Interactomics: Basics & Applications

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Lecture – 21
Basics of Image Scanning and Data Acquisition

Hello, students. In the last lecture, we have had a lab demonstration on how to screen the presence of auto antibodies in the bio-fluids of cancer patients using microarray. In today's lecture we will discuss about various parameter that should be considered during image scanning and processing in microarray workflow. Detection probes have been briefly discussed in our previous lectures. These probes are major determinants of finding obtained from any microarray experiment. Detection strategies for quantifying signals from these probes in microarrays have been categorized into two basic categories the label based and label free methods.

The advancement in confocal laser scanner technology has led to the extensive development of protein microarray technology. As a result protein microarray detection system widely uses label based methods scanners captured the fluorescent label signal using cool charge coupled device or CCD cameras some scanners also employ lasers for excitation and a photomultiplier tube PMT detector. In other cases LED is used for a uniform light source and is detected using a cool CCD in combination.

Today, we have with us Mr. Pankaj Khanna who will be talking to us about GenePix microarray scanner. He will first discuss about microarray scanner as there is a software which controls this and we will further discuss about the data acquisition parameters that should be implemented to acquire the data from the processed microchips. So, let us welcome Mr. Pankaj Khanna for today's lecture.

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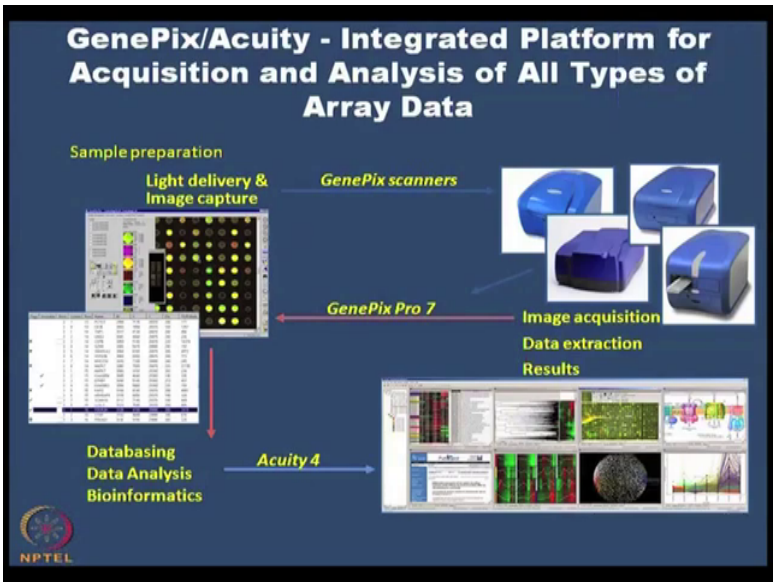

In the form of hardware as we have discussed there are number of different hardware possibilities in the form of GenePix right from 4000B to 4300 4100 these are actually doing the hardware part which allows the lasers to scan the chip. So, once we are ready with the chip so, it will be interface between the CPU and the system which is allowing the data acquisition to happen as GenePix is being attached with a software called GenePix Pro software which helps in understanding how a data is being acquired with the hardware usage.

So, basically another software which is also used is Acuity which is actually a third level statistical analysis software. So, in brief the hardware is being attached with a data acquiring software called GenePix Pro and this GenePix Pro software is now give the results to acuity which can be further analyzed for statistical analysis.

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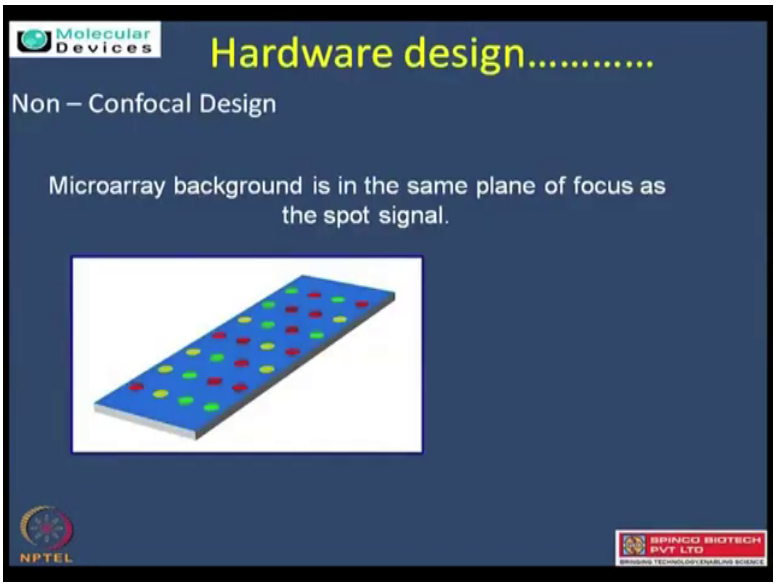

GenePix microarray as such is based on two special designs, one of them is non-confocal design of hardware and second is inverted chemistry. Non-confocal design is like where you have a spots in the same plane where the scanning is done is called as a 2D dimensional. Say when you go for a tissue arrays or analysis where it is very very higher so, in the form of spot size specially at the level of imaging of scanning images. So, there at the level of microscopy people use a technology of confocal microscopy.

So, that they focus the laser at different wavelengths. So, at the end of the day they build a 3D image. But, usually for all microarrays which is the highest is the tissue microarrays it is shown that all the slides are in the same plane which is why it is non-confocal chemistry which just resides in the same plane of the slide. So, that is why we choose non-confocal chemistry

attached with the inverted chemistry of scanning which helps in the best results in the for a signal to noise ratio.

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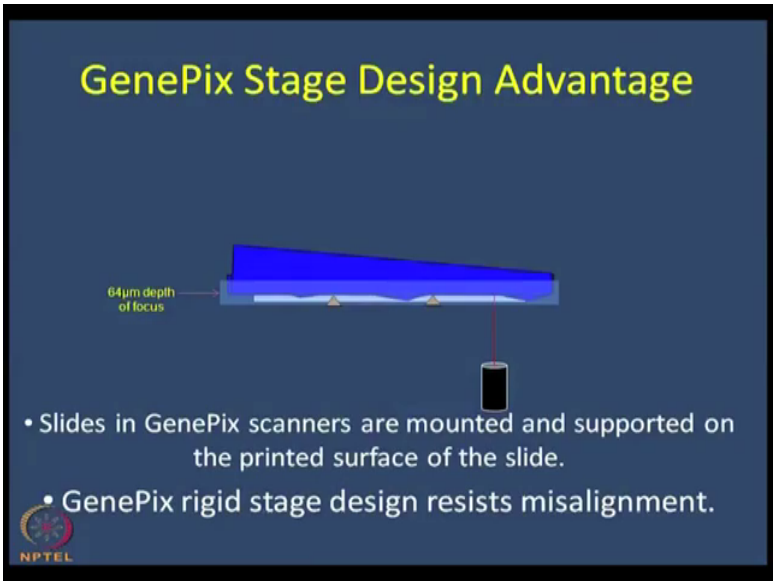

So, as we discussed non-confocal coupled with the inverted scanning how does it help ease usually we see in the glass lights there are lot of small deformities which our eyes cannot see.

Right.

So, what we do is we invert the slide, so that it goes to the level of the same scanning and they are supported at the level of the edges with the controllers which see the deformities at the glasses.

Ok.

So, what it helps is it helps in looking at the deformities and directly allow the scanning to happen on the face of the scanning area. So, what happens is because of the confocal design attached with this inverted chemistry, we get best signal to noise ratio, so that your data is more valid for any analysis.

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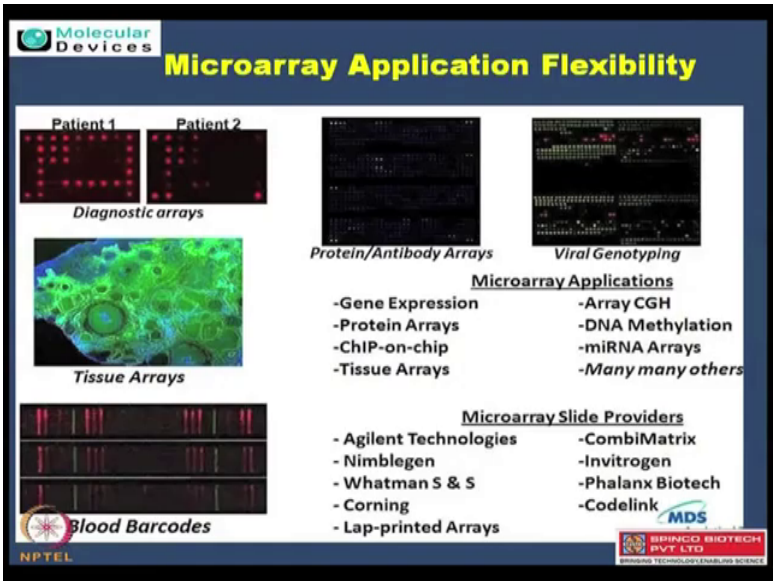

So, classically microarrays are being used for the differential gene expression which usually people categorize in the gene expression format. So, later as the development has happened people went for SNP arrays as well as a CGS which is called as comparative genome hybridizations.

Right.

They are used to look at the chromosomes. Apart from that protein arrays chip-on-chip to look for the control genes they are also being focused and the protein arrays are now really catching up in many sense because the protein is actually the biological function is what many scientists believe.

Right.

And, I am also a strong believer. So, now, people are moving from the DNA to the real functional part how they are directly affected. The major bottleneck was looking at the antigen antibody reactions like the proteins are all being seen there is a no general formula, but now the field is catching up. So, even at the level of protein arrays which is compatible the incoming tissues array which is really in infancy is also now being compatible with GenePix pro.

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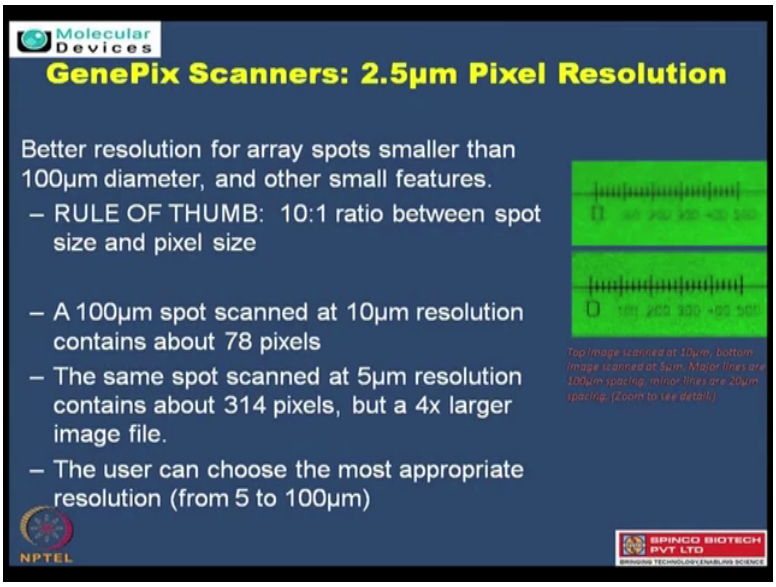

So, our scanners ranges from 5 micron resolution to 2.5 micron resolution. The basic thumb rule for all the resolution is that the amount of your size of your spot be it protein or RNA that should be 10 times that of the resolution what you are scanning with. So, in interestingly most of the DNA is less than 50 microns and most of the protein usually stands at 200 microns. So, essentially the rule goes that 10 is to 1 if you say 10 is a resolution and 1 is the spot size so, that is a major rule. So, just to give you a brief out say for example, if your spot of protein is 100 microns.

Right.

In size, so, what best if you do a 10 microns basically you are getting 78 pixels. So, this is actually 1 by 10 if you do by 5 micron resolution all of the pixilation will increase to 314, but the size of the image actually also increases. So, apart from making more than 10 times the

size of spot, it does not really help at the level of resolution if you increased. The only thing is you are increasing the size, but the data quality is just increased by few very very minor changes. So, that is why the best rule is 10 is to 1 which 5 to 2.5 micron usually is most suitable.

Right.

Where the people are making the slides.

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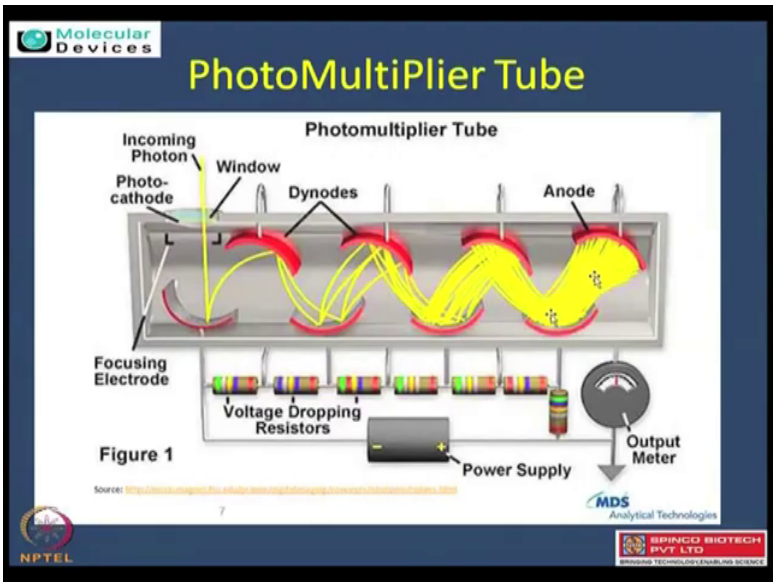

Essentially, because they are all not present so, the data could be right from the 0 intensity which will be equivalent to the background to the very highest intensity. To best way to control the variations is looking at a photomultiplier tube actually that is nothing, but once you

do a fluorescence so, the intensity is coming out of that. So, the very low expressing genes or the proteins will give you very low fluorescence intensity whereas, the high will give very high.

Yes, I just interrupt you here when people scan these microarray slides it always ask for the PMT gain right. So, I think that is what you are talking here photomultiplier tube and the PMT gain.

Yes.

So, just maybe you can clarify here like how one can adjust that parameter of PMT gain.

So, the basic thumb rule there is in any scanning you do not want to see a wide spot the wide spot essentially means the saturation and to avoid saturation exceptional cases for few controls people avoid that happening.

Ok.

So, the best way is to look at a photo multiplier tube condition. So, it ranges actually from something like 300 to 700 which helps one control how much photon multiplication can be done, so the lowest can also be seen and highest does not become saturated. So, this is how the photomultiplier tube is very very essential to control the different kind of variability within the chip which happens due to the biology.

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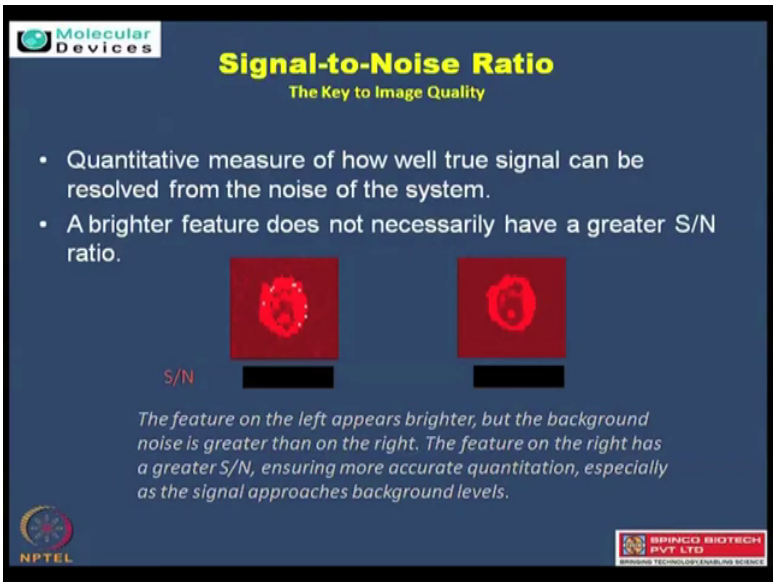

So, the major variations actually come owing to the different sources – one could be technical, one could be biological and another one is owing to the essay or the chemistry.

Right.

So, the technical ones in the form of hardware can be controlled at the level of PMTs and when you are looking at signal to noise ratio the small play of PMT and very less with the help of laser power and then with our own design of inverted scanning.

Right.

As well as the non-confocal chemistry we are able to achieve the best. So, in short if by visibility you may not be able to look like what is happening to the images. So, in the form then here in the image which is being shown the which looks brighter to the eye may not be a true sense.

Right.

So, when you look at even the dull, there the background is very low without looking at numbers if I see them I say this is dull that is bright, but that is not true always.

Yeah.

Because when you calculate reality based on what you have controlled at the level of PMTs and others you quickly come to a conclusion that signal to noise ratio can be seen by eyes at the level of PMT which the laser balance can be seen.

So, in this fashion while you are scanning a slide we can always look at that. So, as you go for the signal corrections so, basically what you are trying to see same terminals are not looking saturations right and then you expect the image is going to be with least background with a valid information which is coming in the form of intensities become a true signal.

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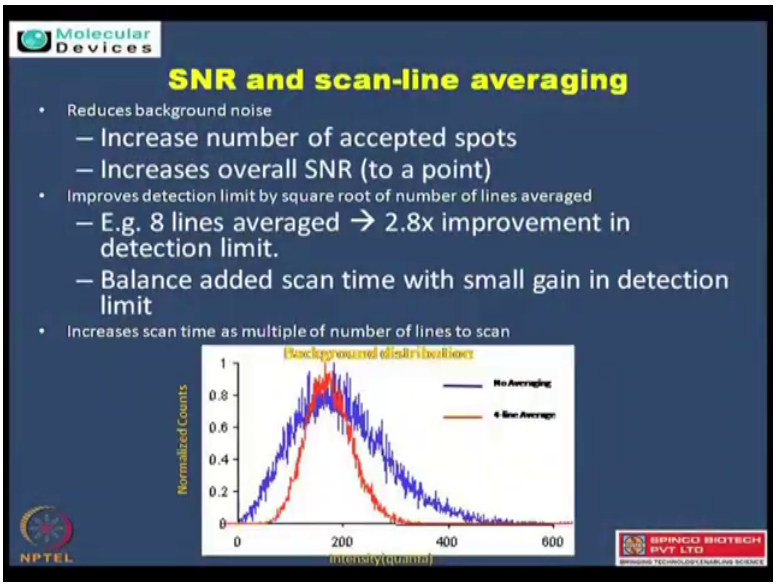

Basically, if you scan once, so image is being collected. So, if you want to have multiple scannings being done, so that all intensities can be averaged upon and get to a signal value, it is being seen that when you increase the number of scan usually people prefer 2 to 3. So, then average them. So, this helps in reducing the signal to noise ratio, in a fashion that you get a real good intensity coming up.

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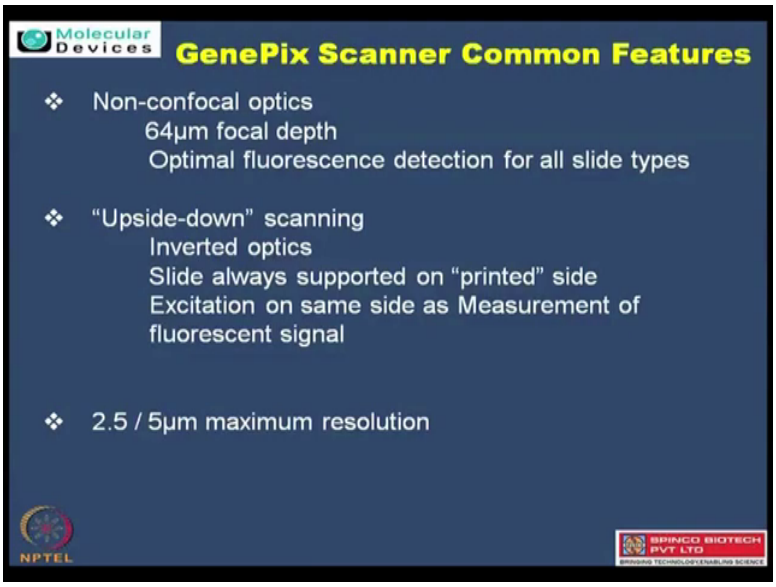

So, the basic concepts in the form of hardware design the basic one is non-confocal optics and another one is the focal depth. The meaning of focal depth is where our laser can focus in the form where it can get or acquired the data and the depth is 64 microns which allows a wide variety of applications possible. So, if you want to use some slight based slides that is having a cover slips so, that the depth is a little bit you know kind of lower. So, you want to focus at the different range.

So, in this fashion the non-confocal optics with a focal depth of 64 is our major feature. Apart from that up down upside down that is inverted chemistry is actually what we are going to use in the form of printed sites being scanned directly and controlled for the variability I have discussed earlier and the resolution at 2.5 or 5 microns based on the system which is available can be also used.

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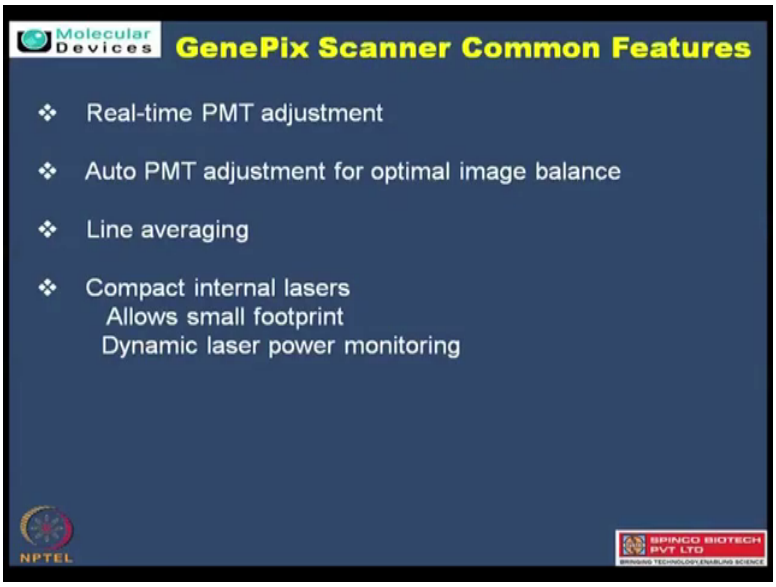

And, real time PMT adjustment PMT we have already elaborated the photomultiplier tube.

Right.

And, the real time is at the level of scanning, we can look at the data when the scanning is live. So, in this form we can control the PMT live in between the scan. And, then auto PMT adjustment for those who are not real bursts of looking at how to control them, we have given auto PMT option. These auto PMT option allows acuity to get the best output, but GenePix pro allows the multiple scan by itself. So, the best PMT suitable for one application can then be seen immediately.

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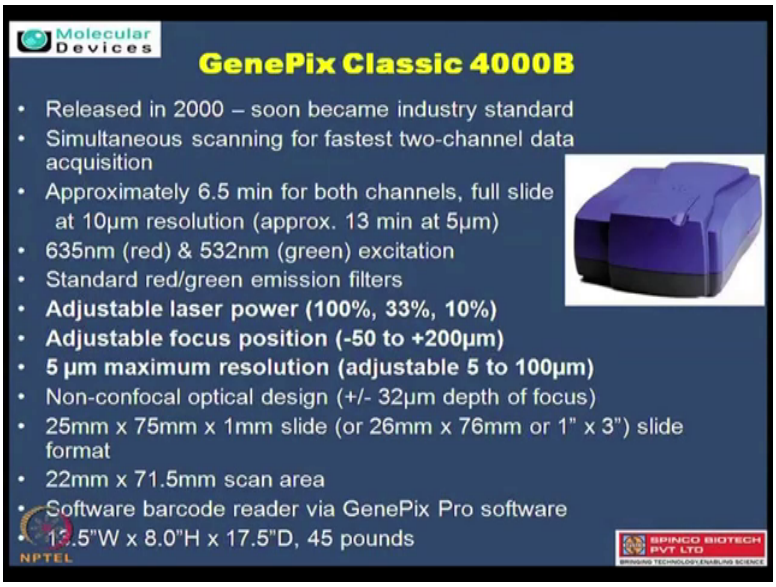

GenePix classic 4000B is having 6.5 minutes of simultaneous scan. The meaning of simultaneous scan is both laser scan at the same time.

Right.

So, you have very less time for scanning. Apart from that it has got two lasers actually 635 and 532 nanometers.

Which is classically used for psi 3 and psi 5 and they are on compatible dice. So, in view of this we have standard green and standard red emission filters to accommodate all psi 3 and psi 5 applications. The laser power can also be adjusted in the form of 100 percent to 33 percent.

Ok.

To 10 percent. So, based on what application and what intensity you want best the laser power can be adjusted as well. So, adjusted focal position is from minus 50 to 200 microns. So, this allows you to focus in a different ways. So, we can have a different slice compatibility coming up with the scanners 5 micron resolution maximum it allows to go for and it can go up to 100 microns of resolution.

So, non-confocal again it is using the non-confocal optical design and any standard slide can be used for the scan. So, it gives a wide applications possible in that.

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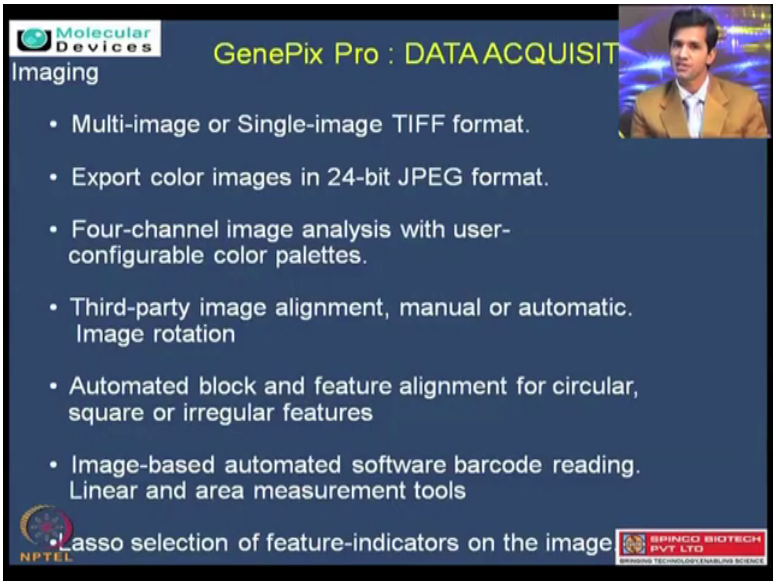

As we discuss in brief like the GenePix Pro software we are going to use for data acquisition. So, basically there are different steps involved what in the usual process of bioinformatics, the first one comes is imaging.

Right.

So, imaging is being done in the form of multi image or single image TIFF format. So, we use TIFF format to create the images form and export these colour images in 24-bit JPEG formats. So, these jpeg formats allow one to only see, but the basic data processing will be done on TIFF format. So, once GenePix Pro is being allowed in a different sense of hardware controls then image acquisition can be done using the number of lasers availability, it ranges from 2 to 4 and many times your application use one laser as well.

Right.

So, based on the channel type laser type you select how many to select for again on your chemistry basis and this third party image alignment and manual automatically being done. So, what this mean is each particular array has got different blocks which in turn contains the features.

So, these features are actual genes or the proteins or the representative of the biological material what you are checking, now this has to be aligned with the annotation informations. So, this can be done by a third party and the GenePix Pro is doing by itself in the form of automation to do the job. So, automated block and feature alignment is possible even the different sizes and shapes of the spot can be handled right from circular square to irregular features which can be handled at the level of image alignments.

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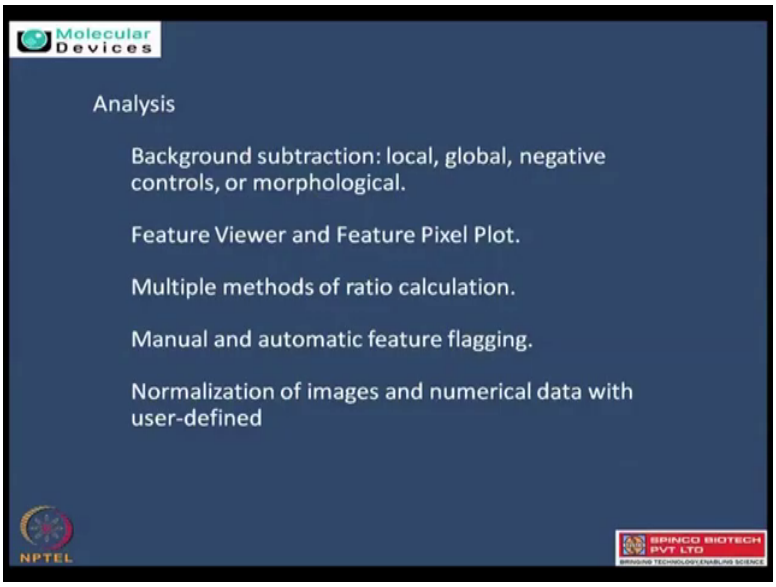

So, in any microarray after the image first thing comes is the alignment which we have just covered in the form of data acquisition because there is a very important part to cover. And, apart from that you have a background subtraction, so that you get a true signal coming in. So, GenePix Pro does help in the background subtractions in a different format and also in the normalization of the features.

So, if you see the background subtraction can be done in the form of local, global and negative or morphological control. So, this negative and morphological controls are subjected to the design of the slide type say negative controls in your slide there should be some spots we should not bind to anything.

Right.

And, leave blank and whereas, the local and global can be calculated in the general space where there are no spots available and the area which is not being spotted nearby your particular feature. So, in this fashion background subtraction can happen. And, then feature viewer and feature pixel plots. So, basically the major thing comes in the acquisition is after acquisition is visualization of the data and this visualization comes in the form of pixels and the plots the graphs.

So, graphs help us in understanding globally what is happening in short. So, it gives you a real image how the things are happening. So, this can also be done and there are multiple ways of calculating in the form of ratio calculations after normalization or during the normalization of the data. So, analysis immediately after those involve few of the normalization process.

Right.

Which GenePix pro can very well handled and other important feature is the flagging of the spot. In a biology we see some spots are really not good or because of some artifact they are not supposed to be taken for analysis.

Yeah.

So, essentially we need to make that spot as flagged as good, bad or absent. So, these can also be done with GenePix Pro software. Lastly, normalization of the images and the different formats is also allowed to happen in GenePix Pro.

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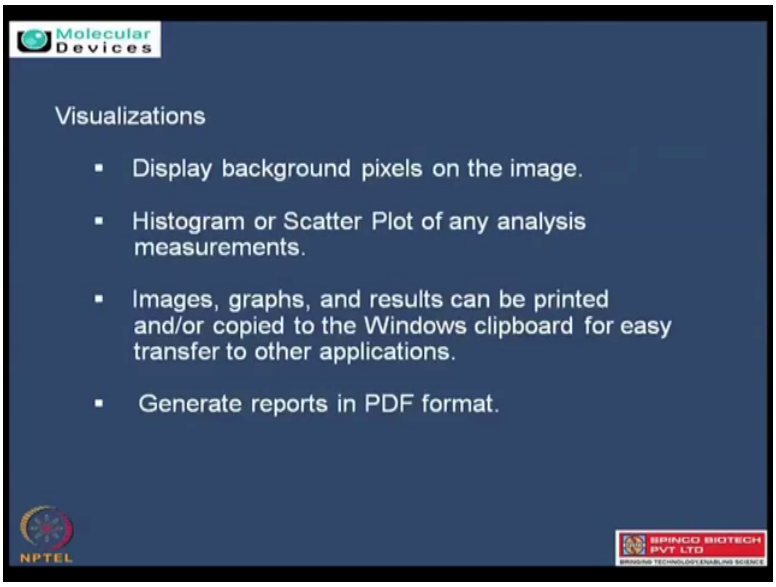

So, once the image is being acquired. So, immediately scientist wants how my data has. Especially few people use even the bacterial controls which is non-related to the biology as such totally they want to see just the essay control.

So, first they say whether the my assay has worked or not.

Right.

So, best way to do for them is looking at the graphs which are being given differentiation in the level of backgrounds, and though also can be done at the level of different histograms, and the scatter plots possible.

Right.

And, these scatter plots can be plotted once against the channel types a laser 1 versus laser 2 or wavelength 1 versus wavelength 2 in classical say psi 3 versus psi 5. So, how these two things have behaved for me.

Right.

So, again different kind of graphs and. So, also the images can also be exported to PDF as well as being visualized in GenePix Pro for your further screening for different QC applications.

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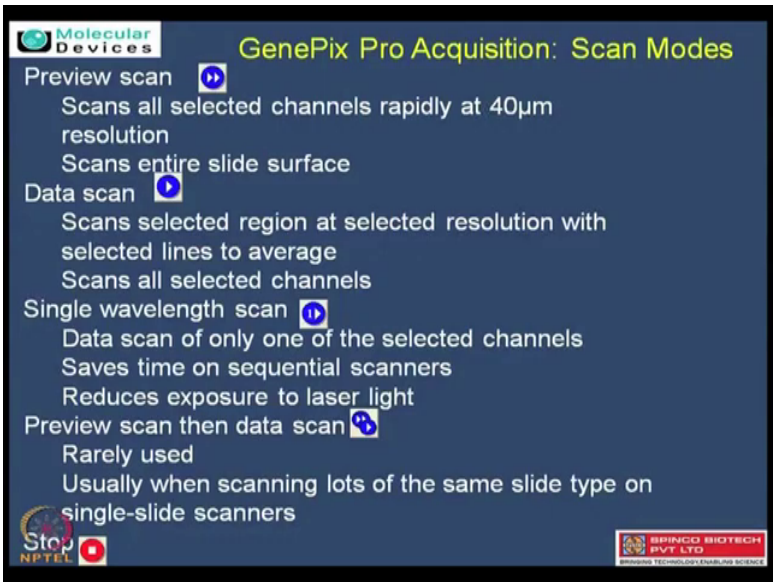

The basic GUI which we are going to see in few minutes actually contains three different areas the first one is an image laser controls. So, there you wanted to see which kind of laser I am going to use and second one is the different features which are used for the controlling of the image and towards the right hand side we have a pain which is allowed or helpful in hardware controls.

So, the basic one is in the hardware control is first as we said that we can look at auto PMT and other adjustments which is being done for that we use preview scan. So, preview scan and that there are different tabs which allows you to go for the true scan and the preview scan and based on the laser which particular scan you are using. So, like for example, if you start with the preview scan you decide on which best pixilation suits me.

Right.

Which different power of laser suits me.

Right.

Once you are able to do these decisions being made you can go for your own data scan, people prefer that because they are sometimes a bleaching effect on the flora flows.

So, they want to avoid the exposure for a longer time. So, multiple scanning is avoided. So, once you see even a single wavelength scan so, if your application requires only one wavelength to be scanned there you can select in the tab only a single wavelength scan.

Right.

And, then so, also a preview scan with the data scan followed by you can also do an automation that once you do a preview scan you see that it is all good, then immediately it can go for that, but very rarely people use that because you see once and then only you want.

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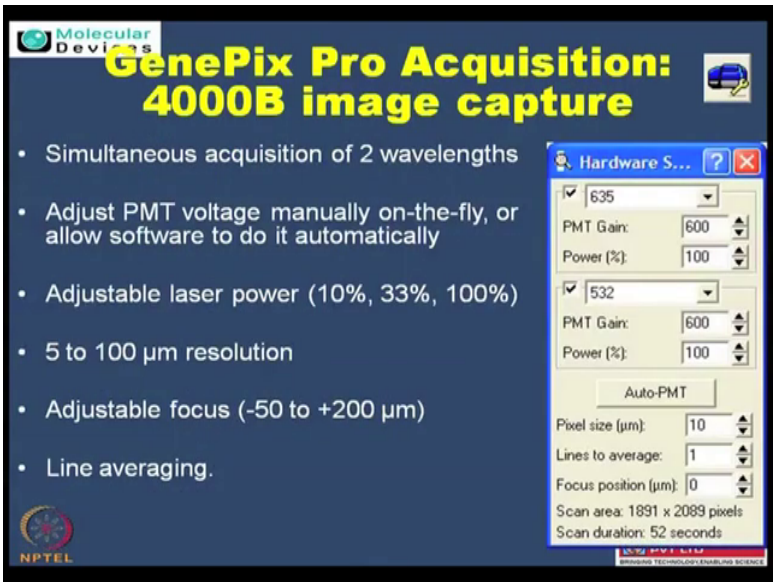

There is a button towards the side where you have a having a control for the scanning time. So, as it is a dual channel that means the two lasers are present in that it is allowed to select whether you want to use 1 and 2.

Ok.

And, then based on one or the user application you select both the lasers and then at the level of live scan you control for the PMT and the mutual resolution you want to use for. So, these are within the same software towards the right hand side you see the pane where you have a hardware control button, there you can also look for all these different images which is being now suiting for your own biological application.

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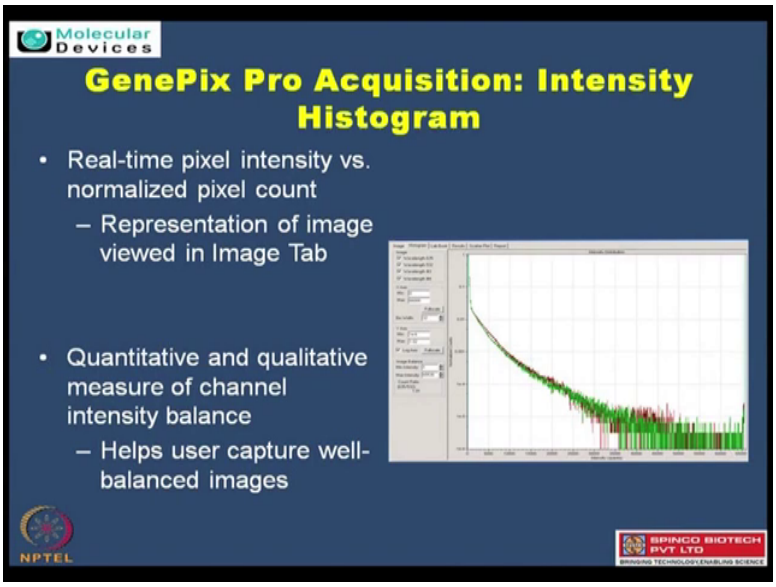

So, basically in the preview scan when you are scanning your live data you can just switch on to the histogram graph.

Right.

There, what it gives is how much red and green channels are contributing towards the intensities. So, you really want that they are overlapping. So, they are really balancing there could be a small variability in the beginning owing to the fact that there are just a background and then the spots coming in. So, there you want that they are really overlapping after a little bit of lag and a few seconds of lag that is it.

Right.

So, once you are able to do then you can see and select whether yes, this PMT is being good for me. So, this is away you check which PMT is more suitable. So, you select a PMT look at them see the overlap wherever there is the best overlap without saturation you want to go over those settings.

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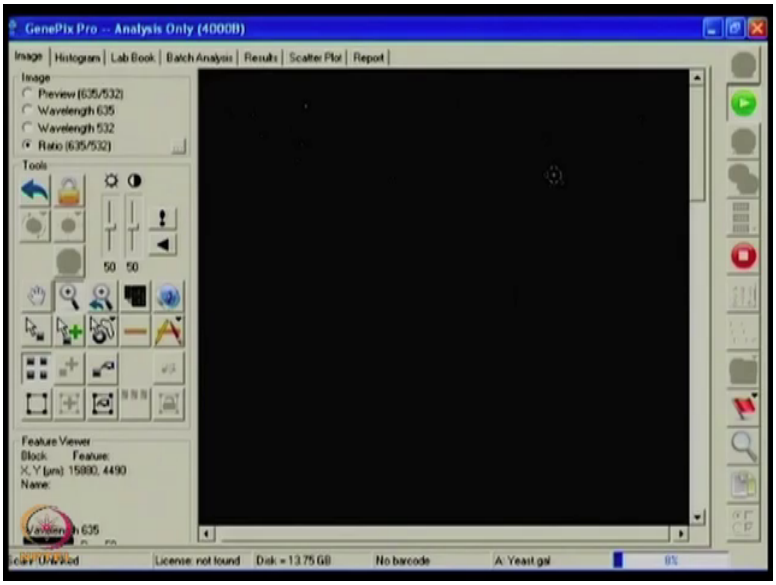

So, what you see now is a GUI or graphical user interface of GenePix Pro software.

Yeah.

On top it is in the form of different tab buttons which allows you in a different work group. Say for example, image allows you in different ways and controlling of the image acquisition and histogram looks at how that image has performed.

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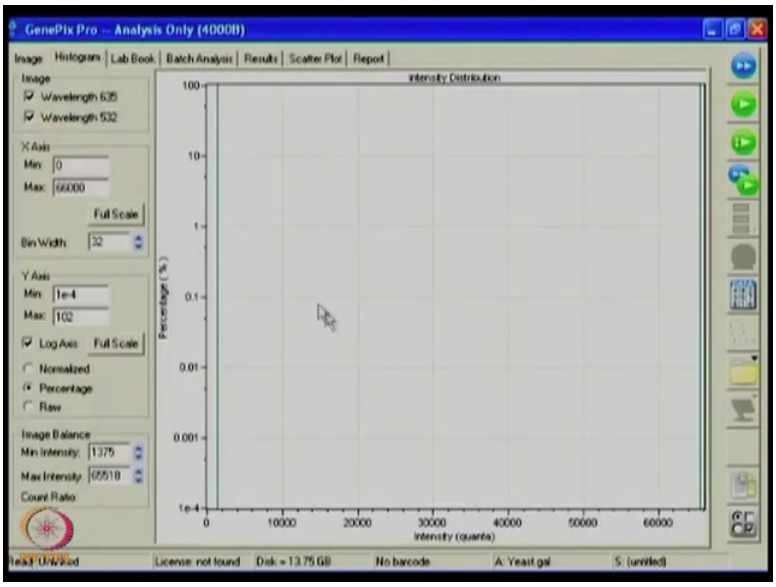

So, this is what we were speaking about in the earlier slide where you can see a live kind of demo which is happening.

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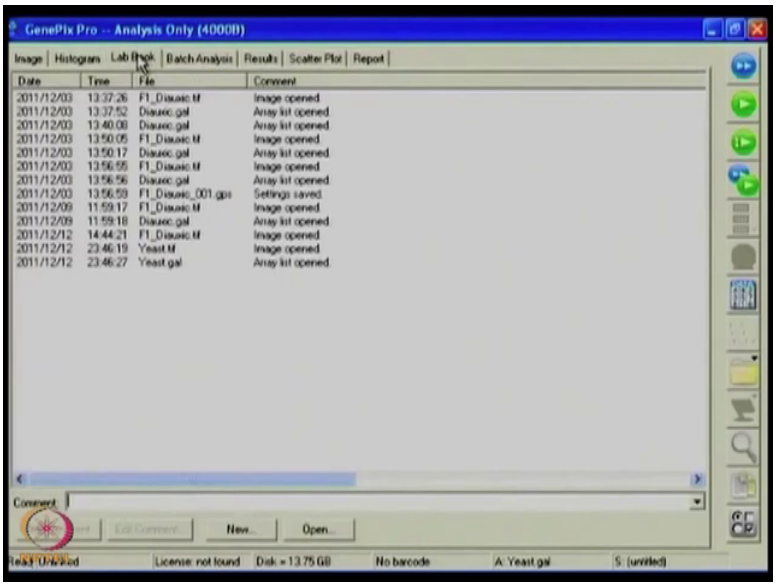

And, then lab book actually gives what all you have done in a different stepwise. So, what is being every movement of viewers in this particular software is being logged in.

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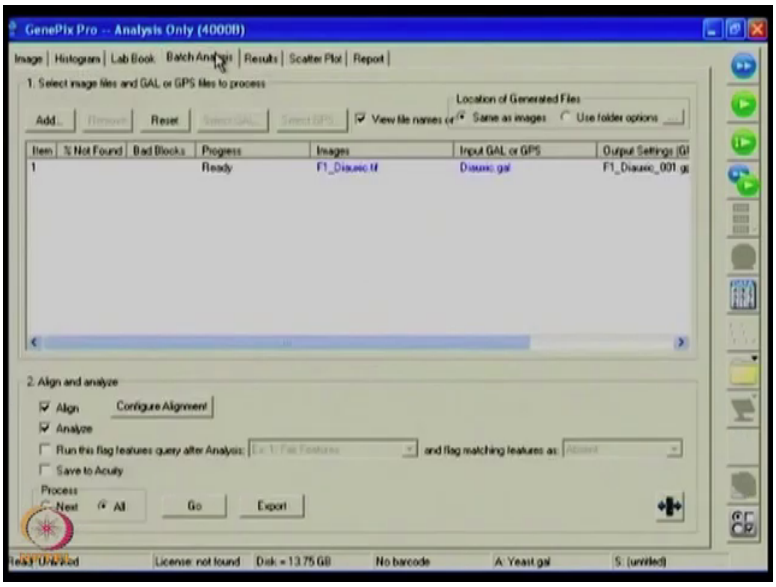

And, analysis can be done in the form of batch form which allows multiple slides.

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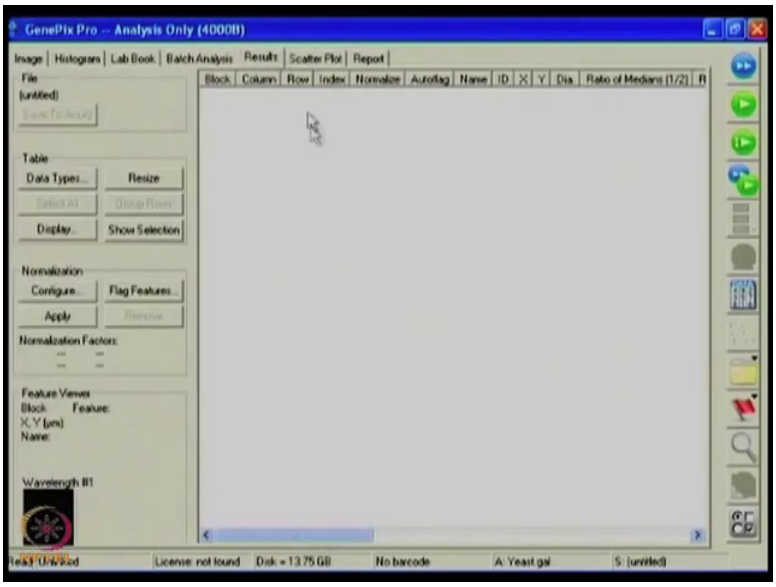

So that you can do alignment and the analysis which can be performed with the batch analysis and when the analysis is over results can be seen.

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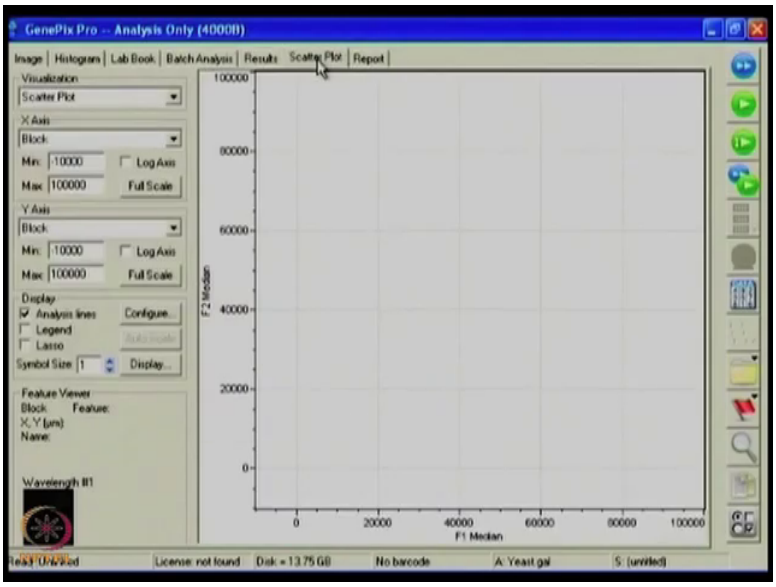

And, scatter plot can be now plotted in the level of this graphical user interface. Once you are through you can look at the reports as well.

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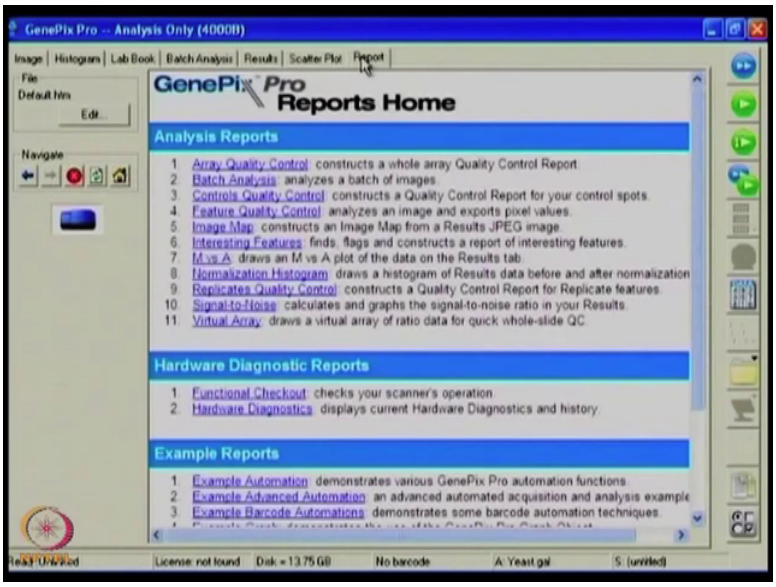


So, let us look at the major function of imaging. So, what and how one control for the best image acquisition. Let us quickly go through like different kind of buttons here. So, now, the imaging can be done at a different wavelengths and the like preview can be done at 635 and 532 in a single laser based the wavelength can be done at 635 or wavelength at 532.

So, even the ratio of the imaging how the both has performed together can be looked at looking at a button of ratio of imaging. So, this one allows you to see how the image is being done after the scan; you can look at one channel preview channel or different channels.

Ok.

And, now let us look at different tools which are available to you while or after the scanning.

Yeah.

So, the major ones are here where you can move across the chips in the form of this hand tool, the plus indicates the zoom tool and the other tools are actually this is one to unzoom and you can also look at the whole image button. So, once you have the image these two becomes activated. So, these two are actually for the blocks.

Ok.

And, looking at the controlling the blocks and these ones are the features. So, many a times what happens usually you get the GAL file which is actually the feature information file. So, GAL stands for gene array list. So, actually it gives the x and y coordinates where each array usually is being presented in the form of blocks which blocks in turn are in the form of features. So, these blocks and feature positions are being recorded in GAL file and then the information or annotation is given to each part.

So, GAL G A L that is gene array list file essentially contains the x, y and the number of columns and so, also the information of each part how they are being annotated and placed on the chip. So, if by chance if you do not know or you have prepared by yourself these buttons here allowed to make your own blocks and we create your own GAL file with the help of the tool which is called as gene array list generator.

Ok.

So, now let us look at the control button which is towards your right side. So, the first one is a preview scan and then you can also have a data scan.

Ok.

1 stands for one wavelength. So, it allows you to take image from only one wavelength.

Sure.

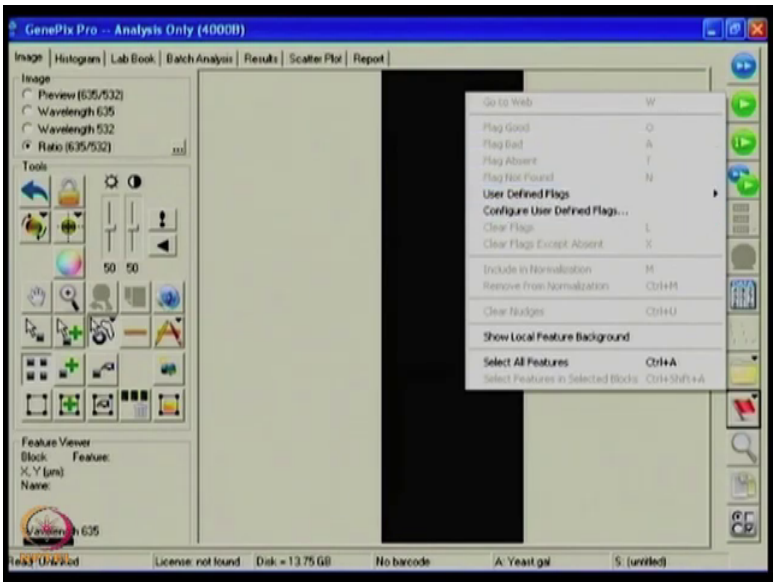
And, then you also have a multiple scan. So, you do a preview scan then you do a scan with this button. You also have other buttons which will light up as and when you acquire the image and this is for the analysis. So, the once the analysis is being done if you click this button the analysis will be performed after the alignment.

Ok.

So, this is actually open button. So, this is like normally your file where you want to open or save your images.

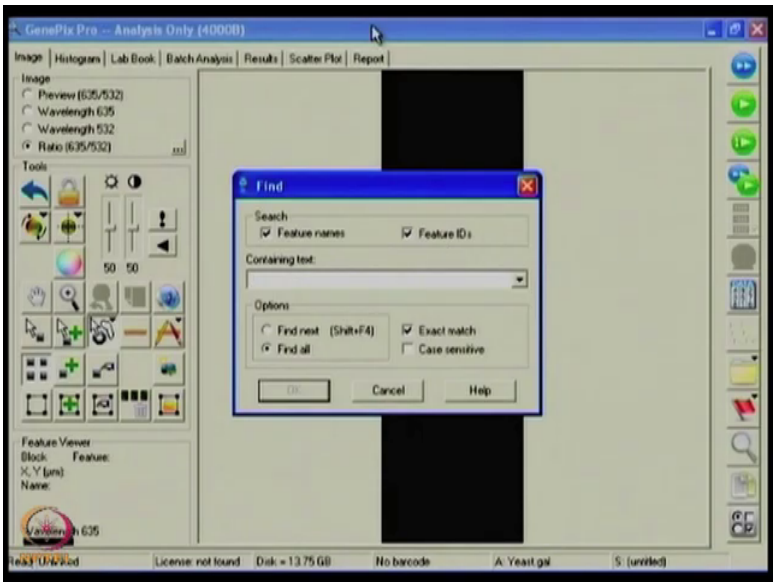
Right.

(Refer Slide Time: 26:17)



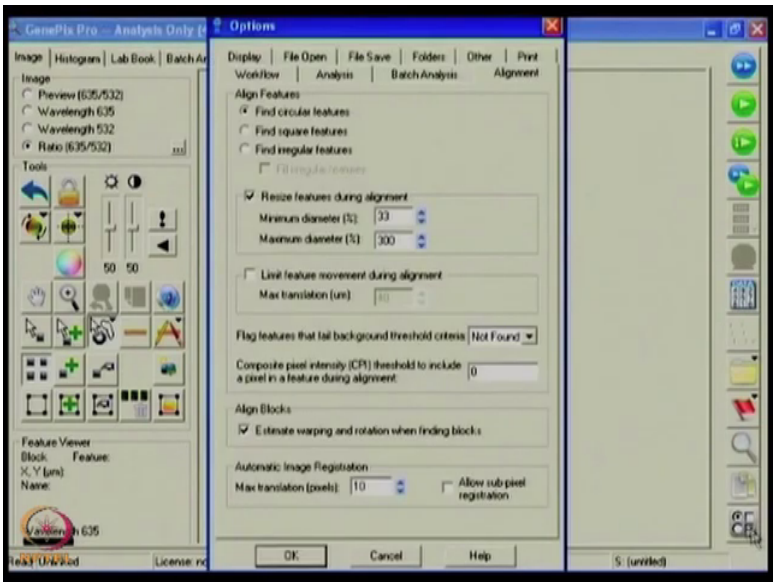
And, this one is actually a flag. As we discuss the different features can be flagged we can look at when the image is available to you can look at good, bad or absent and you can give them a different ratings.

(Refer Slide Time: 26:27)



Here again is looking at different zoom buttons. So, which allows you that if you want to focus on feature names or the feature IDs where you want to go for about particular one. The major one here is particularly this which allows a different workflow controls right.

(Refer Slide Time: 26:38)



Right.

So, this allows in the form a different ways can be had. Now let us quickly go through a one particular scan which is a simultaneous scan. So, both measures would be acquired at the same time.

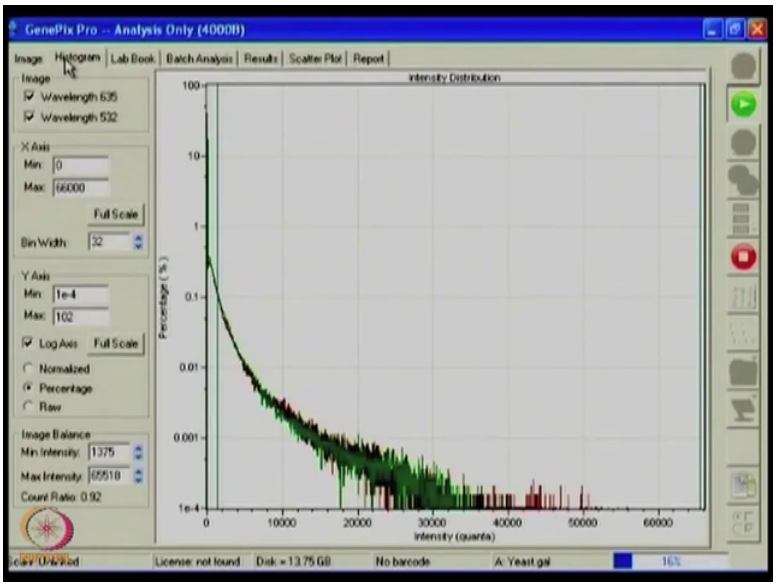
Ok.

So, if I press on a data scan button the image the after putting your inverted slides in the hardware it is scanning. So, you just see on the top which is very less visible. So, let us try to zoom inside. So, if I just put this button and allow you to zoom.

For a reasons here.

You can receive particular how the scanning is happening. So, you are looking at different image type. So, if I click on only one wavelength this because it is live after the scanning you can see it is going for the ratio image scan.

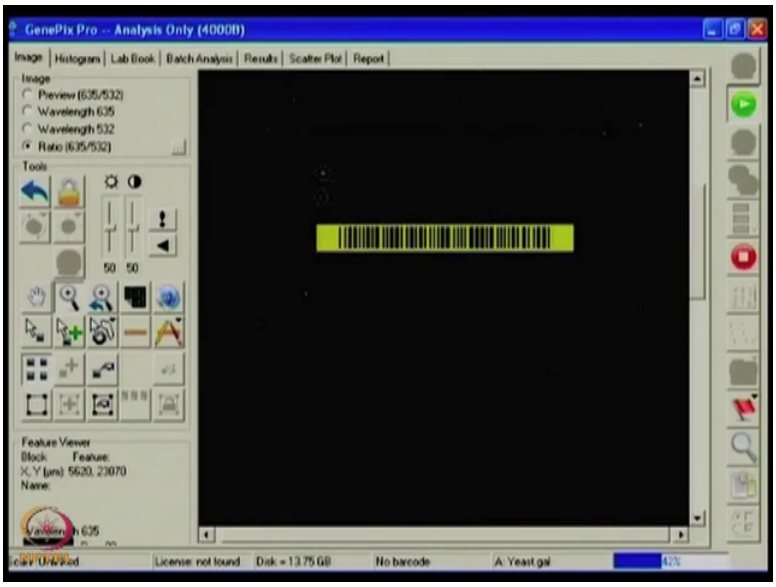
(Refer Slide Time: 27:27)



So, now quickly and a histogram you see it is start coming up because it is scanning is going on live, so, it start reducing the basically as we discussed it should be overlapping.

Right.

(Refer Slide Time: 27:42)



So, my settings are usually looking very nice in this particular one. So, in this fashion the image acquisition is being happening.

Right.

And, you also have you we give you a power that in between the slides usually people keep barcodes.

Right.

And, our system or GenePix Pro is compatible with reading the internal barcode which is being done.

So, that you can have multiple scans also being possible. So, nowadays each slide is coming with multiple arrays because of the variable densities people are.

Right.

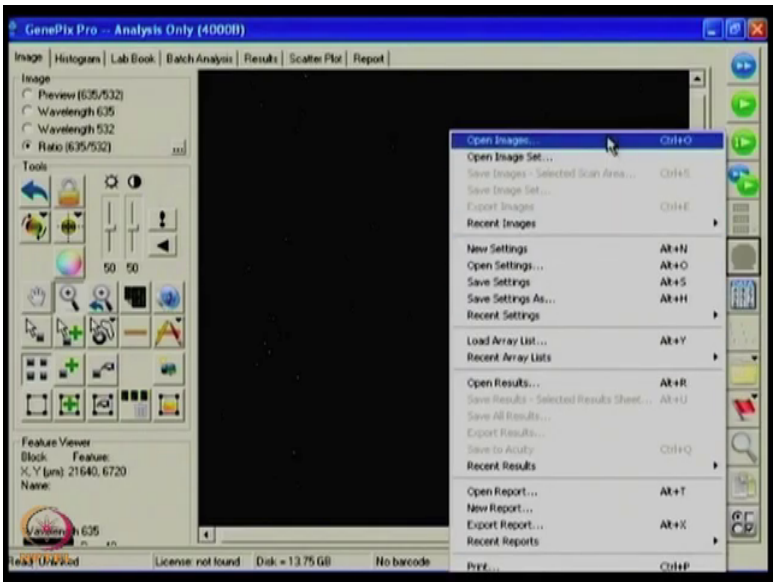
Focusing on the custom type. So, this can also be done with the new software upgrade developments.

Ok.

So, now as the scanning is being performed let us look like I will save the image.

Right.

(Refer Slide Time: 28:30)

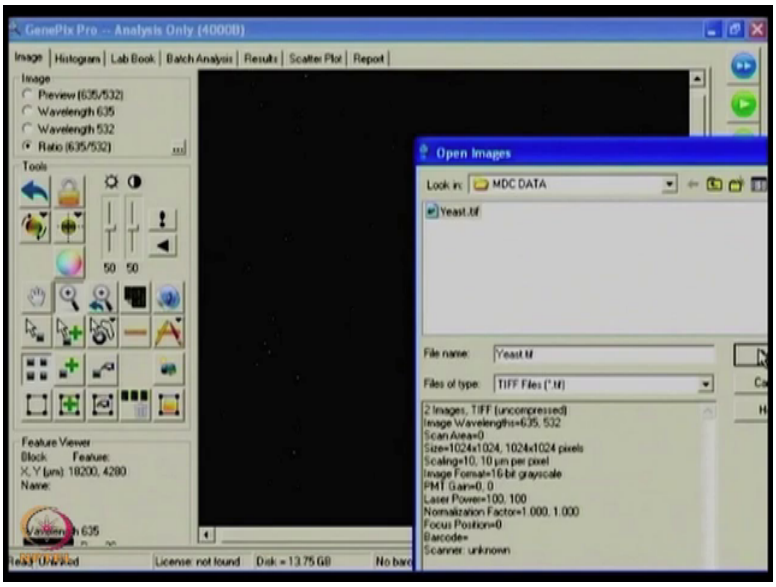


And, once I save the image I would like to see how the different processes is being done.

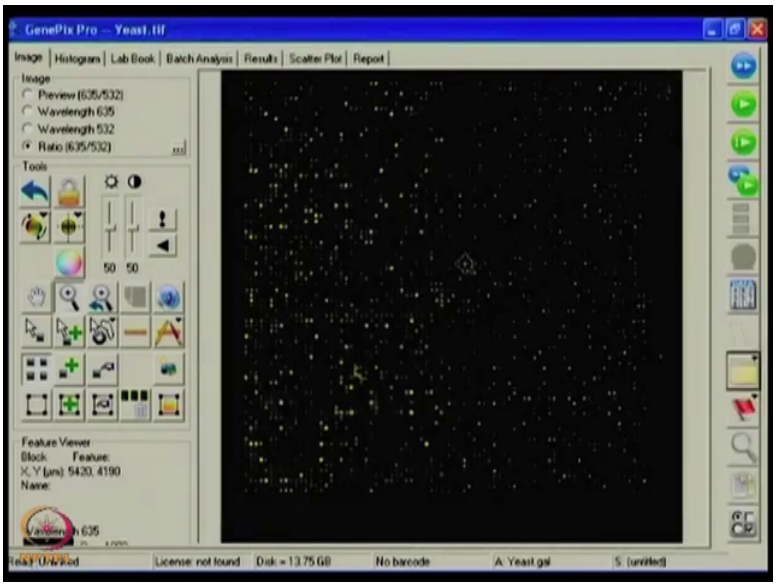
Yeah.

So, say for example, I have saved this image in the form of say this is the Yeast. So, I just want to open an image which I have just saved.

(Refer Slide Time: 28:37)



(Refer Slide Time: 28:41)



So, basically as we discussed each particular array can be divided into the blocks. So, this particular array of yeast contains four blocks.

Ok.

And, each block is having features.

Right.

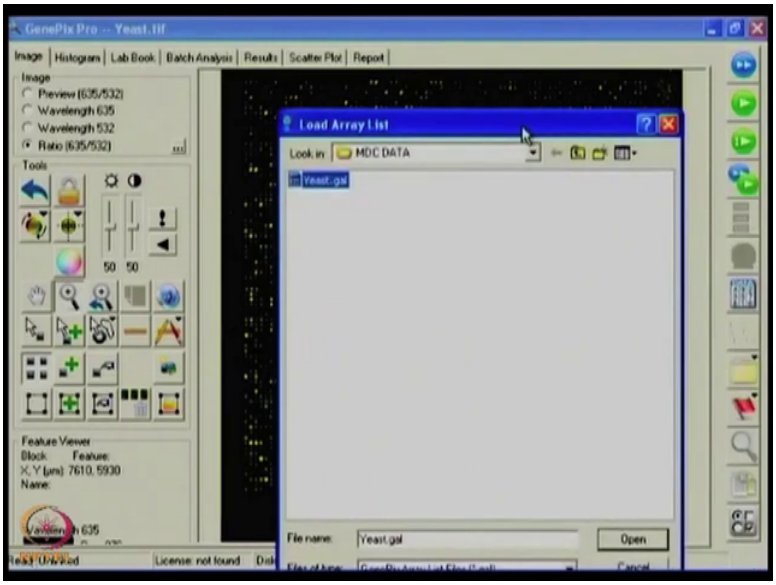
So, number of feature information is given in GAL. So, basically terminology is array, block and features. So, I need to align that GAL information of positions.

Sure.

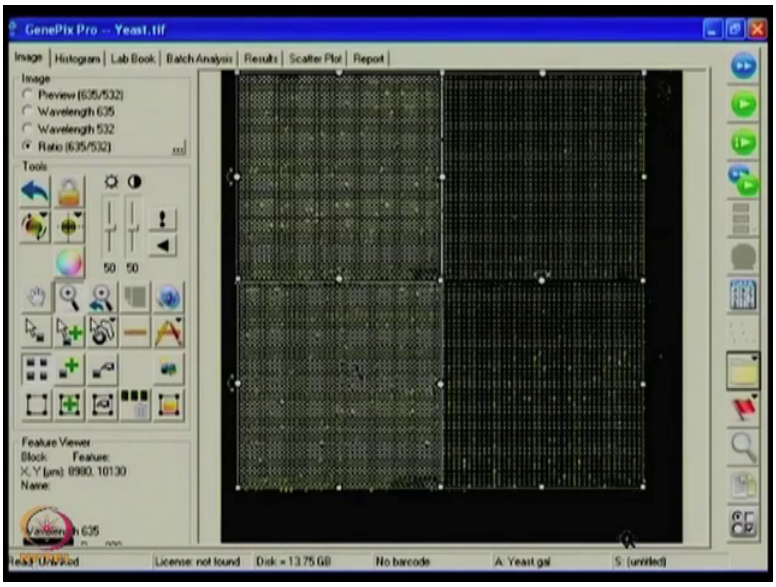
On top of this. So, I have to put a GAL file and do my further analysis. So, what I am going to do now is open a GAL or GAL file which allows me for alignments.

Right.

(Refer Slide Time: 29:19)

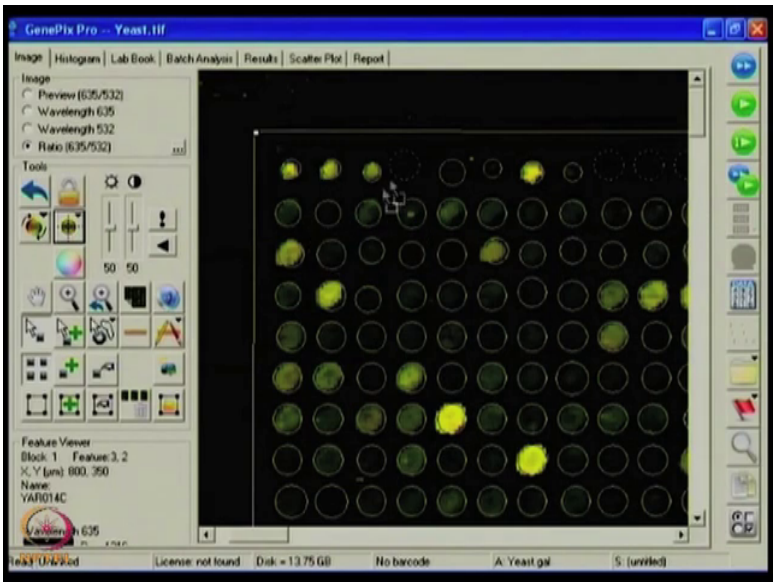


(Refer Slide Time: 29:26)



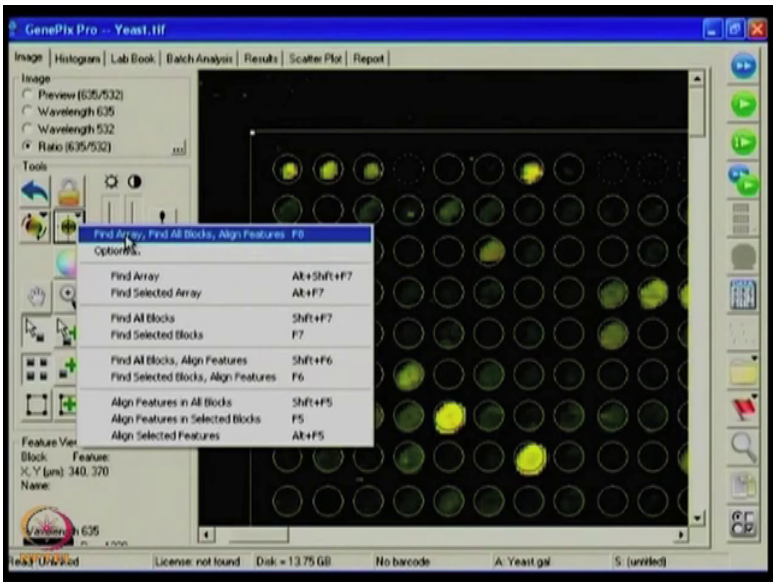
So, best feature of GenePix Pro is its capability of identifying feature by itself it is little clumsy.

(Refer Slide Time: 29:33)



So, let us just see how the zoom button looks like. So, only thing you have to do here is just take your Block and allow it to move to the first alignment and then what you can do is click the button over here which is for the align.

(Refer Slide Time: 29:48)



Align can be done in different ways I recommend to use the first which finds all features, all blocks and do automated fashion. So, if you click once you see software automatically finds all it features.

Right.

Wherever by chance the features are absent or there are some physical deformity it is say it is not present or it flag it as bad. So, as you see you can actually move it, but it does not affect life as long as you have just kept once and the data is being stored. But, usually people ask me is it good idea if I by chance move to do once again. So, it is not a bad idea to do because takes few seconds to do it.

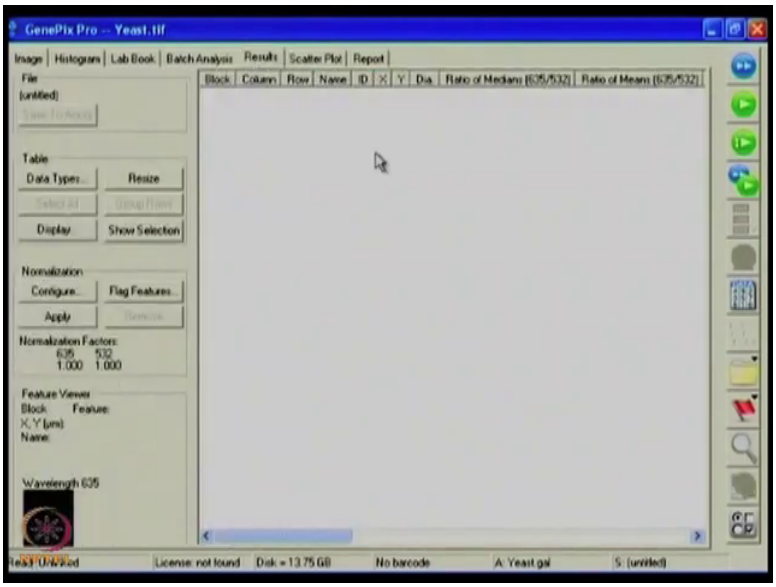
Right.

So, once you have done this particular alignment let us look these two slide which I said we can zoom out.

Yeah.

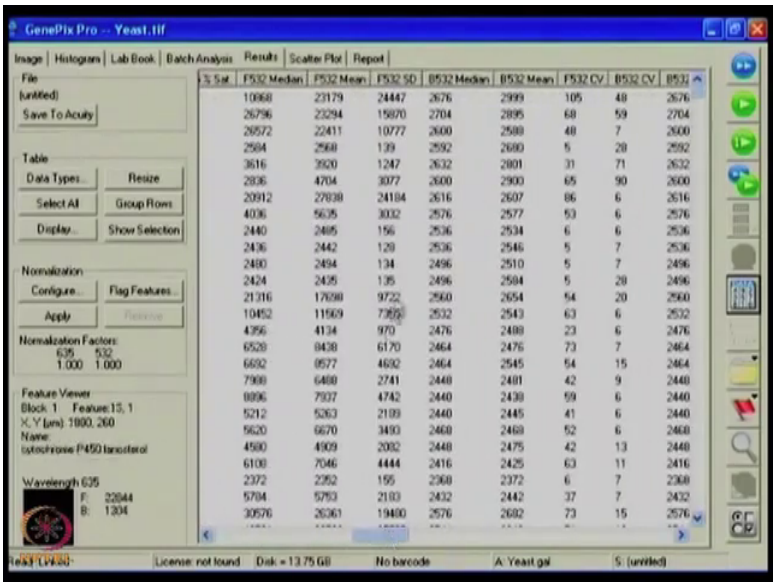
So, you can see whole particular slide now is being scanned and aligned as well. So, it is a very quick process which software performs very easily for doing the job. And, once you have done this you can always hit a button of results now if I go to results actually this is empty.

(Refer Slide Time: 30:46)



Yeah.

(Refer Slide Time: 30:51)



So, if I click on results the results are being calculated and there are some 40 different columns which will be output in the form which GenePix Pro understands different ways.

Sure.

So, just quickly looking it to the major ones. The major ones here are looking at this F means the intensity from different channels 635 or 532.

Right.

And, this background calculation is being done accordingly in the same major range. So, once you do a corrections what happens is you want to correct your intensity mean values with the values of the background.

Ok.

So, this is what is the most important which usually people use for the further calculation.

Right.

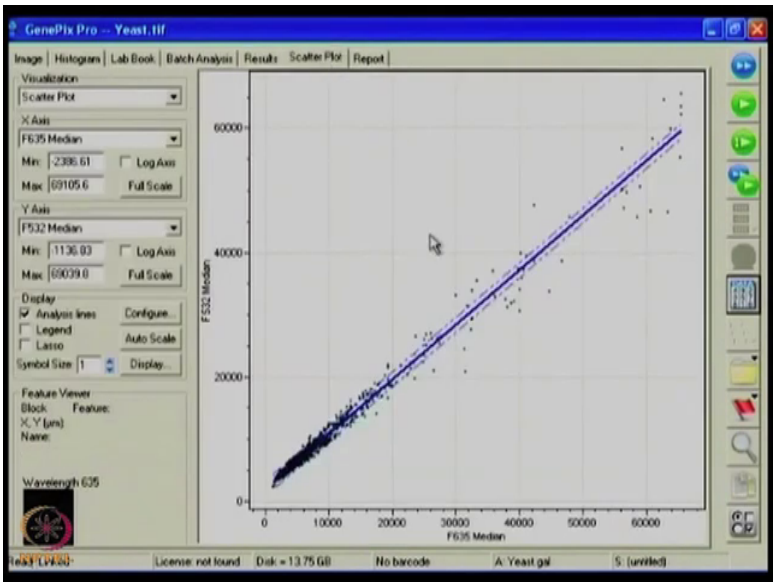
Apart from a ratio of means or ratio of medians which can be calculated again and being presented to you in a different column formats. So, each column signifies different ones like for example, SD standard deviation, CV coefficient of variations and then different channels coming up. So, in this fashion the result should be outputted if you are image acquisition first, controlling the part then allowing you to align and then do the analysis. So, this is a basic steps which anybody or everybody want to do in my career steps.

Right.

So, once you see the images that the people end up in the form of results and you have different columns available to you. So, there different ways people want to visualize how my column because number may vary in that sense. So, the best way to look is a scatter plot.

Right.

(Refer Slide Time: 32:20)



Scatter plot allows you in a different ways what you are plotting at X and Y axis is and here if you see I am just plotting actually towards F635 median over the F635 median. So, you are comparing two different channels how they have behaved. So, essential rule is they should mostly the microarray assumes all the chips are having spots and which are genes which are not varying too much. So, you expect most of them to stand nearby to the origin of the center.

Yeah.

So, this is what you want to look at.

How close is they are aligned.

How they are close aligned.

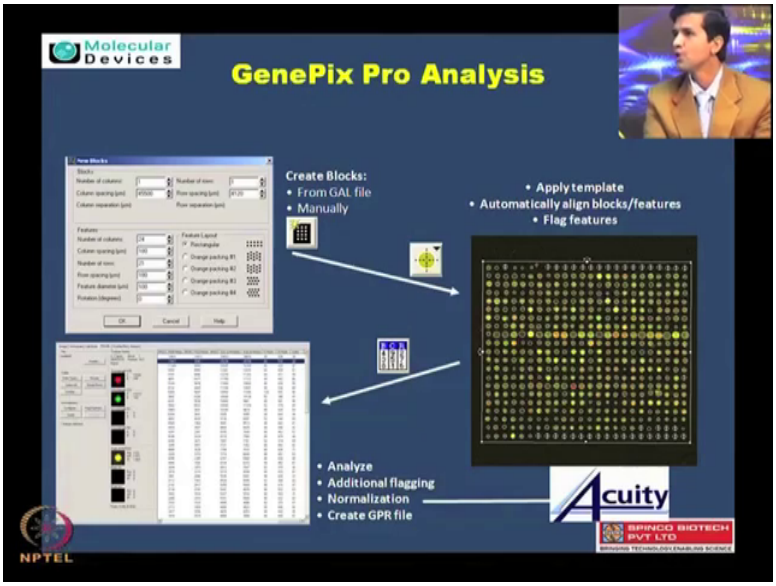
(Refer Time: 32:55).

Yes. So, they should be not too far away from each other so, that they are not nave actually to each other.

Right.

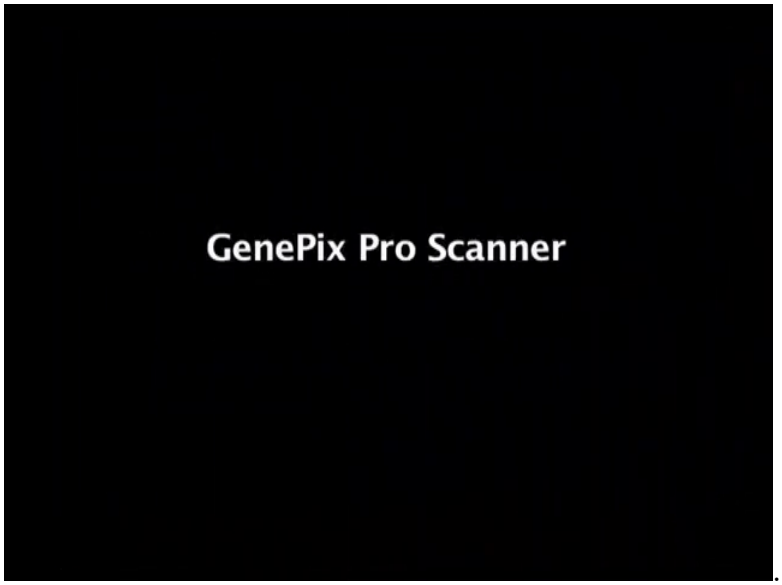
Because you expect there are few differences, but not very very significant which can be seen at very large scales.

(Refer Slide Time: 33:06)



So, GenePix Pro as we discussed acquisition software and the molecular devices recommend acuity software for further data analysis which can be at the level of secondary or tertiary based on that. So, you do statistics as well as visualizations on a single or multiple data to handle that.

(Refer Slide Time: 33:26)



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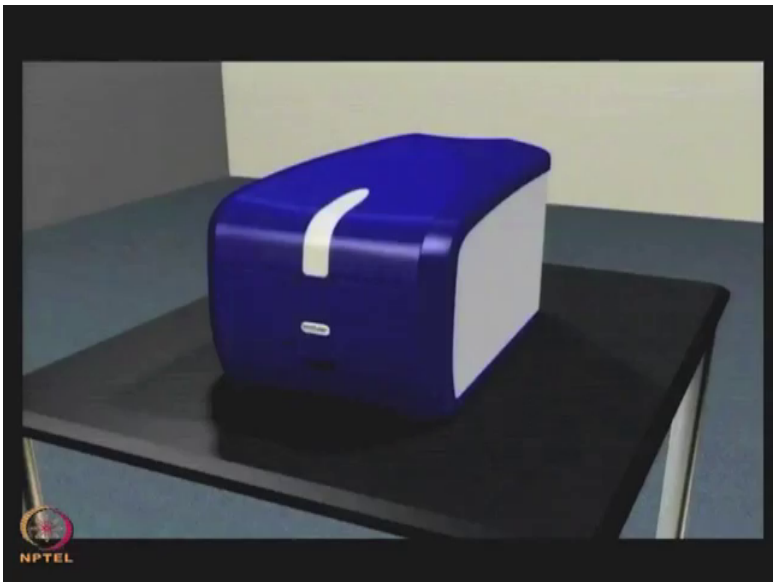
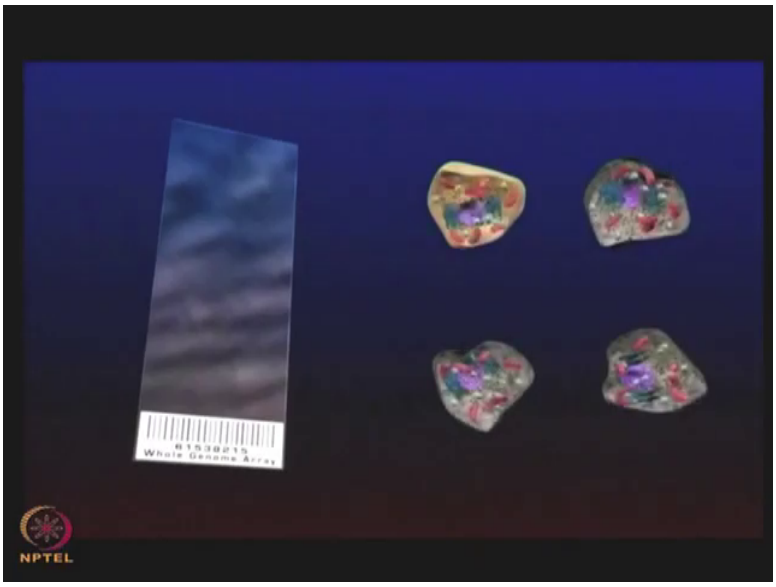
(Refer Slide Time: 33:33)

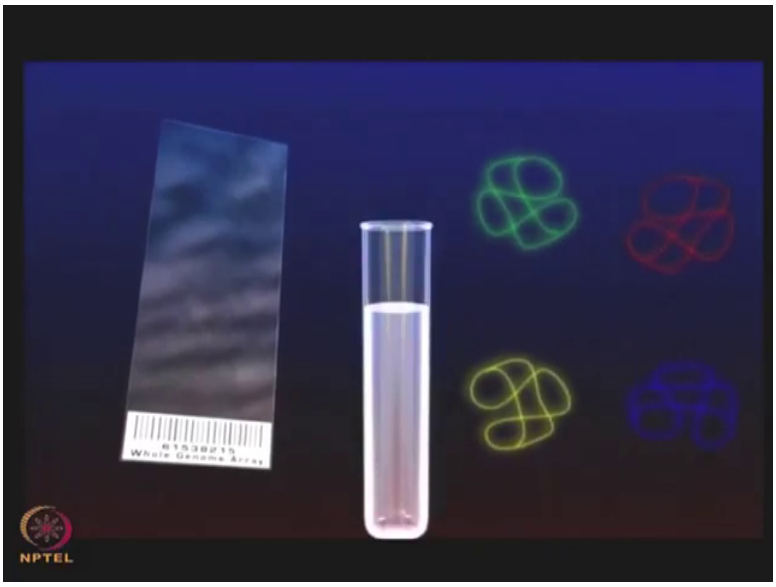


Molecular devices introduces the worlds simplest most reliable automatic microarray slide scanner. Now, you can walk away from scanning while the GenePix autoloader 4200 AL automatically loads, scans, analyzes and saves results for up to 36 slides.

(Refer Slide Time: 33:52)

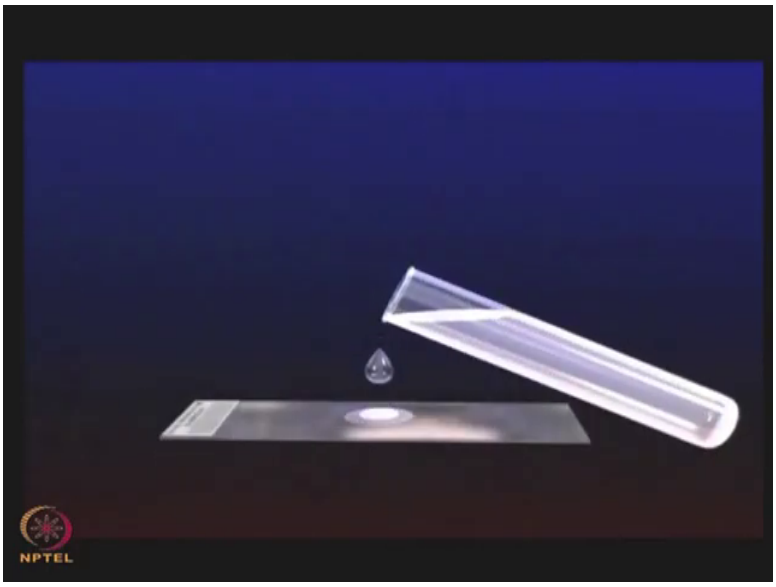


(Refer Slide Time: 33:56)



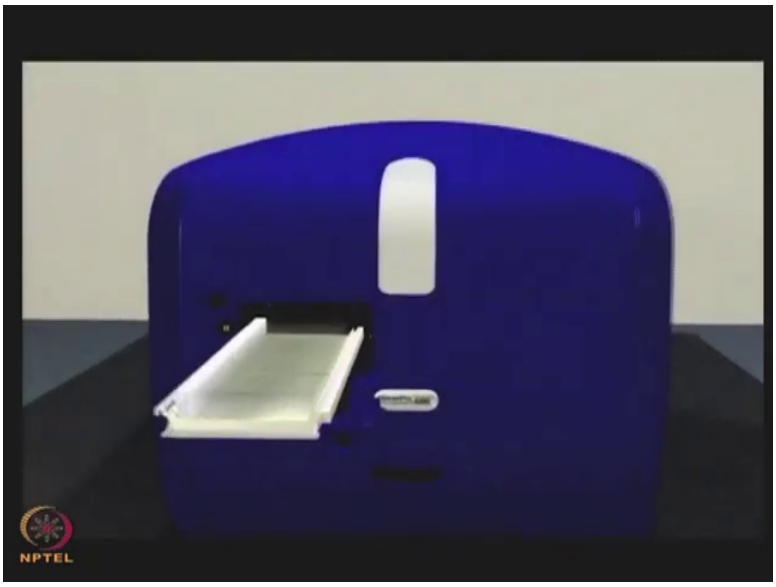
The auto loader accommodates microarrays on standard glass microscope slides labelled with up to 4 fluorescent dyes.

(Refer Slide Time: 34:02)



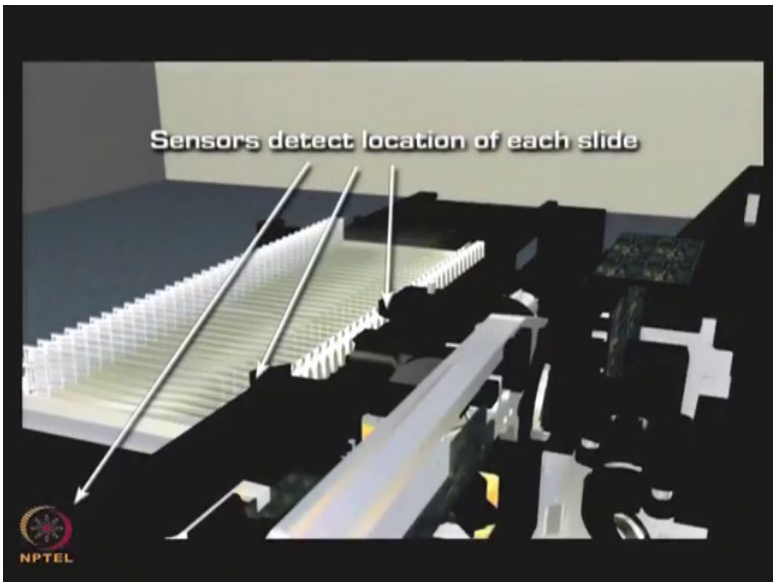
These microarrays can contain just a few hundred spots or tens of thousands of spots representing an entire genome.

(Refer Slide Time: 34:09)



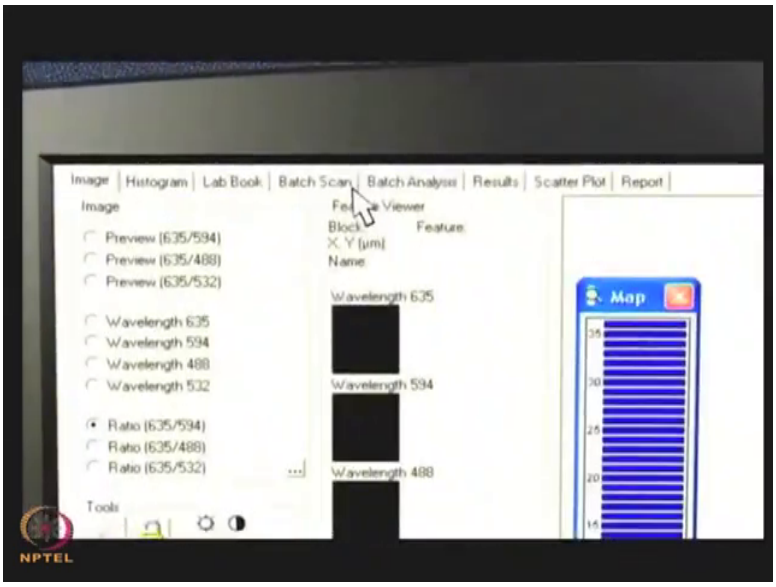
As many as 36 slides can be loaded into the convenient slide carrier.

(Refer Slide Time: 34:16)



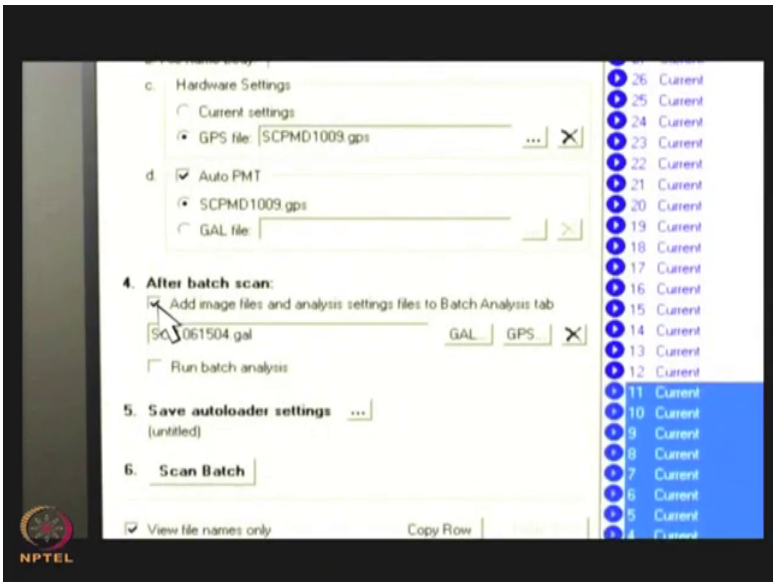
As the carrier is inserted into the scanner sensors detect the location of each slide indicated by a blue bar on the slide carrier map.

(Refer Slide Time: 34:22)



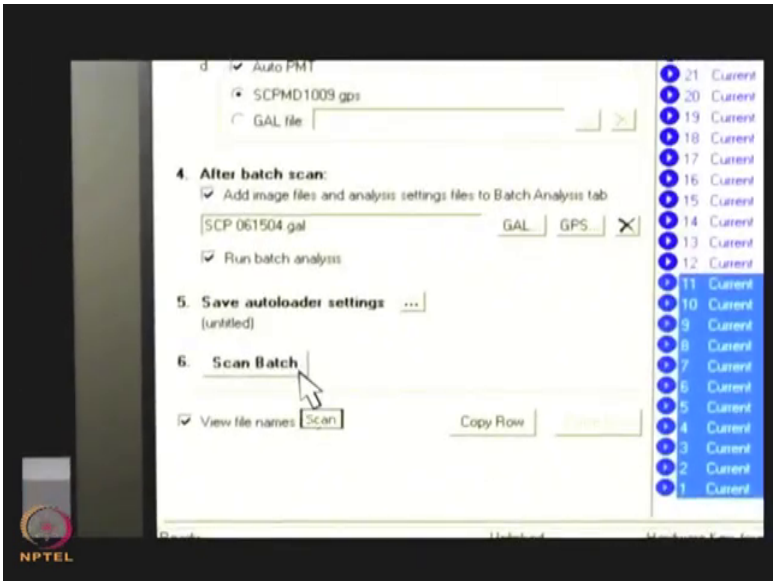
On the batch scan tab in GenePix Pro, you have complete flexibility to define the most appropriate settings and analysis parameters for each slide or for groups of slides.

(Refer Slide Time: 34:31)

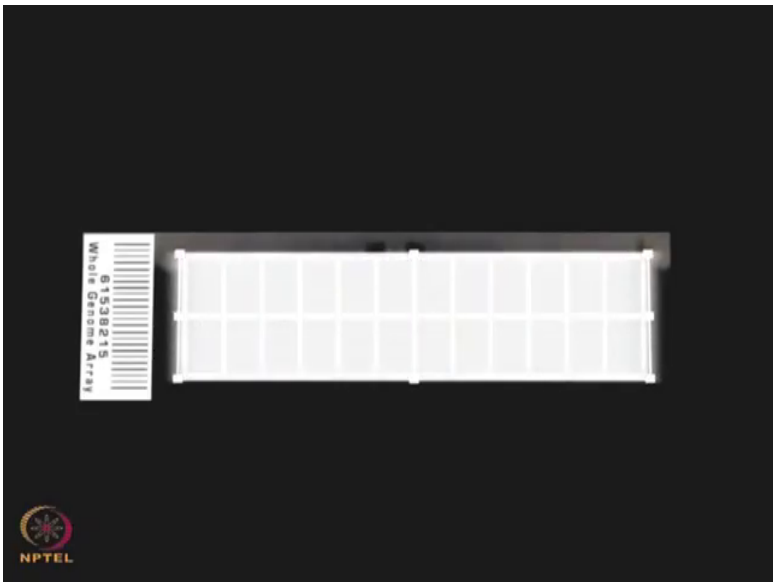


You can also choose to automate scanning analysis and file saving steps.

(Refer Slide Time: 37:37)


Auto PMT
SCPMD1009.gps
GAL file
4. After batch scan:
Add image files and analysis settings files to Batch Analysis tab
SCP 061504.gal
GAL
GPS
Run batch analysis
5. Save autoloader settings
(untitled)
6. Scan Batch
View file names
Scan
Copy Row
21 Current
20 Current
19 Current
18 Current
17 Current
16 Current
15 Current
14 Current
13 Current
12 Current
11 Current
10 Current
9 Current
8 Current
7 Current
6 Current
5 Current
4 Current
3 Current
2 Current
1 Current
NPTEL

(Refer Slide Time: 34:43)

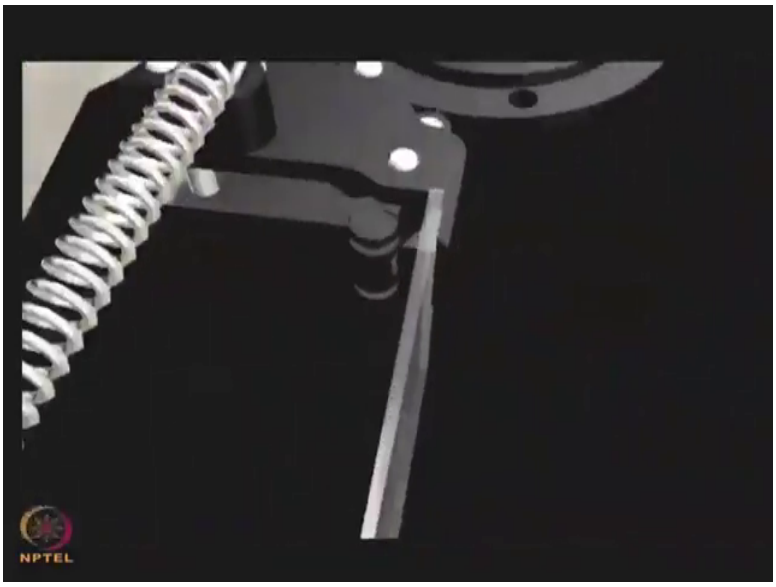


Enter an email address and GenePix Pro will notify you remotely when your batch is complete.

(Refer Slide Time: 34:46)

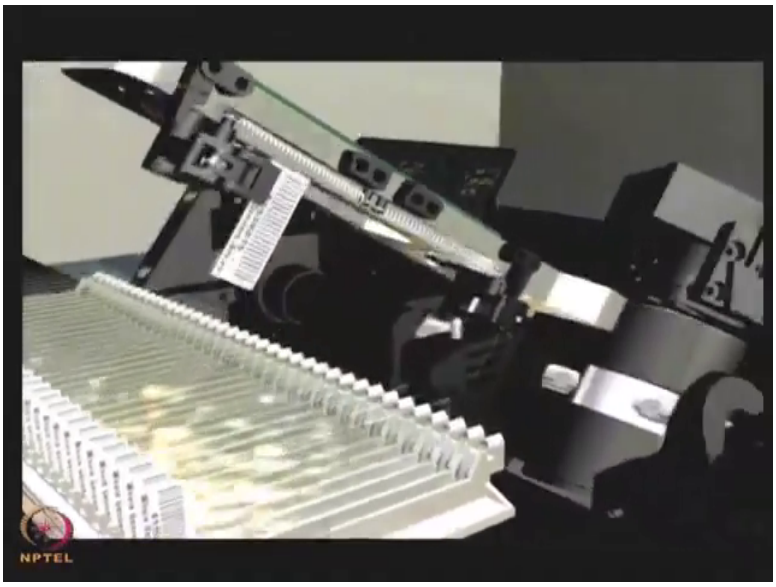
Using the defined scanning parameters the precision robot arm leaps into action and moves to the first slide.

(Refer Slide Time: 34:55)

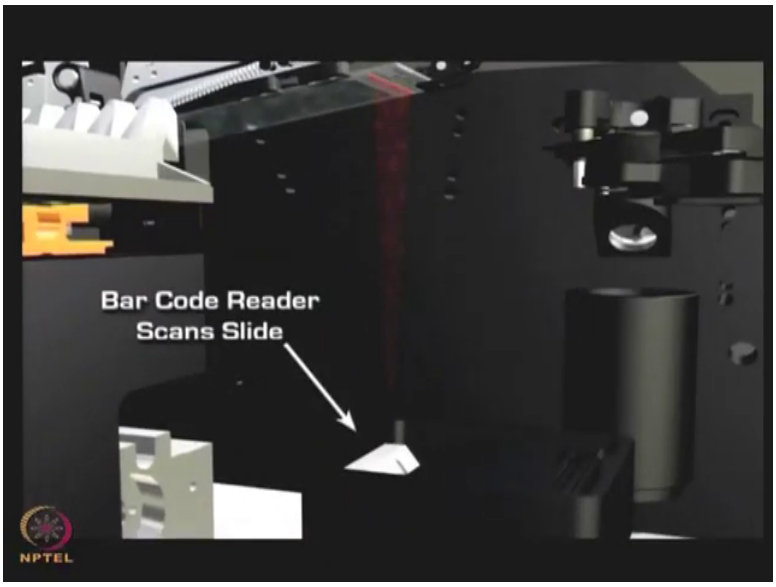


A unique never let go rippers securely clamp the slide and carry it to the scanning area. A barcode reader records the barcode and then the slide is positioned for scanning.

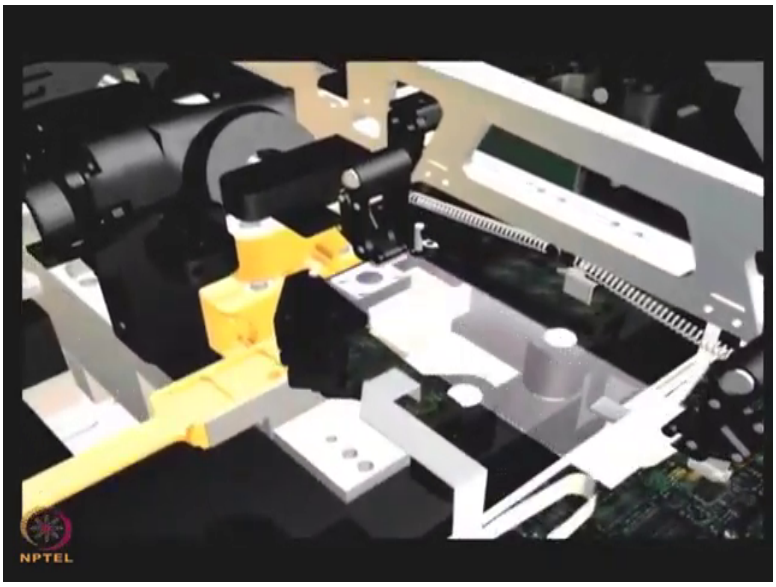
(Refer Slide Time: 35:00)



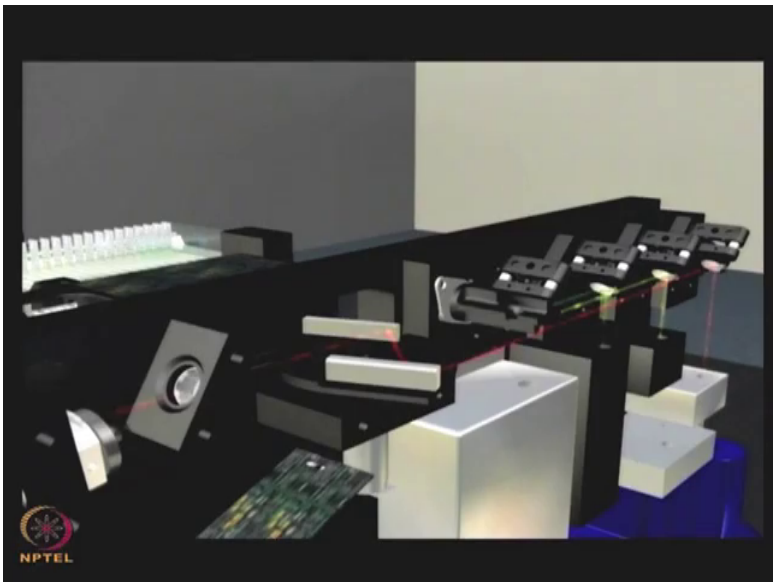
(Refer Slide Time: 35:04)



(Refer Slide Time: 35:09)

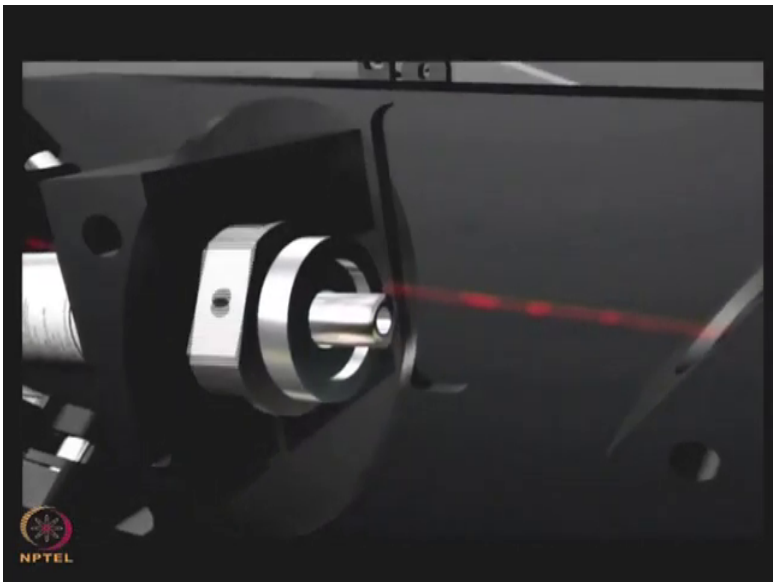


(Refer Slide Time: 35:15)

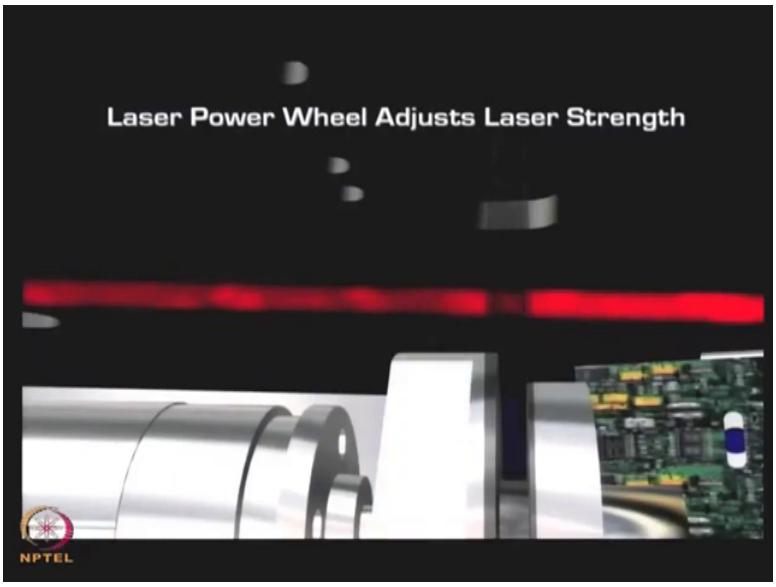


The GenePix autoloader 4200 AL can be configured with up to four lasers.

(Refer Slide Time: 35:20)

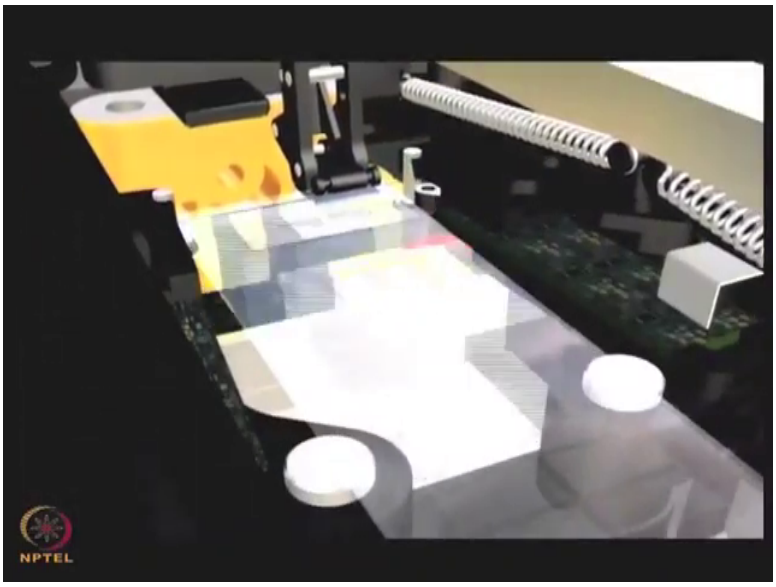


(Refer Slide Time: 35:23)



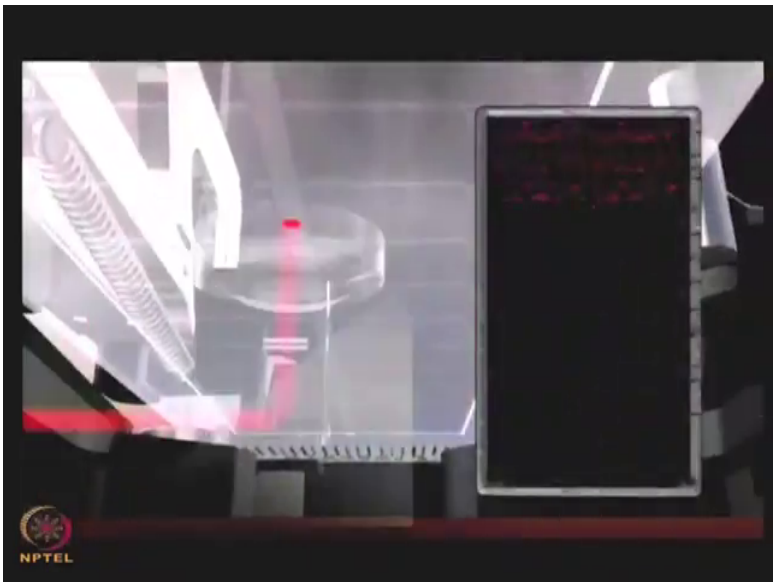
A neutral density filter wheel can be used to attenuate the laser power if necessary for especially bright samples.

(Refer Slide Time: 35:35)



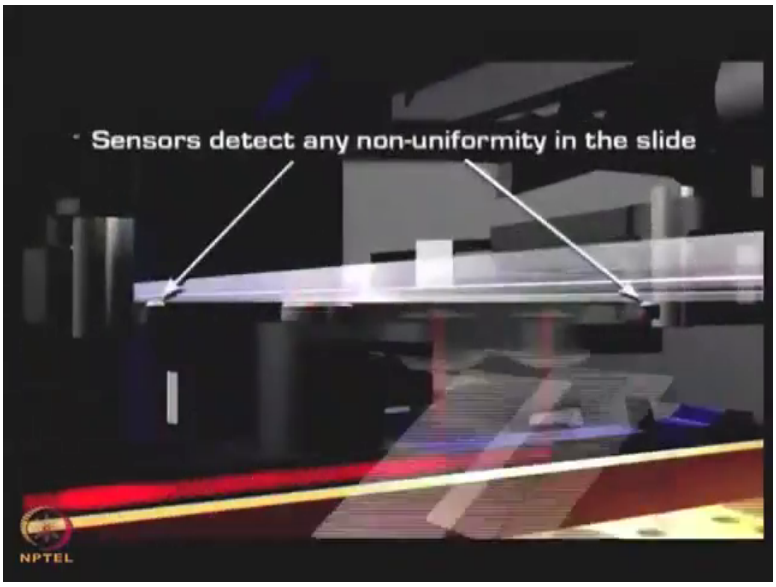
The laser excitation beam is delivered to the surface of the microarray slide.

(Refer Slide Time: 35:40)



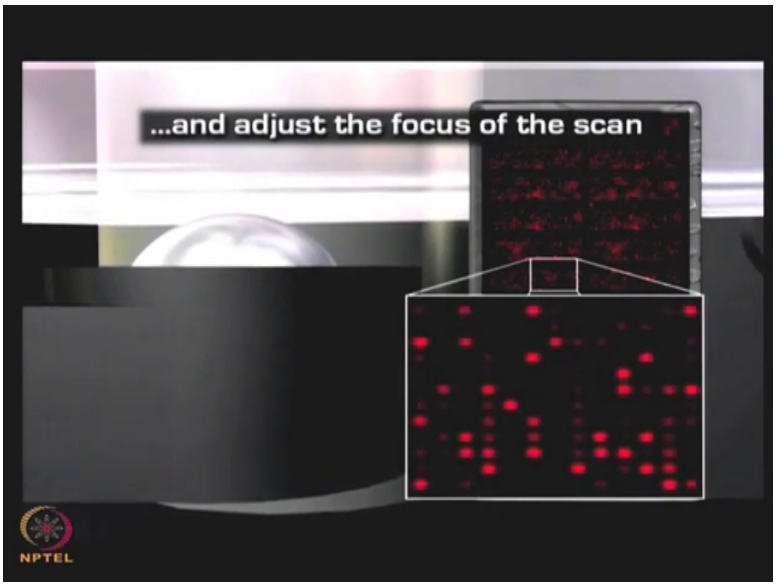
The beam scans rapidly across the short axis of the slide as the robot arm moves the slide more slowly down the long axis.

(Refer Slide Time: 35:46)



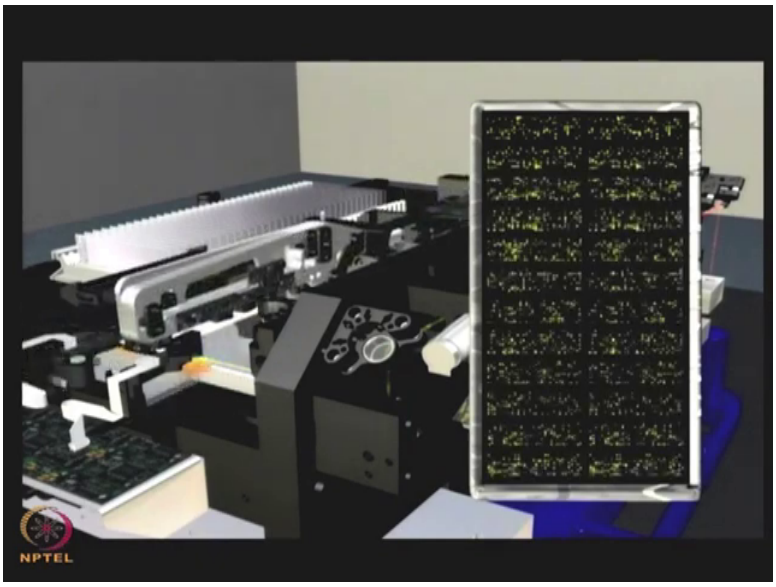
Fluorescent signal emitted from the sample is collected by a photomultiplier too. As the scam proceeds sensors detect any non-uniformity in the slide surface.

(Refer Slide Time: 35:52)



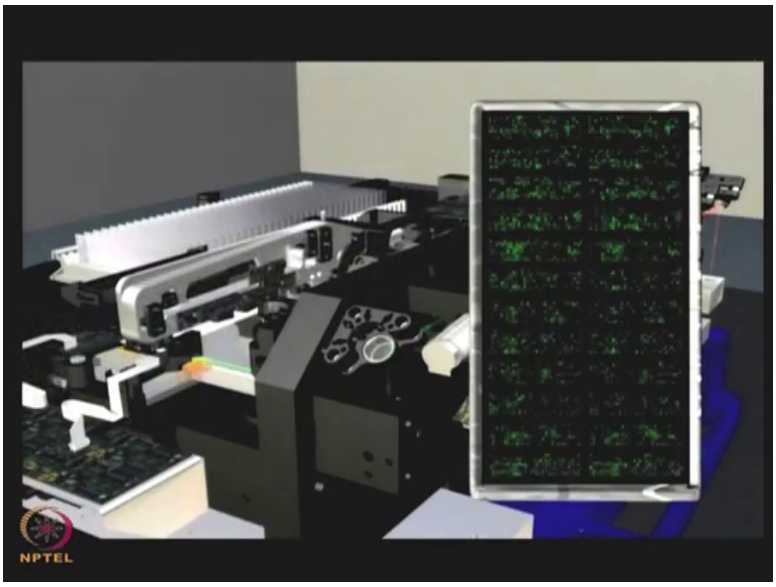
And, the robotic arm adjusts the slide position accordingly to ensure the array surface is always in perfect focus.

(Refer Slide Time: 36:06)



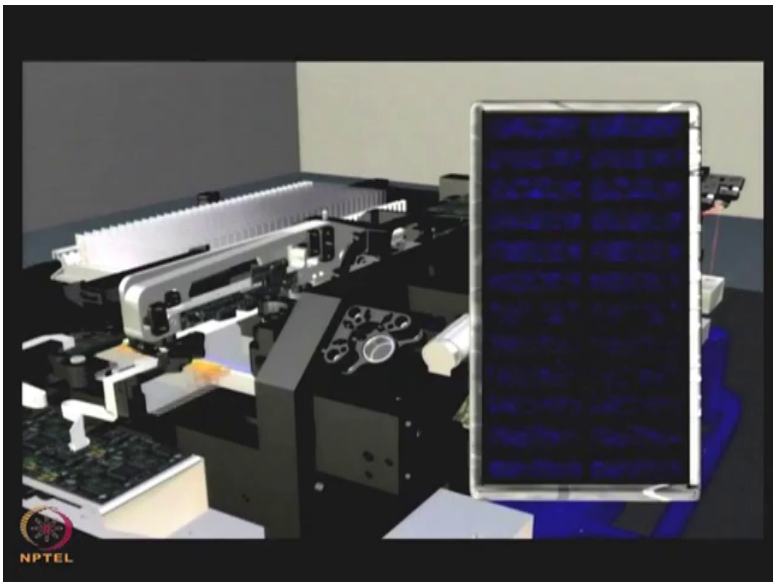
Each channel is scanned sequentially and the developing images are displayed on the monitor.

(Refer Slide Time: 36:15)

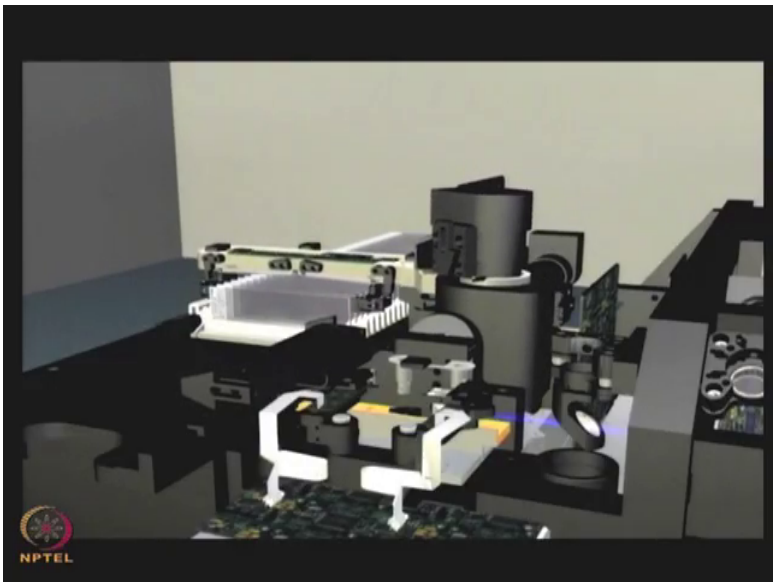


The multi channel tiff images are saved automatically according to file naming conventions specified by the user.

(Refer Slide Time: 36:22)

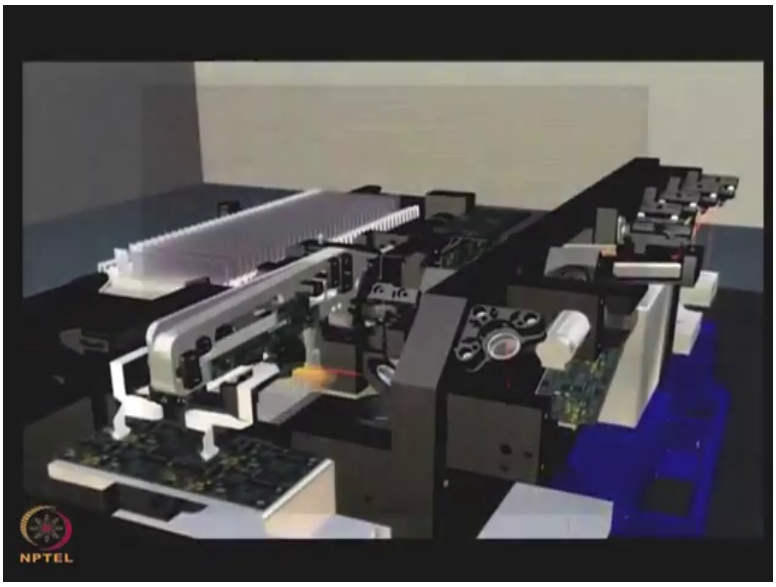


(Refer Slide Time: 36:28)

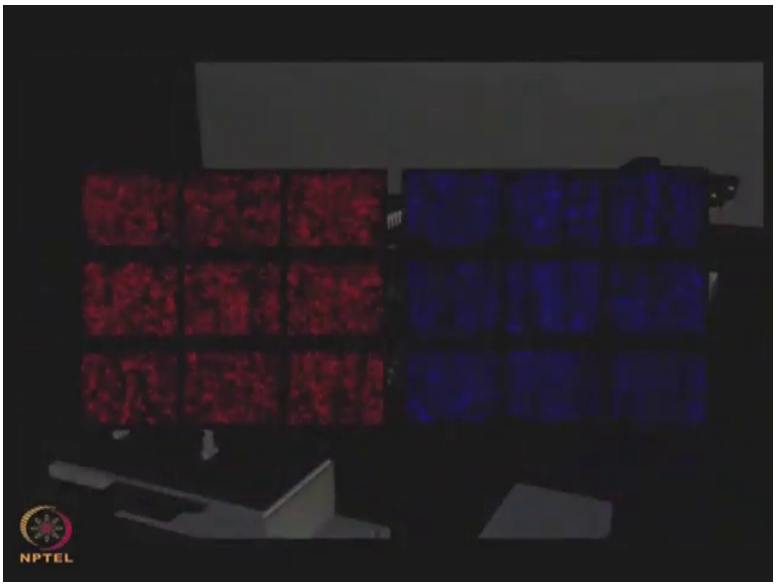


After the slide has been scanned the precision robot arm replaces it safely in the slide carrier before picking up the next slide.

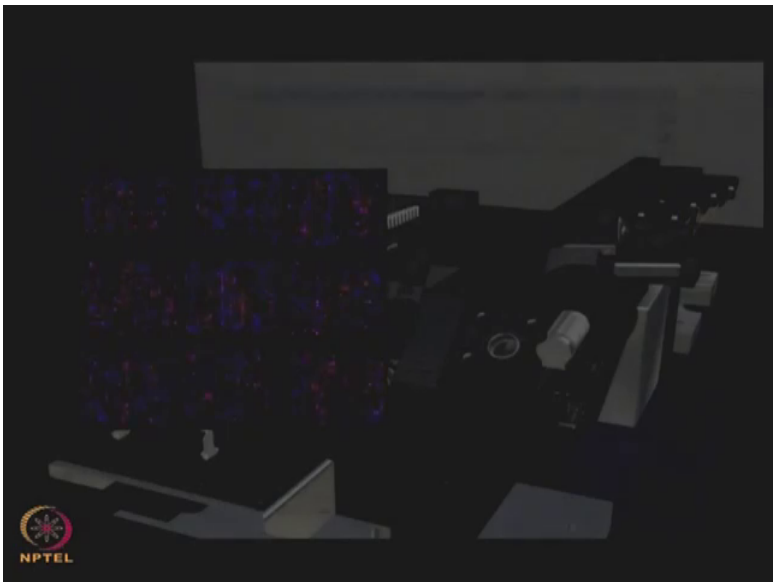
(Refer Slide Time: 36:36)



(Refer Slide Time: 36:38)

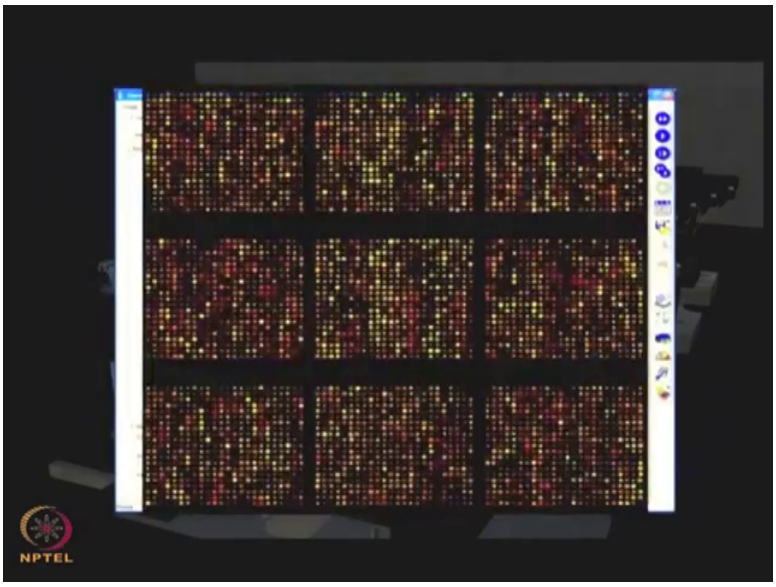


(Refer Slide Time: 36:41)

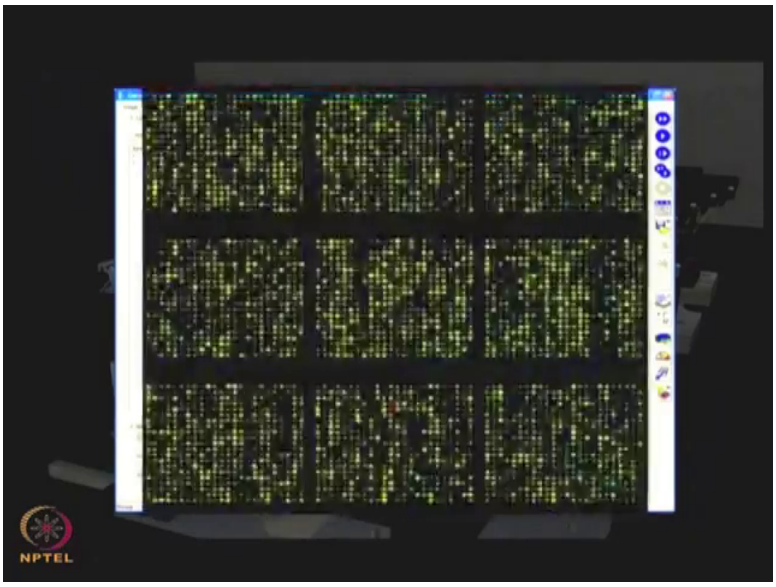


As each slide is scanned a list of each saved image with its associated settings and analysis files accumulates in the batch analysis tab until the batch is complete.

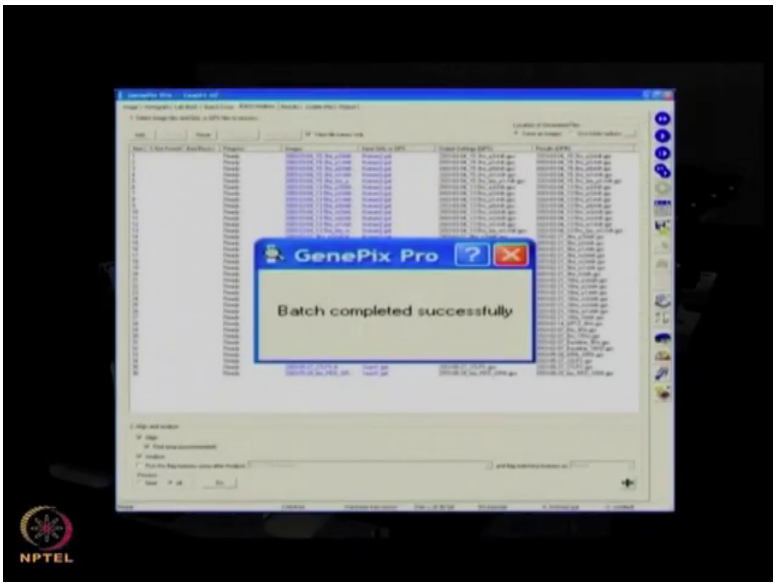
(Refer Slide Time: 36:43)



(Refer Slide Time: 36:46)



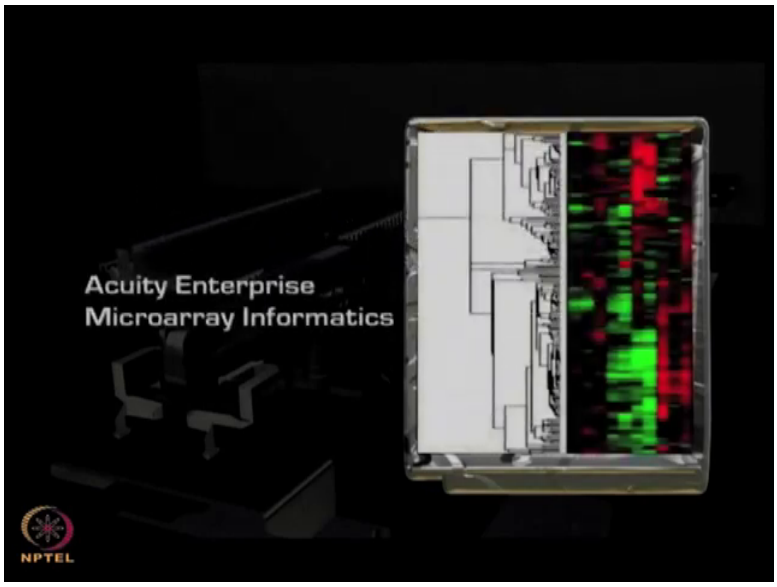
(Refer Slide Time: 36:49)



(Refer Slide Time: 36:52)

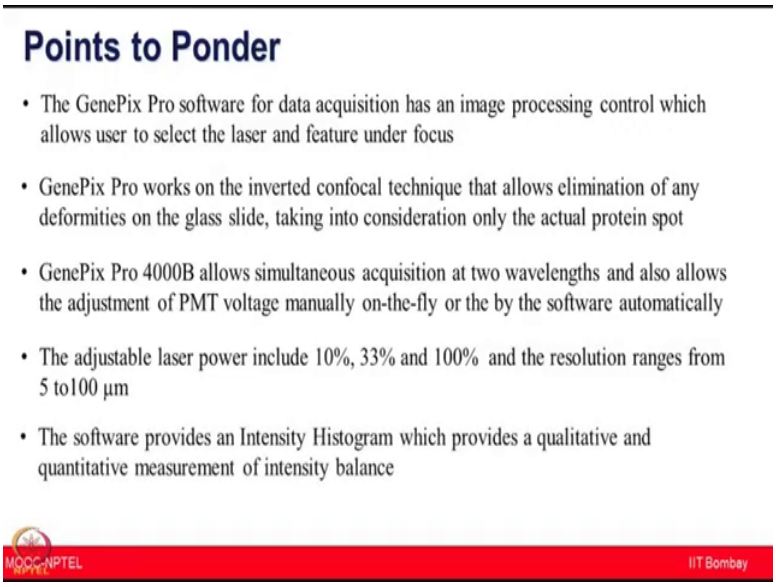
GenePix Pro automatically finds the spots and calculates up to 108 different measurements for each spot the results are saved as a GenePix results or gpr file.

(Refer Slide Time: 37:10)



gpr files can be saved automatically to the acuity database for statistical analysis clustering and other advanced investigations.

(Refer Slide Time: 37:17)



So, I hope in today lecture and demonstration for various parameters. You are now familiar with the basics of a scanning parameters, image processing and data acquisition in a protein microarray experiment intensity of a spot is a representative of the interaction between the sample and analyte. To achieve this target proficiency in image processing and data acquisition is required.

As discussed the artefacts due to the contaminants such as dust particle or even very high background on the chip. These issues make automation in image processing and data acquisition very challenging. Researchers have devised several segmentation algorithms to reduce the manual interventions. However, you have seen there is the degree manual flagging is necessary to mark the low quality spots. The data would now be pre-processed for background correction and normalization.

You also seen the demonstration of one such software GenePix Pro microarray image analysis software for data acquisition. And, you will further see how it will be used for data analysis in subsequent lecture. There are several commercial softwares like proto array prospective software and many others as well as there are several open source software I recommend you to play with many softwares and try to explore the features.

While the basic steps remains the same many softwares offer some very specialized features which also includes same time the data visualization, data plotting and a better and effective ways of data representation.

In our next lecture we will continue our discussion on microarray experiment workflow and how to analyze the microarray data obtained from the images generated from today's lecture.

Thank you.