensional electrophoresis:
Image processing and data analysis

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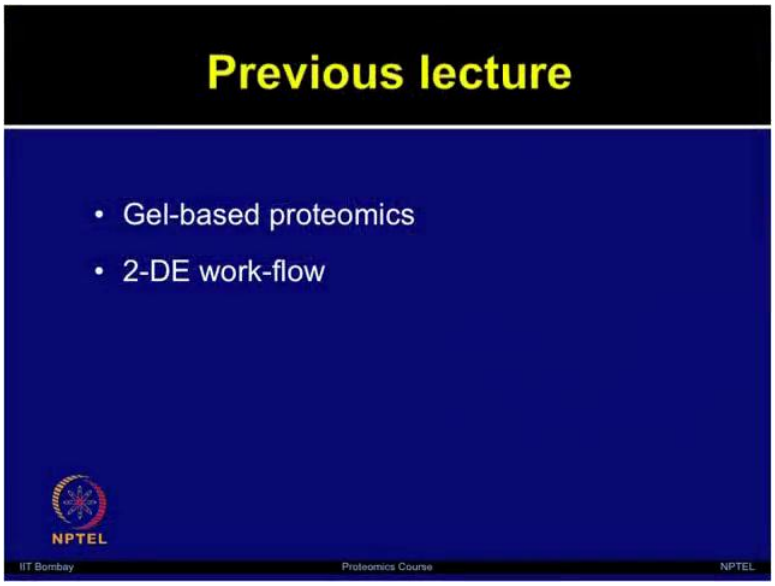

Welcome to the proteomics course. In today's lecture, we will talk about two dimensional electrophoresis, image processing and data analysis. So, as in the last few lectures, we have discussed about two-dimensional gel electrophoresis and in fact, various type gel based proteomic techniques such as, s d s page, blue native page, we have talked about different type of new advancement like offgel fractionation as well as two-dimensional electrophoresis. We also discussed in very much detail about two-dimensional electrophoresis workflow. How can you perform the two dimensional electrophoresis based experiment step by step, various details integrity of performing protein extraction, different type of challenges associated with the sample processing, followed by how to perform iso-electric focusing, and then how to do SDS page analysis, in between these two, what is required to makes these strips compatible for doing SDS page, which was discussed in the equilibration step, and then the second dimension separation using SDS page.

Now, once you have run all of this performed steps and run the gel, after that you want to visualize your spots. So, you need various type of staining techniques. So, we discuss

different type of staining methods, which are available. And then once you stain, those gels you can visualize those spot, you can see how many protein spots are visible on the gel and then those spots can be further scanned for image processing. Now, a good scanning image processing and followed by a data analysis to obtain some meaningful information from these gels is very challenging.

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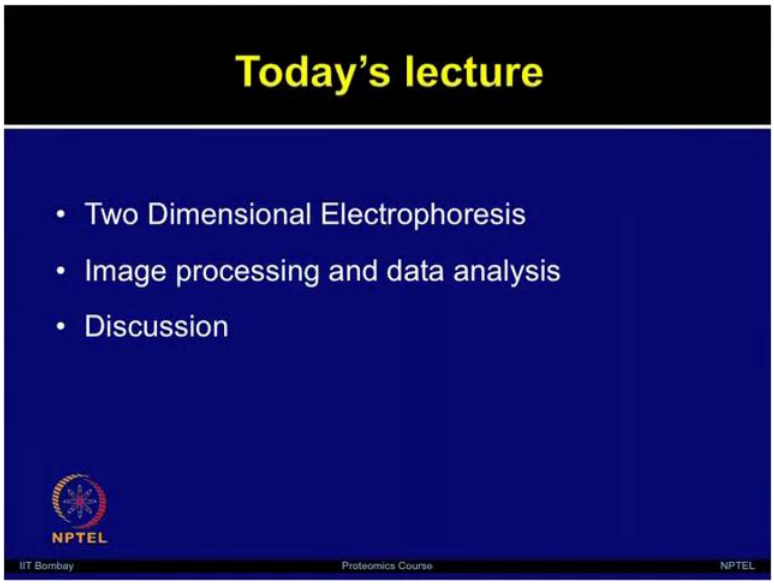

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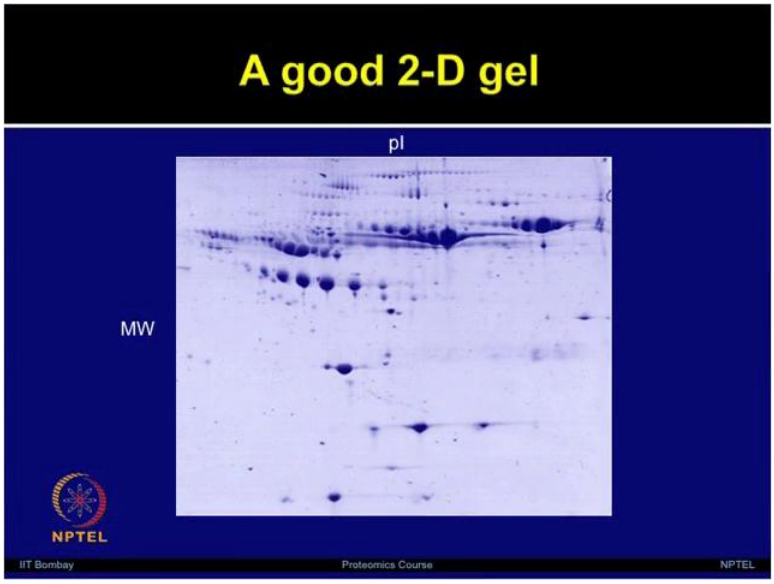

So, in today's lecture, continuing our focus and theme on two dimensional electrophoresis, we will discuss about image processing and data analysis, various type of challenges

associated with the image processing and what are the steps required to perform data analysis. Now, when you have run a very good 2-D gel, you will expect an image, representative gel shown here, where you have well resolved spot throughout the gel; this is a gel image shown from serum protein extracted from human and those proteins spread on the two dimensional gel.

Now if everything goes well, you can see the well resolved spot throughout the gel and the whole proteome can be well separated. But many times this is not the case, many things go wrong, many times the salt will have some interference, nucleic acid contamination will have some effect and you can see various type of artifacts, you can see different type of vertical as well as horizontal type of streaking on the gels, these small details make big difference when you are doing the gel analysis for the image processing part. So, how to analyze two gels by using software, is not so tedious, but when you are talking about many gels, where you have large number of controls, large number of treatment, then it becomes very challenging, because all your gels should have run at the same time, with same (( )) migration, but many times that is not the case, you cannot run fifty gels or hundred gels in a given day.

So, there will be some manual artifacts, some day to day variations. Now on one hand it is good idea to keep all your parameters very consistent, so that the gel migration, all the protein resolution will be of the same parameter; at the same time even if all the gels have not run exactly same length, still the software's can help you, for the image processing, you need to do the various type of image processing, before you analyze your gels.

Now, we will talk about different type of image processing tools, how to first prepare the images for data analysis, and then how some of the commercial available software's can be used for obtaining very meaningful information from these gels, whether you can see some sort of differential regulation of the proteins, up regulation or down regulation or you may have some unique proteins emerging on your sample type your treatments. So, these types of analyses were performed on the software. And then followed by, one need to the statistical analysis to obtain confidence in the data, how meaningful this information is and whether I can proceed with this spot, for further protein identification using mass spectrometry.

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So, all these details require very much practices, one need to really spend hours on data analysis. In fact, running gel sometime is much easier as compared to doing very meaningful data analysis. So, to discuss about details for various challenges, which are associated with analysis of 2-D gels, the image processing tools; as well as what are the details required for image processing by using various commercial software. 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 Health care, who will discuss how to analyze gel by using image platinum software.

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So, during this course of discussion, we will talk about stepwise procedure, how to take this scanned images, the raw tifffile and then align those, make the (( )), and then followed by do the spot detection and various type of manual refinement for image processing; and at the ends we will discuss about, how to obtain meaningful information by using various statistical analysis. So, I will have a discussion with Doctor. Srinivas now. So, today we have Doctor. Srinivas, an application specialist from GE Healthcare, who will be discussing the challenges of image processing and how to perform the two dimensional electrophoresis image processing and data analysis.

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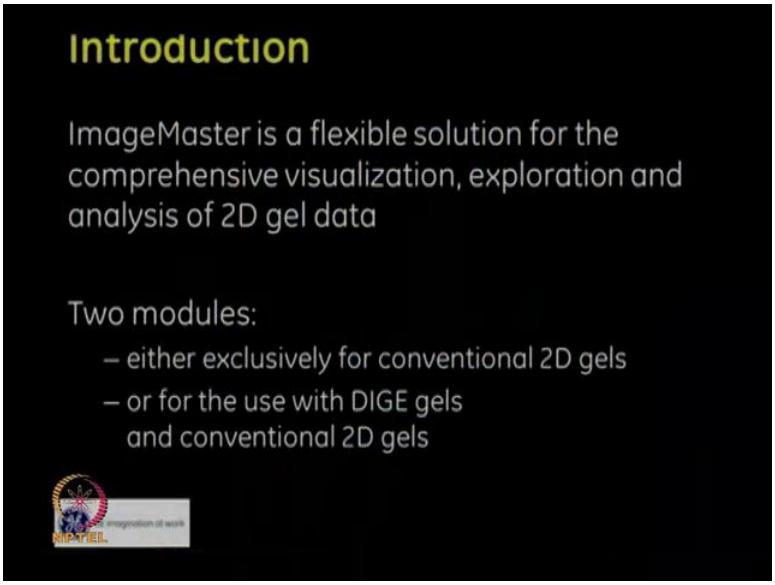

Today, I would like to explain about the workflow of image master platinum, now when you can see it is very important software to analyze 2 d gels especially when we have the multiple number of gels, it is very impossible to analyze manually. So, this software plays very key role in the analysis part. So, as you can see majorly, there are two modules are available, the excel image master is a flexible solution for the comprehensive visualization, exploration and analysis of 2 d gel data; two modules are available in this software, the first one is, image master 2 d platinum, and within this we have another module, image master platinum which is enabled for the dice application also.

So, it means that the same software can be used for the traditional or regular two dimensional gels as well as, it can also be used for the advance techniques such as difference ion electrophoresis.

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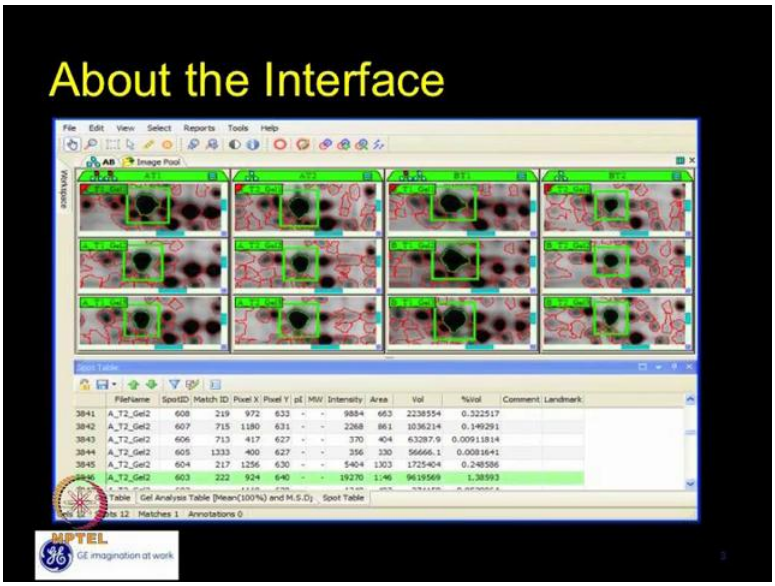

Yes, it can be used, but up to some extent, you can able to use, if you want full-fledged dice analysis, one should have the decider, this is what for conventional 2 d electrophoresis, image master platinum is the better tool to analyze the gels. So, if you can see the overview of this particular i m p software, there are three major parts one is the complete icons, where you can see the complete file tools as well as, edit view and these are all the views and after that you can see image view, thus these are all the images at time, which you are visualizing almost all 12 gels at time which we are visualizing at particular place and the third view which we can see, all the total number of gels which is present here. These are all the major visualization part in the software.

These are the three major interfaces here and you can see even, the workflow in the left hand side, once we go through the software analysis demo, there we see the complete details for this particular feature also. So, automatically software can walk through this stepwise.

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Then, if we can see the complete workflow of this particular software, first we need to add images then, edit gel images, the editing is very important because, one during scanning one may have some extra portion of the gels as well as, some streaking of the gels at the cathode end or anodic end, these all should be removed from the gel. Or even this can come from the staining artifact or even some dust particle, the scanner. Sometimes, air traps in between the glass plate and in the gel.

So, it should be taken care. So, for that edit imaging is very important then visualization of image then, construct match hierarchically, there is a procedure how to create a match set and how to create a class set, there are two differences we will discuss more in our software demonstration then, processing gel images, where we can detect the spots and whether, we can edit those spots like how these are all the original spots or these are all the some dust particles or else if there are couple of spots, which is coming and showing as a single spot in that case, we can edit that particular spot, we can split them into two different spots and somewhere if the boundaries software itself gives very nice boundaries, if you are not satisfied with those boundaries, you cab able to split easily, you draw yourself, but on the boundary you would like to draw, this is what we can do.

Then, data analysis once you did all these things, you will match your gels and after that you get some statistical data like a nova and with the help of these you can exactly see,

which are all the up regulated and which are all the down regulated and which are all the similarly present and these all you can see actually in this output.

So, I guess preparing images for analysis, that is very crucial because, although there are lot of claims from all the software that, its very automated procedure, but regardless of that, I guess all the software pick up lot of artifacts, even the small dust particle and even stains will be directed as a sort of a small spots on the gel. So, one need to have both automated as well as manual interference and a balance of each to ensure that; whatever spots are picked up those are the real spot. And I guess that is, where you mentioned different type of spot boundary which can enable us to remove the spots or add a spot or drawing the right boundary. Even I will go through each and individual those will be very critical. We will discuss those in more detail, in the image analysis workflow, first we will go through the add images.

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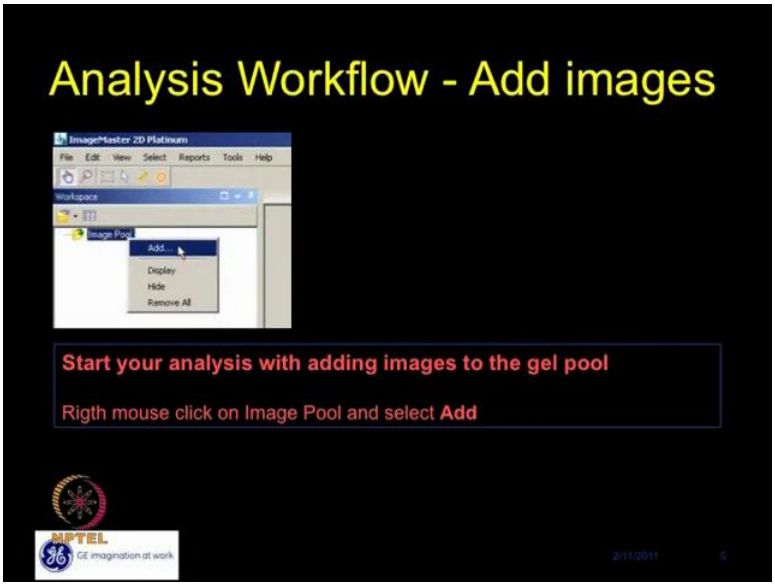

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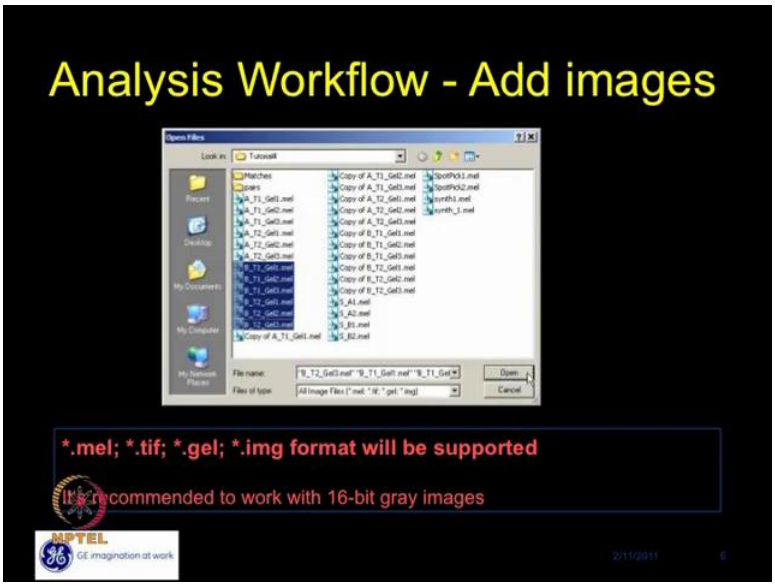

How one can add images to the software, there is a option when you open image pool, as you can see here you can add any image, which is stored in any area of your computer like once you add them, you can see it goes to that particular area and if the software can able to analyze dot gel, dot Mel, dot tiff kind of images, it very easily it can be analyzed. Various types of accept the simple jpg or uncompressed tiff, these kind of gels we cannot able to edit, but other than that, dot tiff, dot gel and dot Mel. So, it requires high resolution images. So, that it can have more information with this image at least the image should contain 16 bit resolution.

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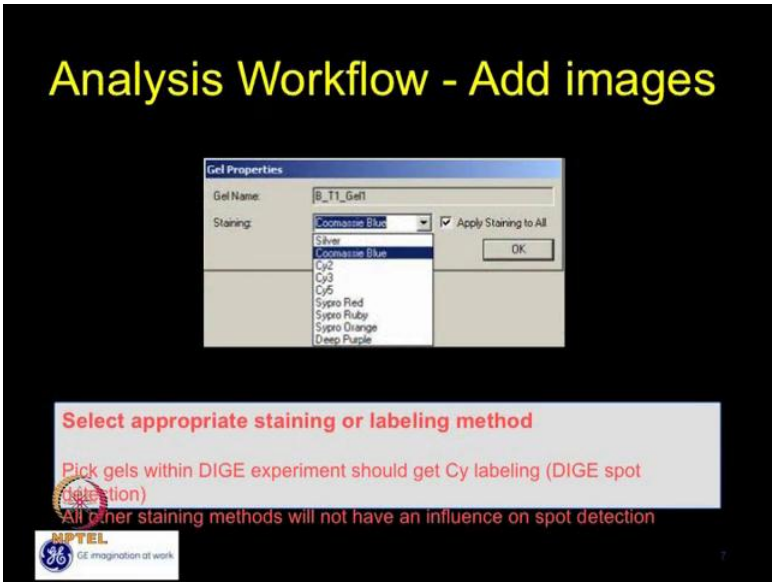

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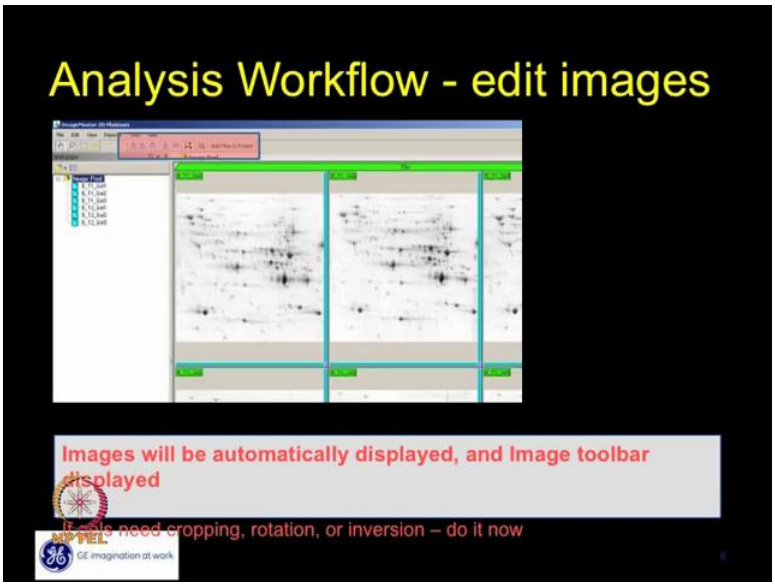

This is the important thing in this software, during adding your images; it will ask you what stain you did for your gel. So, we need to mention our color especially, they are in within the software, we incorporated different staining those are like comas sine, silver or different fluorescent color which you have to mention, once you mention that, then you can open your images now. So, what is the use of defining the different type of stains is that going to change certain parameters based on what type of stain, we are going to define. By giving this particular staining one can give a pseudo curve for these images. So, it will help you to view the images, this is what it.

So, if you have a stain with the comas sine, you can see in the blue background and with the silver, brown and different type of stains as well as different fluorescents colors like r b g. It has stored tiff image in the grayscale and that we are going to analyze. But rest of the things is just for visualization; whatever the editing of the visualization that would be only for your visualization, there is no change happening in data. This is the major important thing; I think that is very important, for people to learn even, if they are playing with the image that is not going to change the raw data, because all are just helping you to visualize the image and align it properly.

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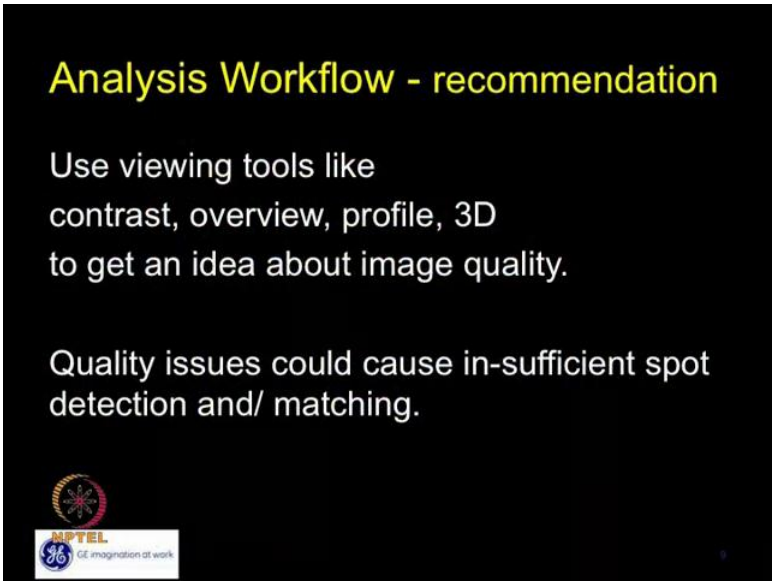

And during your analysis workflow, there are few recommendations, which we can give those are like use viewing tools like, contrast overview, profile 3 d image as well as, to get an idea about image quality, if once you can go through all this parameters, definitely you can decide this image can be analyzed or this cannot be analyzed. So, once you get an idea of this. So, you can go ahead in your analysis of your data.

So, it means the user has to thing for each spot, if there is any doubt whether, this split spot or real spot, one need to look at a three-dimensional profile of it. And also look at the contrast and just try to change different type of parameters. To ensure whether one is really handling a small spot or an artifact, even as we know, if any dust particle give you a spike and 3 dimensional views and one direct protein can give you, the perfect peak sort of thing. So, this is able to differentiate and we can decide.

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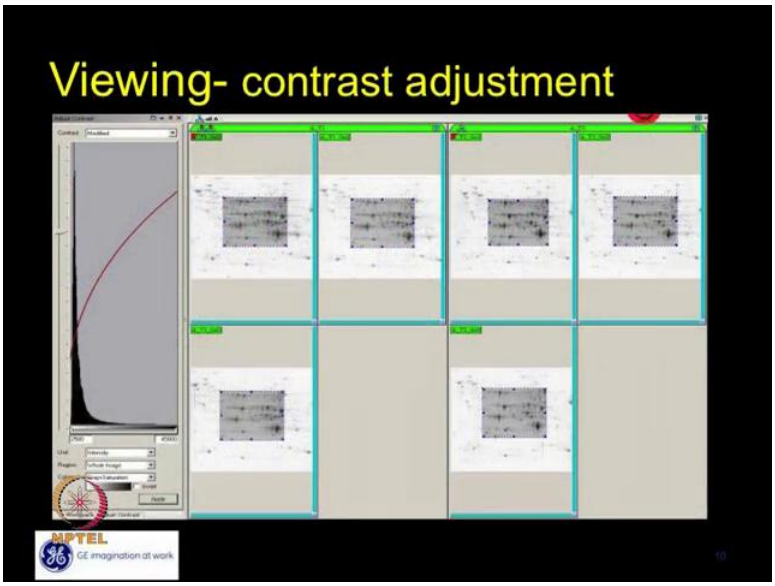

This is what we can do, how to adjust the contrast in our workflow you can see, there is an option on our from view, there is an option to change the complete contrast and brightness whatever, you want in that case, you can select any one of the box from the select tool, after selecting that particular box tool, you can change whatever, you want from the x axis where can see from y axis, you can see that is a contrast and as well as brightness. So, the real changes you can see, in this particular block.

After changing whatever, level of changes you want for visualization once if you think this is perfect view then you can press on applies. There is a potion like apply you can just click on the apply button then, it will the same parameter will be given for gels this is what it can be done. So, if you have a match set of let us say twenty gels. So, if you adjust it for one kit can be applied for all gels. Applied for all gels the same parameter can be applied for the all twenty gels.

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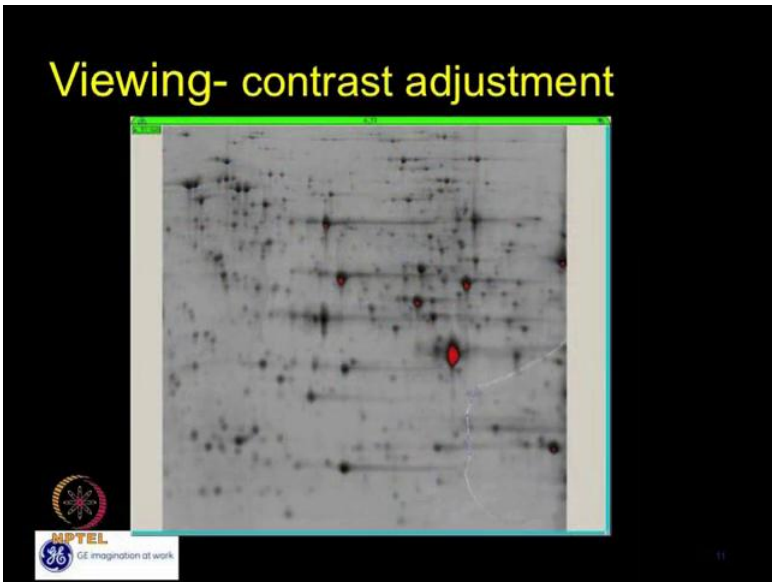

Exactly and after doing a after setting this your contrast adjustment everything, you will see the final image like this. Still there you can see the saturation like in red color. So, this can be removed like again readjusting the particular. So, these red dots which are indicating for saturation are actually generated by the software. Software is indicating that this contrast parameter is not suitable for perfect, so you may need to change little more.

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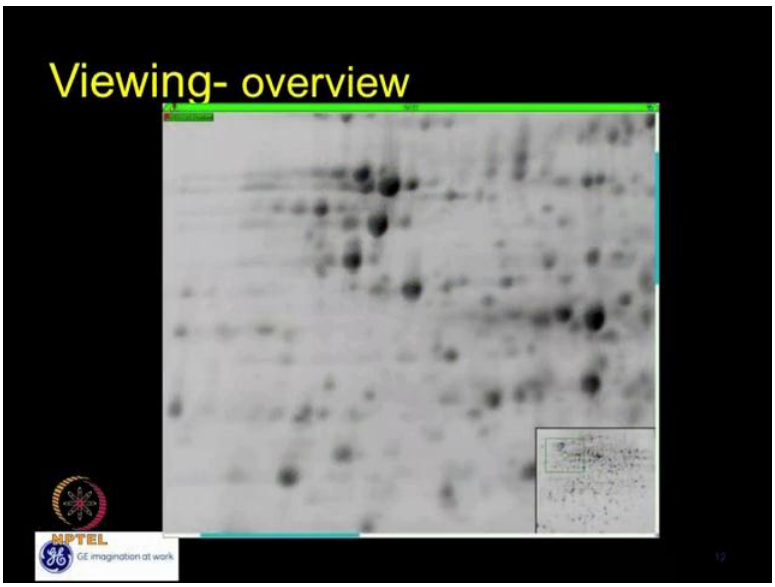

There is a zoom option in this you can see that particular area directly in a big scale. So, that you can see if is there any lower bounded portion or if there are multiple spots binding

together and showing as a single spot in 3D view as well as you can see like this also. You can decide actually here when manually one can draw the outline with zooming, that particular image. So, this is very helpful tool again. Yeah there is another tool in the software like profile view, there you can select the particular profile, where you goes into a particular spot. It gives you that particular profile of the x axis as well as y axis. Yeah any other spot it just wherever mouse cursor goes there it shows that particular.

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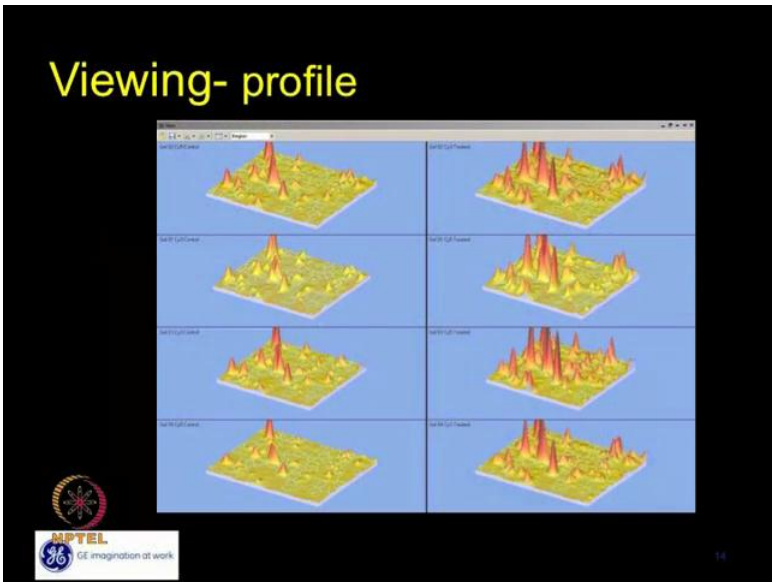

Another 3D view of a particular area here earlier which we are discussing a particular spot as a 3D image, but if you want to see a particular area also you can see as a 3D view. This is what you can able to easily identify which are all the dust particle which are all the real proteins spots this will helps you.

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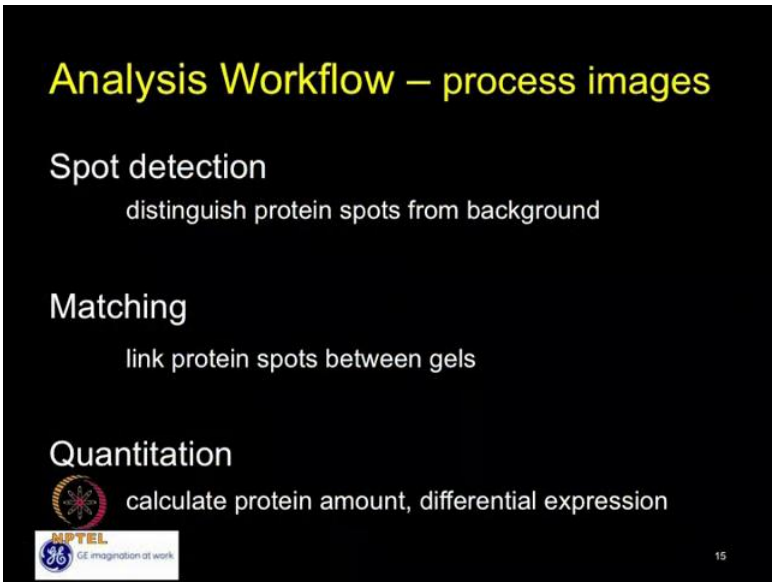

Now, let us discuss about processing the images and here the first thing we need to click on this spot detection, once we have to detect the spots even after the spot detection, we need to match them. So, this processes automated the spots detection process

The spot detection process anyway we will go more detailed in the next slide. But I am just saying there are 3 major steps in here that is the spot detection matching quantization at least three are the major steps. When we can see the spot detection here, there are three parameters those are like smoothness it gives, when you set up the lower smoothness it over the spots like it try to split the spots as many as possible. When you try to give high value it is under splitting. So, one can go through your image and how it is splitting you have to set that particular smooth value. If you adjust trying to detect the spot so, first of all you need to give certain commands and parameter to the software. Play with the numbers that are optimum numbers like if you can say smoothness two to three is quite enough. There is no much variation in this, but whereas, saliency this is very critical parameter where it takes the 3D image and it sees the exactly how slope is that particular protein, if you are giving the highest slope then it will take the very frivolity.

Then when you are giving the lower value, it will take the lower probability so, higher the value higher the probability. What about minimum area? Minimum area actually most of that can be removed, it can easily remove the dust particles because any protein having the minimum area above five at least. So, we are pretty five as a minimum area, most of the

times. Ninety percent cases we can put five as a minimum area. So, it removes the dust particles into consideration only proteins that is what we put minimum area. So, I guess more clear when we will really look at the demo of the software when you can actually show that, by changing the numbers. What is the real output?

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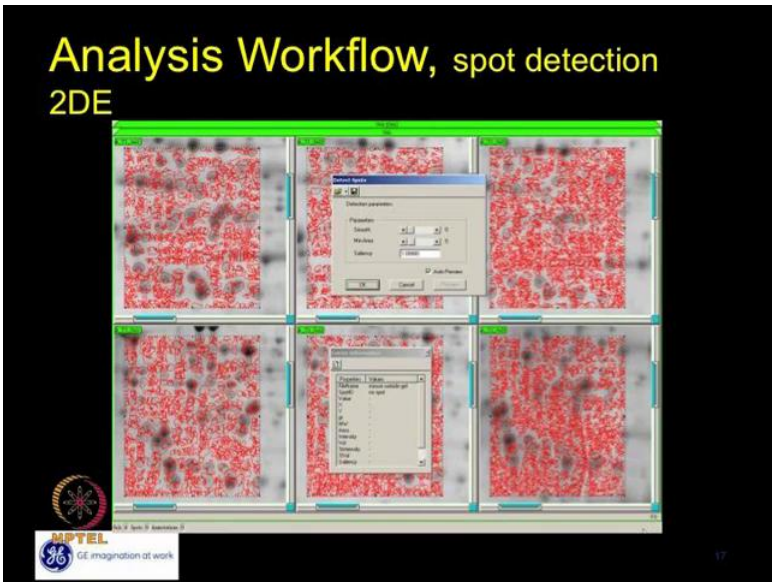

Real output you can see easily during our spot detection. It looks like whether it is splitting or it is this optimally splitting, but everything you can see on the screen and you can decide whether this parameters are or not you can one can decide easily this is. Suppose that boundaries are software generated and these are all the parameters which you can see, are talking about the smoothness saliency and the minimal area.

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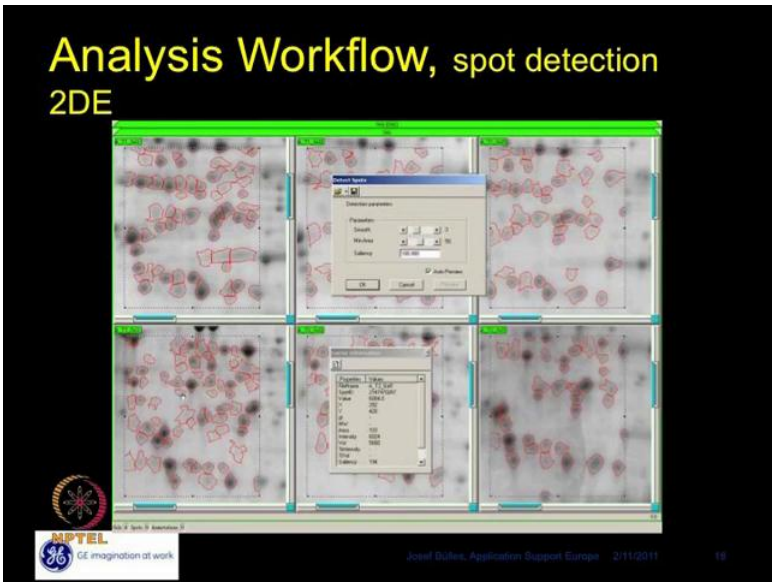

Minimal area this three spot detection once you put this, earlier we can see these are very detector; that means, they have given some wrong parameters after that what they given some optimal parameters. Now you can see its perfectly detected and perfectly boundary. So, I think main thing is first of all one would like that software should detect as many spots as possible in very accurate way, and then whatever the still leftover, because I can see in the slide in your first image. There are some spots which we are not able to detect by the software. So, now those spots can again be detect by the manual way by adding the spot.

Exactly, even there is another option I think that there may be removed that particular area that is what it is not at all detecting this particular right handed area because there is another option exclude area. So, we one can exclude that particular area in analysis.

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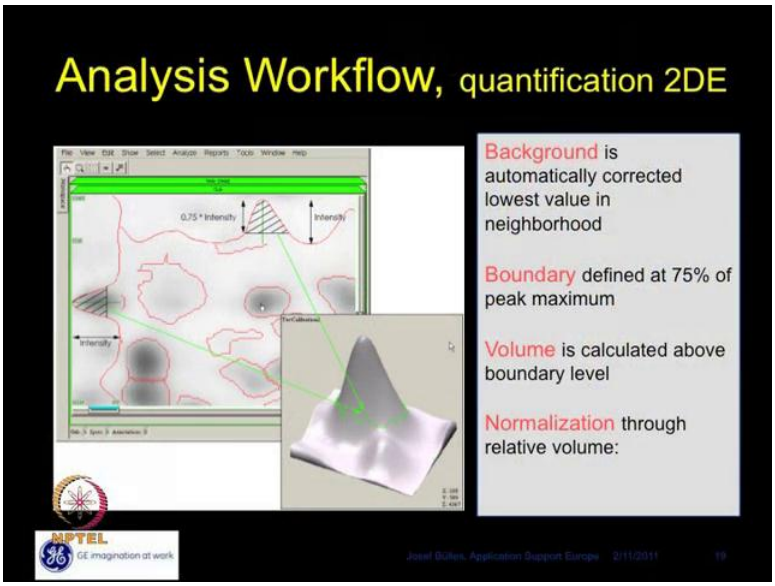

Yeah in this experiment they may be interested in this particular portion only that is, what they included. This much data what happens in this 3D view. Very clearly you can see this is background automatically removed here. Actually, what is the boundary which we are putting when you can see the 3D? It is not put taking the bottom of the 3D that is the protein peak, it is taking some portion it is leaving some portion and it is creating the particular boundary. So, that the total volume going to take above the boundary only it is not taking the below the boundary. So, that we are directly eliminating the whole background this what it will help you. So, I think in the slide of the quantification of two d e the way it is shown that the green circle which is defining the spots

Right spot boundary is discovering almost seventy five percent of the maximum peaks. Exactly, this whole spot are seventy five percent of the peak maximum. Because you expect at least 15-20 percent will be the background. So, then whatever we can put this particular seventy five percent for all spot. So, now, here it is going to affect in your analysis assuming 20-25 percent in the common background for all the spots, which will be helpful in analysis workflow now, we can see the quantification of the two d gel electrophoresis. There are basically four parameters which we are seeing here this background is automatically corrected lowest value in neighborhood boundary to define at seventy five percent of the peak maximum. So, one can remove easily the background, then volume is calculated above boundary level only where you can see a green line boundary above of this.

Only we are calculating as a volume then normalization through relative volume; that means, this relative volume is the completely as all spots from all spots it is reducing this twenty five percent. So, remaining whatever the value is relative volume this is going to be analyzed actually, compared analysis workflow quantification of the two d electrophoresis gel is one should look about the background of the gel as well as boundary of the gel volume and normalization.

Then background is automatically corrected lowest volume in the neighborhood; that means, actually when we are boundary define at seventy five percent of the peak maximum then volume is calculated above boundary level. So, that we can you can remove background very easily then normalization through relative volume you can easily normalize yours spots. So, in this slide what you have shown the green circle which is defining the spot boundary that is actually shown seventy five percent of peak maximum. So, its means you are eliminating the background from 25 percent but should keep kept consistent for the whole spot. Yes this is the normalization which we are doing right. So, that there is no artifact analysis and then one can remove the background artifact and then only look at the peak volume and the correct 75 percent of the peak.

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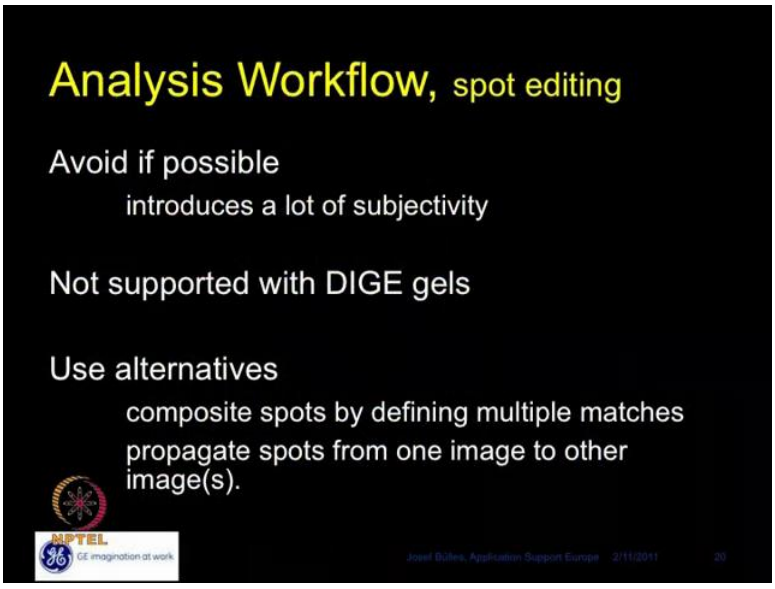

Correct most of the time, if it is possible avoid spot editing image well, you can do this part editing, but when you are doing the number of gels more and more in that case if manually you are editing in that time we do not what are the parameter. Whether the boundaries are

the splitting this you cannot do for all just equally. So, try to avoid this thing initially, but if it necessary definitely you can do this then introduce because introduce a lot of subjectivity and not supported with d gels it is not all supported with d gels then use alternatives. This is like composite spots by defining multiple matches and propagates spots from one match set to another like once you are matching. If there are all not matching perfectly and the same gel you can try to match another time, third time, fourth time and fifth time like try to match as many as possible and you can introduce lots of like four five landmarks. So, it can easily match as well as it can easily detect.

Your recommendation is that one need to relay more on the automated in another, and just because the number are more it very tough to do the all the quality control check with the manual way by defining various types of landmarks and by repeating the process several times.

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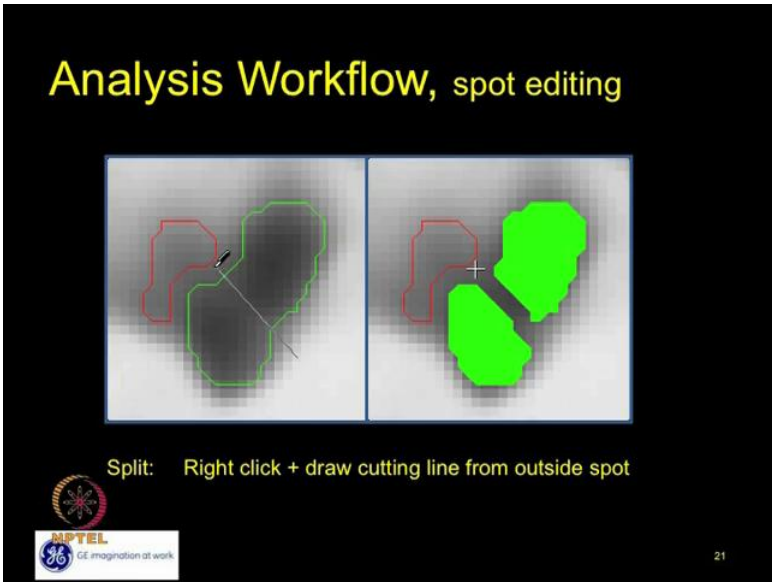

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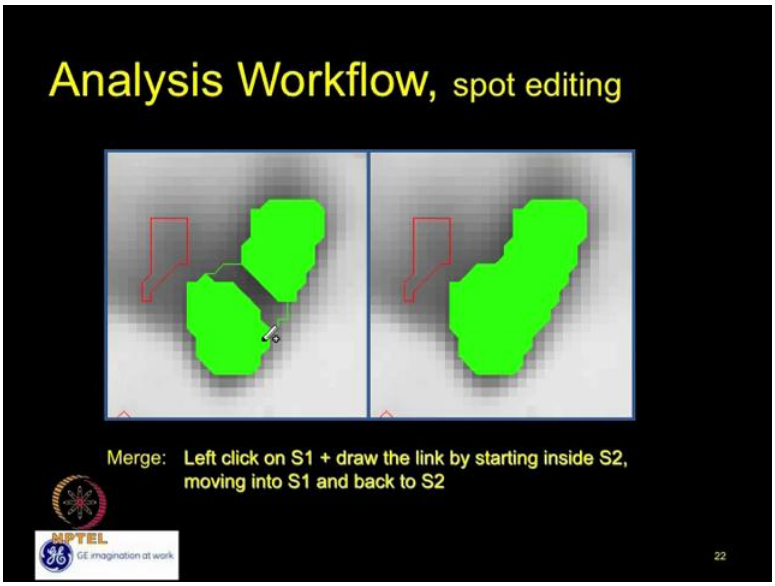

This is what we are recommending then if you would like to edit your spot how to edit the spot first. First of all this is the way which we can split or that particulars there is a tool for spot splitting then you just draw wherever you want to split, then it can be created as a two spot now. Then in this way you can easily edit spot as well as same if you want to merge two spots then by this way you can draw line from both sides. So, that it can be merged together. So, these are manual tools which can be used for splitting or merging the spot.

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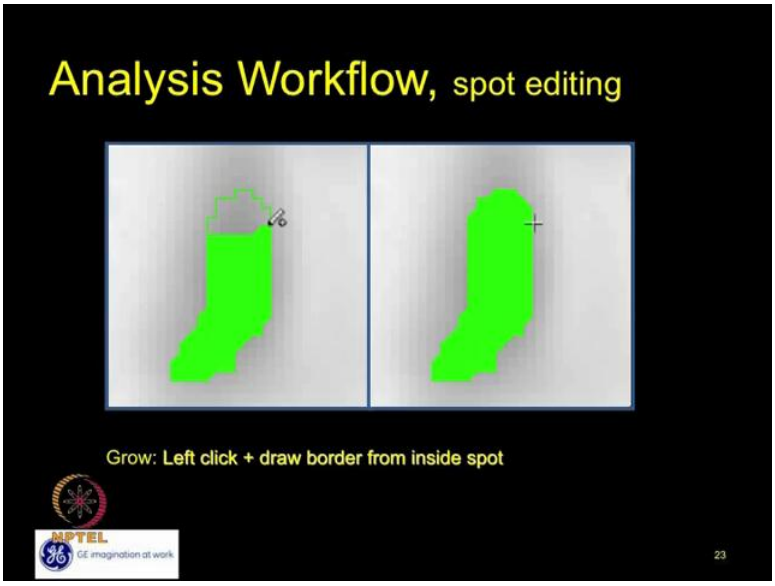

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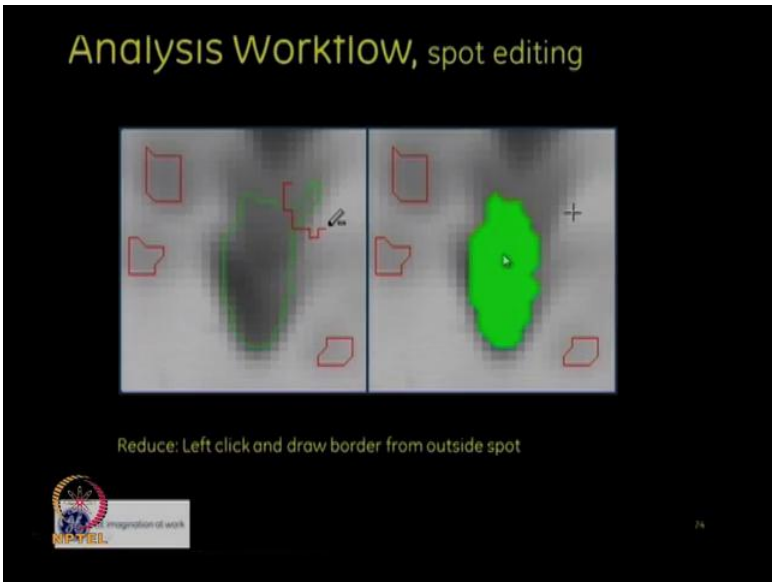

Then another option in the spot editing if you are not at enough satisfied with your boundary, you want to enlarge this particular boundary then no need delete and retrieve. The whole thing instead of doing that you can just mention what is the area exactly would like to extent to this spot. So, that in this way you can expand your volume of your spot the same way if you want decrease some portion from your spot like where is whereas you can see there is a tail sort of thing, but you do not think so, this may not your protein portion. So, one can easily cut that portion. So, they can reduce the spot size also this is the thing which we can do. Defining the accurate spot crucial and different type of software view like 3D views and profiles can actually help one to take that decision.

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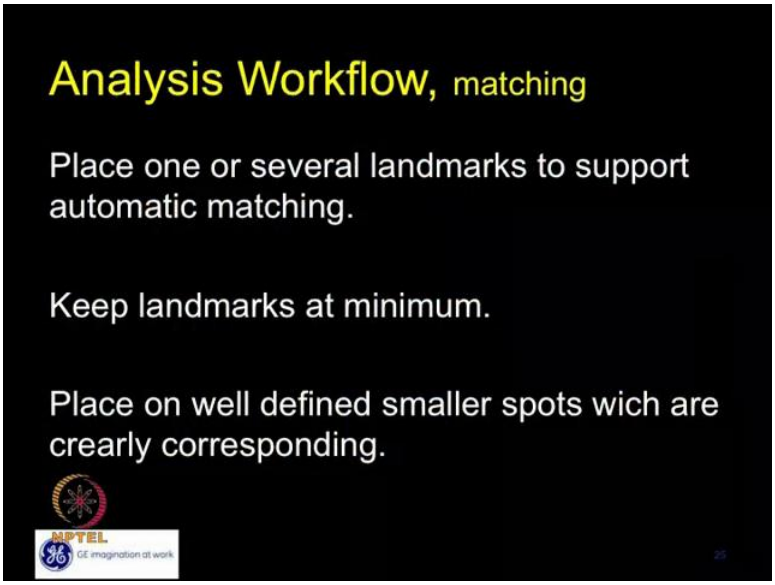

Once you finish off whole your editing process now this is the time to match whole gels. So, we can place one or several landmarks to support automatic matching. So, your once your matching it can be matched, but if you put then landmarks sort of thing it can be very useful to matching, I will show you images how it affects the matching then keep landmarks at minimum. If it is possible try to minimize two three four, otherwise it will again create some discrepancy in your spots actually. So, that one can actually a line all the different gels by using these landmark

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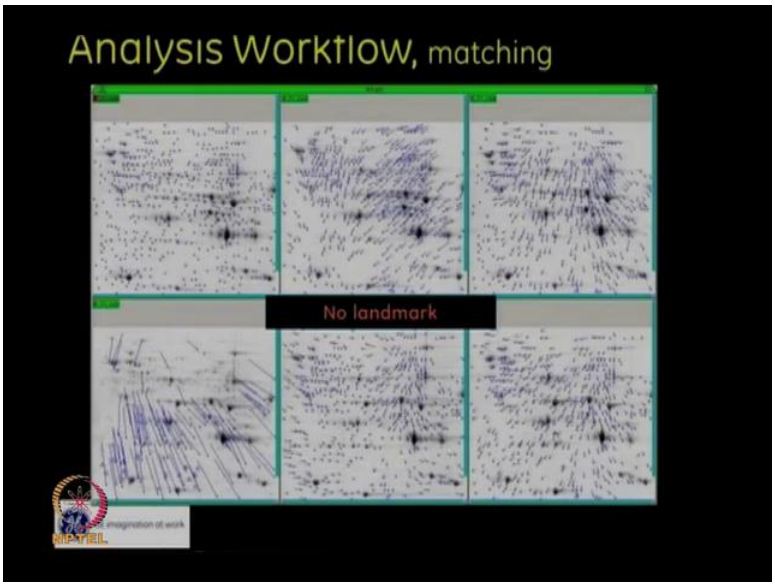

Yeah exactly place on well defined smaller spots which are clearly corresponding actually where I can see a small spot. So, that it may not take it is a little bigger spot it may not take that particular area into second into this another gel also. So, in that way if you minimum as possible it try to putting a small spot, this is another important thing, if you match this kind of vector sequence actually these are all the vectors, which you can see exactly what does it mean exactly if this is deviating from the difference gel to that in remaining gels how it deviating that particular protein, whether it is a once side deviating or it is differently deviating that that you can see exactly here.

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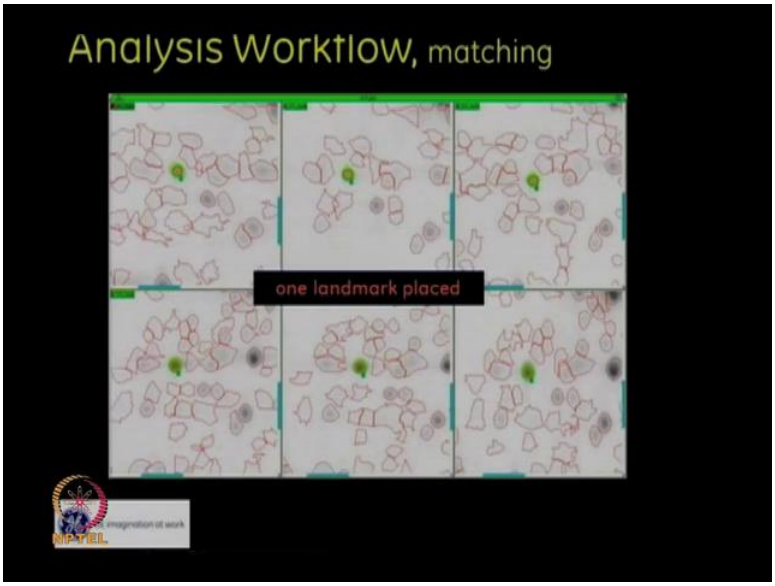

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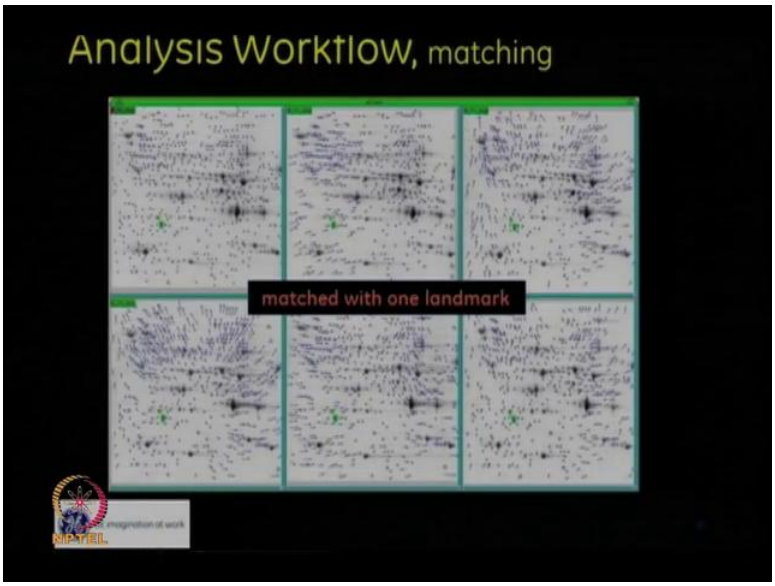

Matching it will show no landmark at all, we see once we put the landmark how drastically these changed there is no landmark. So, this is another thing matched with one landmark. So, again the vectors have now look well organized in the similar orientation.

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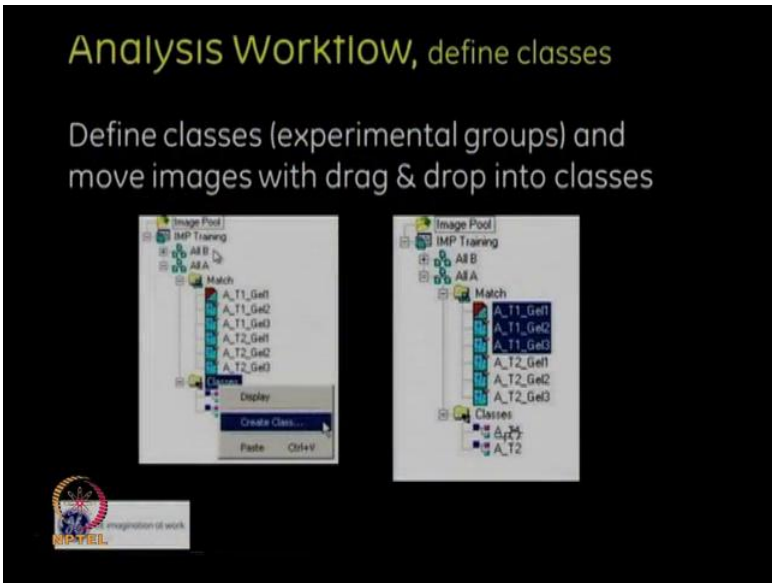

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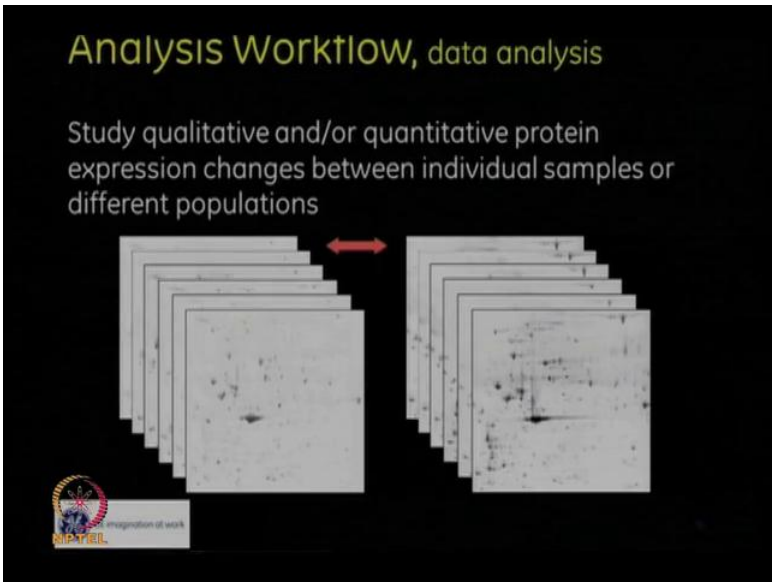

As compared to once we have seen earlier where there was no landmark after matching everything. So, how to design your experiment here everything how to match the how to create the match set this we will see more in our analysis our software analysis. This is what looks like from image pool we have added our images and after that we have create. We have to create match set and we have to create ours class this will more detail we will go during our software demonstration and these are all useless workflow. After data that is the once we get the complete data from that what are the flow regulation as well as what is the statistical parameters then you can compared all the parameters and you can select your interest of protein. This is what you can do finally, image master platinum software demonstration shall we start.

Sure. So, I guess you gave an overview of the whole data analysis which one can perform by this software and different type of challenges one may face during the analysis as well as. What are the different types of parameters one can actually use for a proper image processing, but I think, it will be really useful to see the software layout. And how one can really take these images of analysis? So, yeah please go ahead and show the demo on the image master platinum software.

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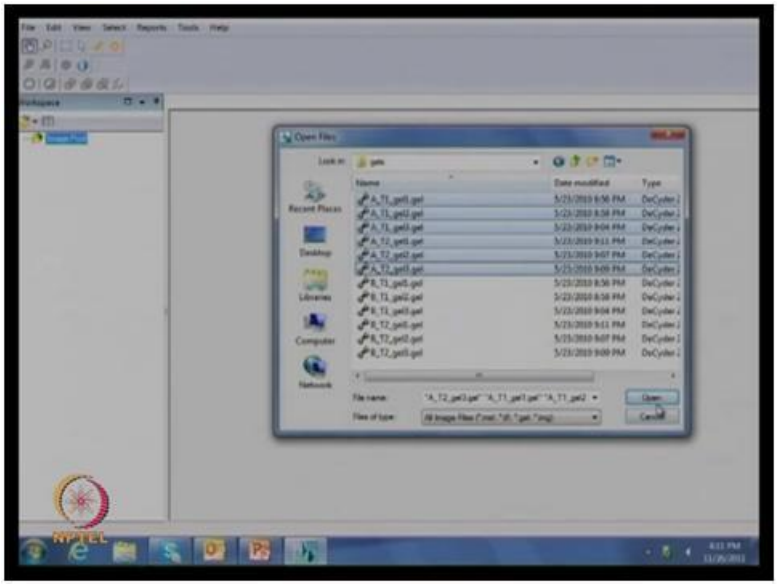

Yeah this is the soft layout where you can save your in your ppt. This is the original software layout now here, there is an option like image loader like add it wherever your gels stored it go to that place and add the gels like as I have multiple gels here. I am going to add few gels through our software to analyze like gel one, gel two, gel three and gel one, gel two, gel three so you process six gels.

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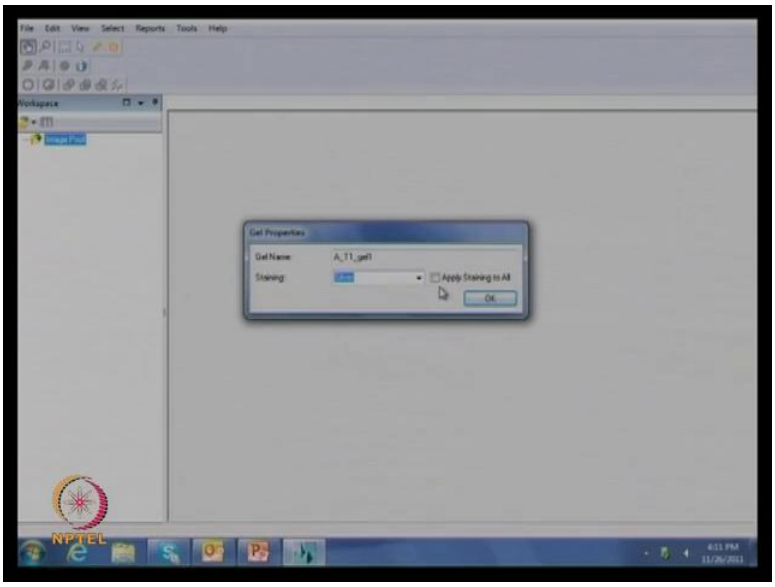

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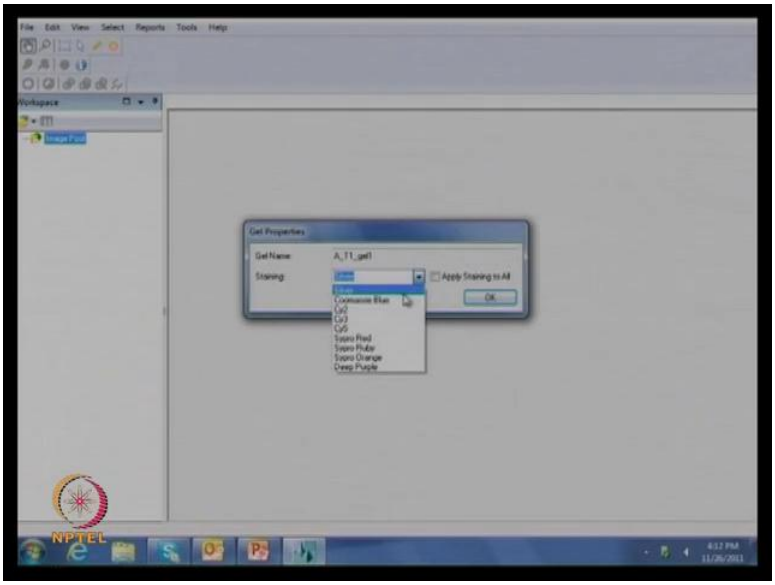

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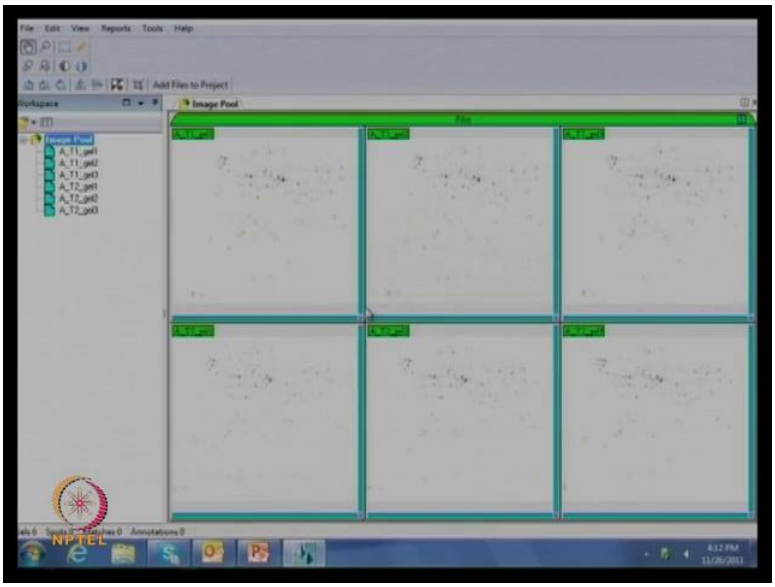

Six gels at a time these are all the six gels basically three of two replication like three replication for each gel actually; that means, one is control and another is the in that way now as we discussed in our ppt this is the thing which is asking for particular staining here. We can give coomassie or sliver or different fluorescent. So, as this stained with sliver I am giving this sliver and I am applying this same color of remaining the gels now click on now as you can see the six gels at a time in this after that now you cannot see the proper spots in all images it is very bright images of each you can see. So, now, we need to edit the images for our visualization. Can we change the contrast and brightness differently?

Yes different features. So, this is the button where you can adjust contrast and brightness. So, as I can show here if you now we cannot see any differences now after changing also, but one can have to press on apply another feature is available here.

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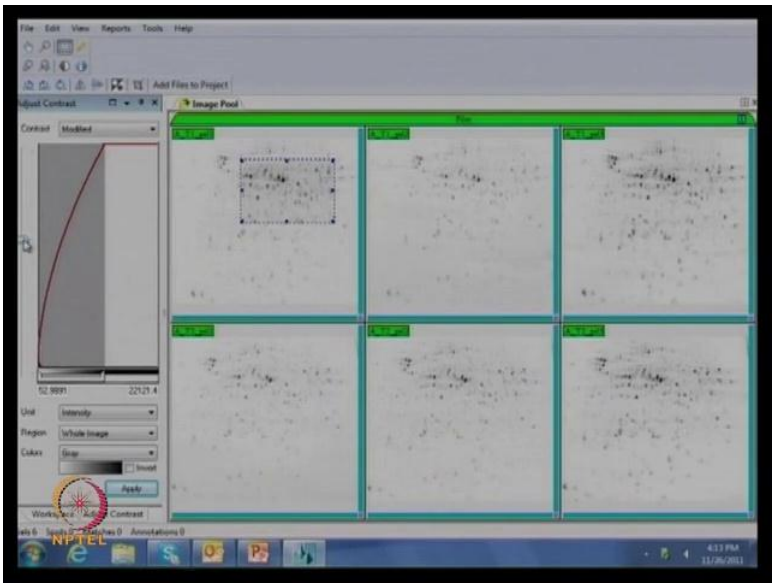

More spots more shop otherwise there is an option you can select this particular area then change the parameters. Now, we can see real time changes in that particular spots but is that we are going to apply only in one gel.

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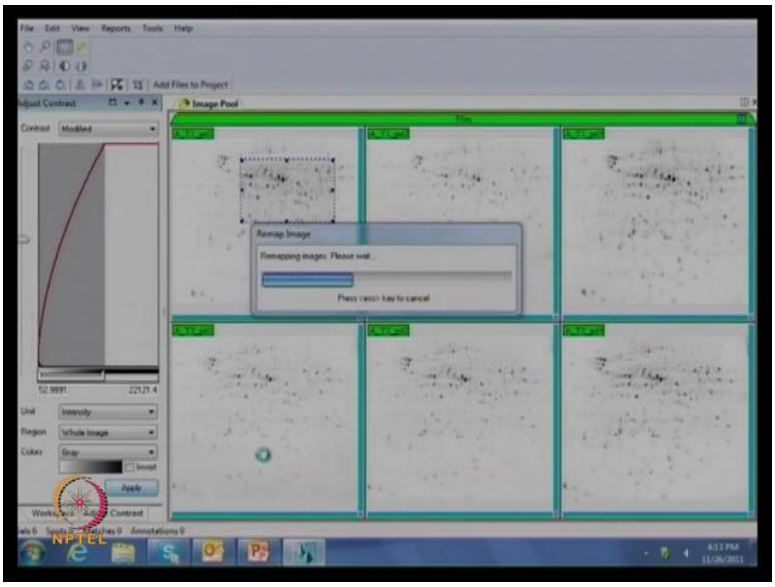

No its not where we select that is you can see now after you are satisfied this particular parameters then you can apply so, it can be applied for all gels. So, now, we can see more spots very clearly here throughout the gel. So, this is the another option now your spot this visualization every it is clear after that now we should not disturb anything you know original images. So, we need to make a copy of these particular images. So, so for that we can crop and create a new image because in our as we as we have some artifacts in our gels we can remove all those artifacts like there are some gel migration issue. You can crop the images and then you can store a new copy of the fresh image without changing your original image. Otherwise once during you analysis you have changed all the things in your original image and you may not you may losing your entire data.

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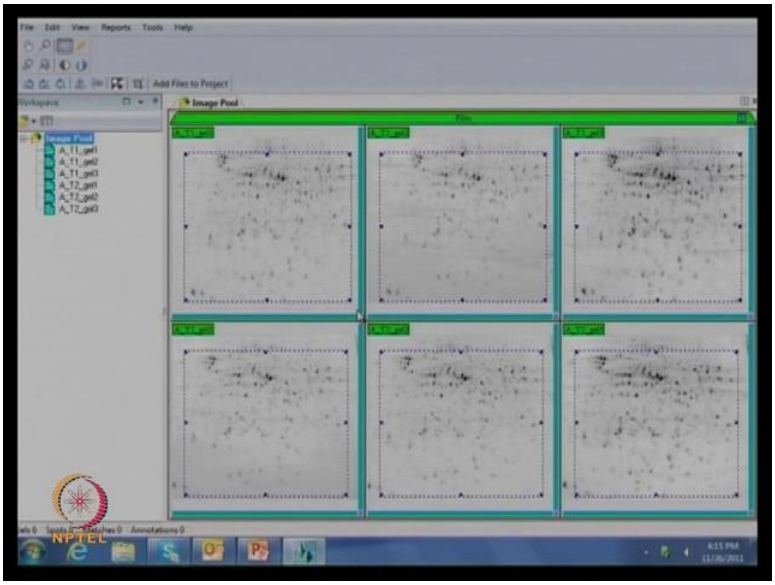

This should not happen. So, we are what we are going to do we are going to crop them. So, when you are selecting in this particular gel it is going to select in a in a single gel instead of doing that single selection just press on shift and control and select a area which can be common in all gels so, that at a time you can select in all gels. This is what you have to do then you can adjust them the same way. So, that after pressing control and shift in the keyboard you can easily edit all boundaries at a time otherwise if you are editing a single boundary at a time we do not know we are we may doing some wrong in image to image. All the processing will be uniformed.

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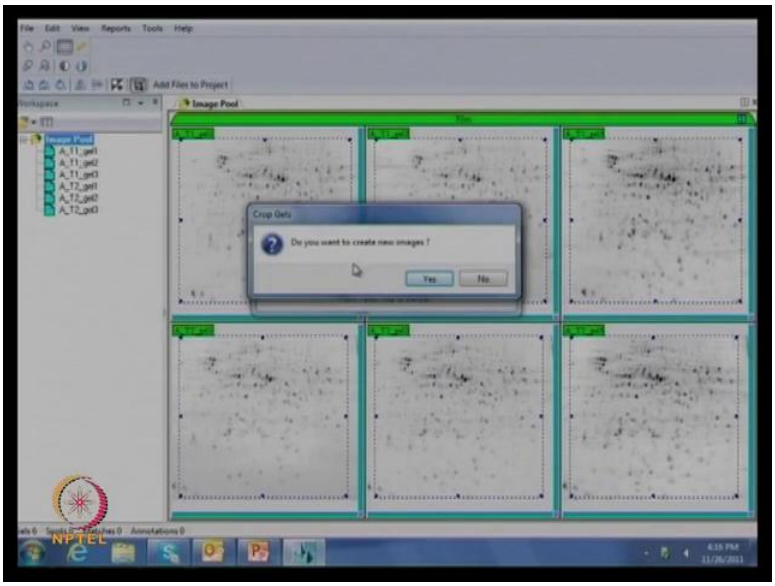

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This is what we will prefer, then you can crop them, then do you want to create new images yes we would like to create new images it is a very quick process. So, now, we can see all edited images here with extension of bracket one. So, same original images it has been stored. It has also created a certain new images with the crop. New images with the crop after that you just close them then open what are the edited images.

So, now we do not need to select all the 12, but we can just processed images so that one can create a grid lay on the gel. Form the view option they can create the gridline. So, that

one can easily judge whether my all gels are showing their reproducibility or not in which grid I have the number of spots I have and remaining. First it would help to decide which landmarks were need to use in the parameters. This is very helpful tool again and these are all the another option is there if you want to remove in this click the same way and another which we all can talk is show profile once you click the profile wherever you goes your mouse cursor it shows that particular spot.

X axis went to y axis you can see for all gels the same thing here. So, this is just initially we can see the gel quality exactly this is what it is showing then you can remove that particular tool. This much is initially then now we are going to plot design or a experiment how exactly we have our design of our experiments and how we can manage our gels right from the project we have to create a new project. If you have any description about your own experiment may be you may have some you may have something about specific details for the particular project.

Particular project you can enter here you can type here and it can be stored within this project and click IIT demo already exists. So, I can give IIT demo one. So, now, in this IIT demo one can create the match set here by clicking right. So, match the two gels a t one and two this is a t one is the control and a t two is the treated so. we have control and its three replicates and treatment its three replicates giving a t one if you have again say same description you can give for a t one and then create another match set give a t two. Now you shift the gels, where a t one gels to a t one folder and a t two gels into a t two folder right. Those three images from one replicate you are moving in one folder.

Yes this is where, you can see one gel is a red half and blue green half and the same well here in the a t two also the same way you can see; that means, this is gel set as a reference cell and remaining two gels going to be matched against this reference cell. Any gel can make as a reference gel there is an option from the view you can sit you can change the option. Change the reference this is what now display, them now you have all of your gels here oh once you have the gels we can directly detect the spots now.

From this button I can detect them now the three parameters smoothness saliency and minimum area the smoothness. As we can see if you 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 a inversely proportional for the splitting.

So, it is average value from one to five actually so, but one can set as a two to three it is a substitute this value is sufficient for to start actually and saliency again this is also again one can set up to hundred and fifty, but to start very good value is almost all fifteen to thirty. I am just giving fifteen for this initially then minimum area five is quite good enough if you think in your spot there are more spectacles there are much there are.

Artifacts are there you may increase this also in that case you can be remove very easily more artifacts. So, in this case otherwise five is fine value. So, all the three parameters can actually accept those, and then detects the spot. That is must on we 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 one. Only we should zoom in the region and see like whether I am going to do the same zoom. This particular region sees how this spots are boundaries as well as how the detection is there. Can you apply the same zoom in parameter for all the three gels?

Let us select one region and then apply them for all because it has properly defined the boundaries. And let us have a spot where one can actually now tweak around to get them, where as you can see there is echo and see there is a protein, but it is showing as a protein one can delete this kind of spots also. Right one should have to go to edit this part there is an option like edit enable. Go there and just delete this part wherever this spot you'd like to delete this particular spot I am not interested in this then I can delete it.

That, but before we take the decision can we have just have a look on the 3D profile. So, it is what you are interested in discussing that is highlighted in green. Yeah in 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. Treatment may have that spot appearing due to the of the protein or it is totally shut down, but as, but it is not the case exactly here because these are all the three replication of the same protein.

Let it be we can then finally, we can see the statistical data whether it is there in both gels as well as treatment also. So, that one can go with the statistical parameters. Now, let us look at in detail about one of the real spots. Otherwise I can visualize this thing I can zoom more so, that one can see more. Now, this is the intense spot, Intense spots which you are visualizing as another boundary they are marked for seventy five percent from the top.

Now, let us say it became actually low mount and let us say if in a region we have missed out some spot like software has missed out. Let us say this is the adding like let me zoom let me select a particular area interested in this particular area and selected everywhere, let me zoom that now you can see this particular area in all gels. This is what if you want today if you want to include some spot wherever if you are interested you can definitely add like this kind of extra portion. We can easily we can delete actually like we can reduce this particular portion. It is better ways to remove this spot and then draw the spot.

Yeah that is also better way, but both ways you can do actually yes we can delete it or you can redraw that is both well both as well as you can do now we find all spot boundaries are and everything is fine no we can match these three gels how reproducibility of our three gels of reproducible of all three gels this is what which we can do here. So, come to your original state without zooming and match them this is the match option. Now you are applying the same analysis parameter around both control and treatment.

Yes exactly now we are matching only control gels only. Only one group how our reproducibility is there within our group. Within one group this is what we have to check initially. So, if you have suppose may be instead of three replications if you have maybe five replications may be six replications out of five six replications one can remove that particular gel and they can take remaining four or five gels.

So, if there is some time during various experimental run if there is one or two gel which is very bad which are going to affect the overall reproducibility. So, by looking at this set of parameter one can decide out of six gels may be one of that will be very bad and is going to affect our statistical parameter. And then select those one which are having the very good number of matching.

Yes that should be done and now we can see in these two gels there are few vectors which you can see this, we can enable to remove by adding the landmarks. Now this is the landmark option 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 this same position in remaining all gels.

So, from 146 it has increased to 746 by adding a landmark and now vectors are matching well. Yes see right, but you can see if there are few vectors there, but still there these are all moving at a same side so; that means, there is not like a irregular. Reach very carefully. So,

if you can add some more one more landmark here it can be removed very easily. This is what we can do now we can save these particular things and apply the same parameters to our treatment gel also. So, you can see no need to remember also the same parameters if you can detect your spots they automatically it is the same parameter earlier which you are here which you have used same smoothness is three and saliency fifteen minimal area is five. So, no needs to change anything then click on ok. So, there is a long process of analyzing it or rather I think it is sort of quite quick.

It is very quite quick than it is completely finished now the same way you can you can go through each and individual gen by zooming and 3D view and you can select which are all the spots and which are all the hot spots then you can directly match them now I am doing this say matching twelve hundred and seventy four matches I have been done. So, these gels are more reproducible; that means, as compared to. So, this is very fine gel so, now the replication which we did was confirmed. This replications are now one can analyze in the class analysis in between these two classes like inter classes and intra class say sort of things.

One can actually now do the statistical analyses So, now, I am crating the classes here otherwise before that I would like to go to one report point what is the gel table that is the these are all the gels which are present here shift a you can select all the spots and go in go to spot table see now. So, what parameters are displayed here in the spot table? This is the file name and this spot I d has given a particular spot I d for all proteins and this is the pixels. Let me this is the match i d; that means, out of six cells this match i d it is giving one second.

This is the file name the this is the gel number then spot i d is which is given complete number for all spots then match i d then this is the pixel size and x axis and pixel at y axis then if you can able to give the p a also the and you can calculate the p a 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 saliency of that spot all these parameters for all the gels. That can be obtained from the software.

These are all the parameters which you can see in this software. So, one if you find if you are satisfied with the all these values then you can go ahead your next level of analysis that is class analysis now I am going to create a class here create a class this is the a t one

another is the a t two which is control entreated now again I can shift these gels into at one to a t one another match set, which I am creating. So, where can we easily shift before going to class? See you are moving all the six gels into composite max set. So, all six gels are here and now we can create again the same way classes.

This is a for class analysis now we can see both gels together now three gels from the control and the remaining three gels from the treated. This is what you can see now it is behaving it is taking value as a single gel of eighty one and single gel of the eighty two now it is going to average of these three gels and now it is another averaging this remaining three gels. So, a single value you can get from the control another single value you can get from the treated once we did the same thing then quickly max them again one can remove this all this factors by adding the again or particular landmark so.

Process what we did earlier now go to reports where you can see the analysis classes go to table now you have the match i d that match i d is the particular spot number as well as the maximum area of this complete max sum. What are the maximum area as well as a t one value and a t two value? So, what are the values in each six gels for each spot? For each spot exactly this is the averaged value again. The ratio values if you can see this is the ratio value which is giving up regulation or down regulation. So, this is the anova value which we can see on the bases of this anova value we can easily select our interest of protein as well as you have the fold relation also this is one folder up regulator this is one point two folder down regulator the same way you can see the whole values now no need to go each and individual spot you can filter that easily now. What we are going to do is first at least anova value which, we are going to take the maximum we are going to take is may be 0.05 as the statistical significant value so, those many spot it can be highlighted.

So, this is the spot which is already undergoing this particular anova value, threshold and it can see all the spots which you want to have this particular anova value those are highlighting here now. It can very easily one can very easily go through that and this particular table you can easily export to excel also from their you can have your complete data also this is what the complete analysis software gives you the output. So, again it is very useful to see like different steps what are required for performing this analysis and also the software gives lot of options for doing different fold change statistical analysis, of how significant data is and then one can actually still go back to those spots which software's

says significant and look at manually each spot to verify. Very useful discussion and demonstration on the software for image processing on two dimensional gels. Thank you.

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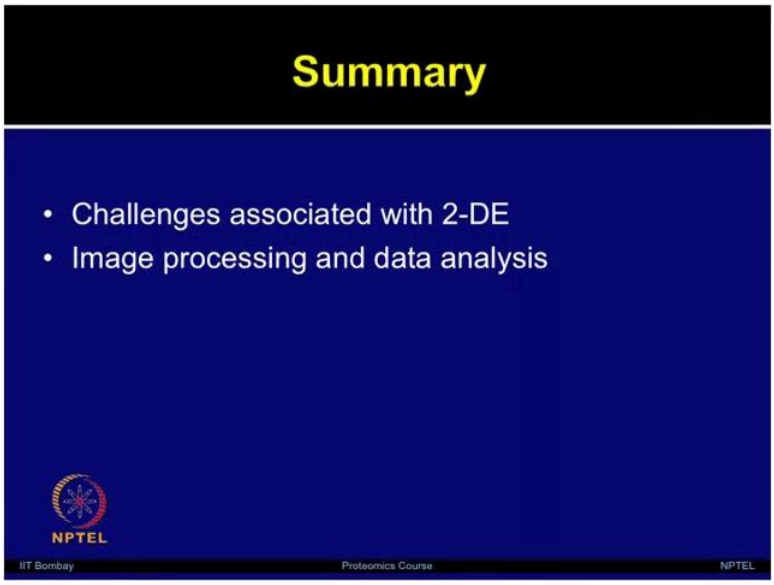

And we will continue our lecture flow for further two dimensional difference in the electrophoresis in the next class. So, discussion with Dr. Srinivas on image processing by using image platinum software I hope it was useful for you to understand the details required for image processing and how various type of parameters were need to pay attention during the analysis. So, in today's lecture in summary we discussed about the challenges for the image processing which are associated with two dimensional electrophoresis how one can process the images very efficiently.

How one can analyze these images stepwise by using various type of software most of the software work with the similar principle and to give you the feel for the data analysis. We showed you a software demonstration during the discussion with Dr. Srinivas from the GE health care software image processing by using image platinum i m p seven at the end you must appreciate that one need to do different type of statistical analysis to ensure that the identified spots can be really further pursued for research; and those are going to be further processed by using mass spectrometry. And if they can provide some meaningful information, then one need to have full confidence; that is why we need to do a statistical analysis beforehand.

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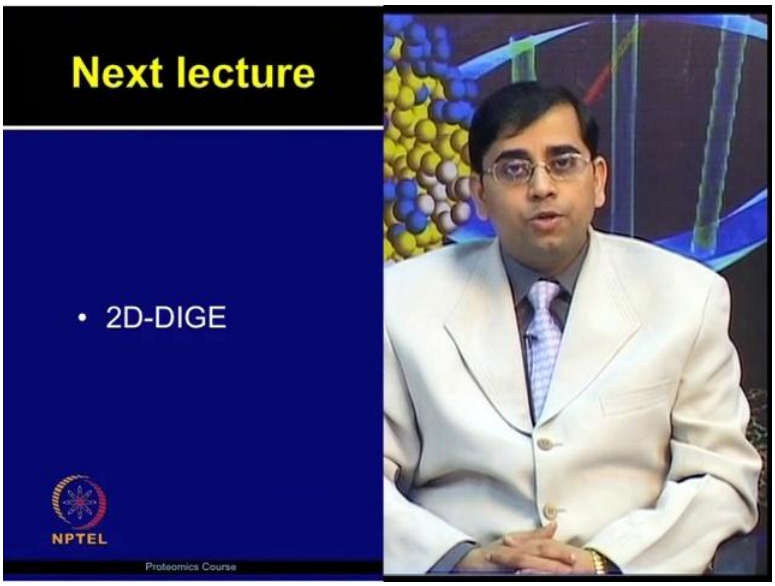

So, I would like to acknowledge Dr. Srinivas from GE healthcare for discussion on image processing and data analysis as well as giving us a demonstration of the software for 2D gel analysis in the next lecture, we will continue our theme on gel based proteomics and I will introduce you with a new technology on two dimensional difference in gel electrophoresis or 2D, and then we will continue our lectures on 2D and it is image analysis.