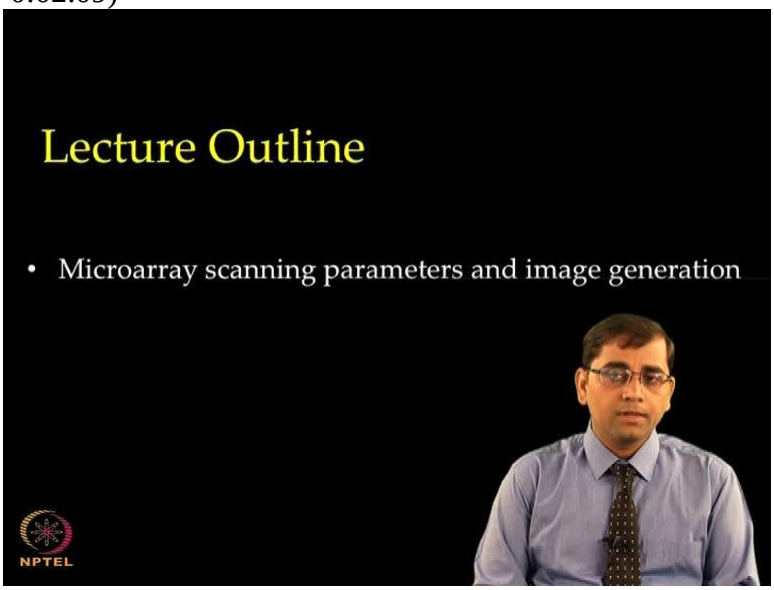
Interactomics: Protein Arrays & Label Free Biosensors
Professor Sanjeeva Srivastava
MOOC NPTEL Course
Indian Institute of Technology Bombay
Module 7
Lecture No 33
Basics of microarray image scanning

Welcome to the mooc interactomics course. Today we will discuss about various parameters that should be considered during image scanning and processing in microarray work flow. Detection probes have been briefly discussed in our previous lectures. These probes are major determinants of finding storm any microarray experiment. Detection strategies for quantifying signals from these probes in microarrays have been categorised into two basic categories, label based and label free methods.

Due to the strong advancements by DNA microarray technologies in the area of confocal laser scanners it has been modified and extended extensively in protein microarrays as well. Consequently label based methods are most widely used protein microarray detection system. Scanners captured the fluorescent label signals using cooled charge coupled device CCD cameras.

Some scanners also employ lasers for excitation and photomultiplier tube, PMT detector. In other cases, LED is used for a uniform light source and is detected using a cooled CCD in combination. Today we have with us Mr Pankaj Khanna who will be talking about GenePix microarray scanner from molecular devices. We will first have a discussion about microarray scanner as well as the software which control this.

(Refer Slide Time: 0:02:09)



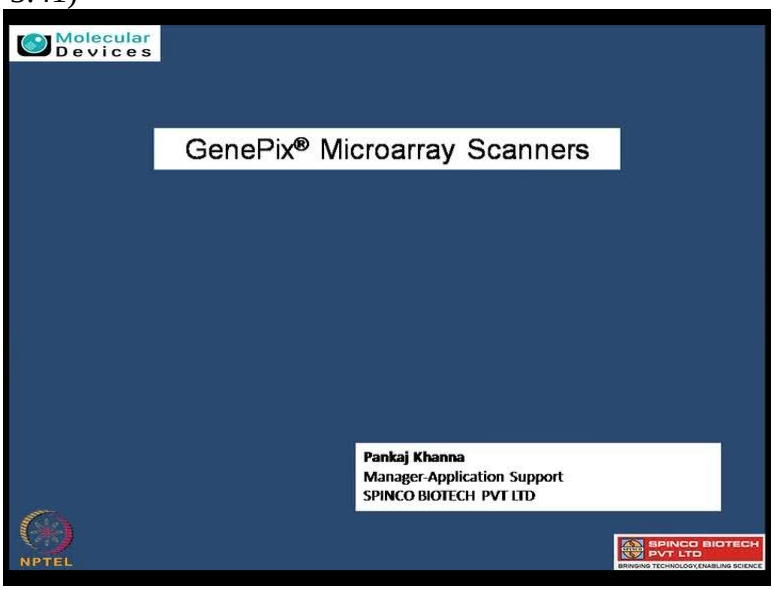
We will talk about various concepts as well as live demo of software interface in the subsequent lecture so that you get better understanding of how this whole process is controlled and can be performed. This demonstration will help you to get a unique learning experience through the user interface of this specialized software.

Prof. Sanjeeva Srivastava: This is my pleasure to introduced Mr Pankaj Khanna manager application support from Spinco biotech private limited. Today we will talking about GenePix microarray scanners which is from molecular devices and Spinco is distributor for the same. So, we will have discussion about microarray scanner as well as the software how to control the hardware. So will talk about various basic concepts as well as a live demo of the software interface so that you can get a better feeling about how this whole process is controlled and performed.

So, Pankaj welcome to discussion about microarrayscanners and the software.

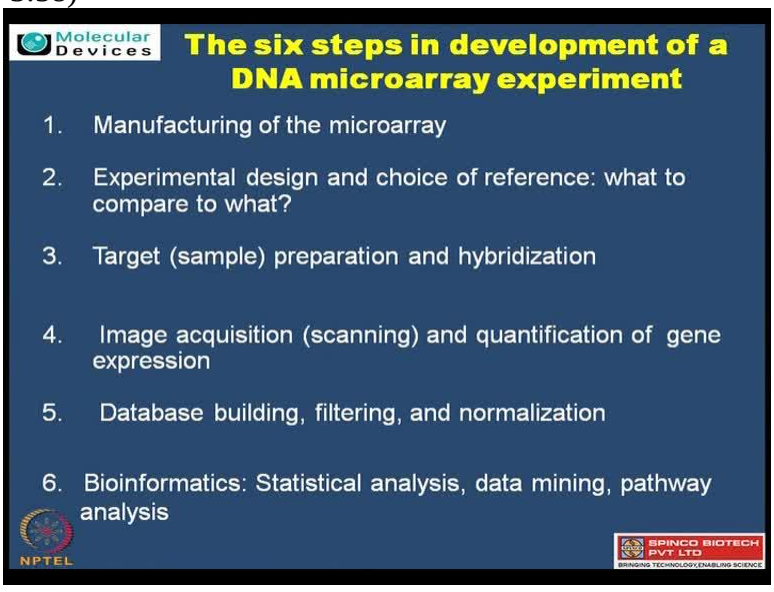
Mr Pankaj Khanna: Thank you Dr Srivastava its my pleasure. At Spinco biotech we are having molecular devices as distributors for last many years and we are dealing with the scanners for many years at the level of microarray.

(Refer Slide Time: 3:41)



As you have already described that this is use in the firm of microarrays. We are dealing from the image acquisition to the level of data analysis for the microarray applications. So, lets go through little bit on what is GenePix actually can do in firm. So…

(Refer Slide Time: 3:58)



Prof. Sanjeeva Srivastava: That it this proteomics course and I have been talking more onto the protein microarray and things but this whole technology got initiated from the DNA microarrays. So, just so that students have some idea about how the DNA microarrays are performed just give

some brief steps about overall DNA microarray technology and also like how and what GenePix can help in the microarray process.

Mr Pankaj Khanna: Sure. Sso basically the DNA microarray steps are having divided into six basic steps starting from the manufacturing of the microarray. So, based on the slide and your biology the manufacturingof the slide is done. So if you want to go for RNA expression chip you have RNA expression chip made. So if you are using a protein expression chip you are using a protein expression chip. So after the design is being decided the next step is the experimental design which usually scientist think based on the biological question what they are asking.

Prof. Sanjeeva Srivastava: Right.

Mr Pankaj Khanna: So, based on that they choose which references, which controls to be studied and group them. Once you are ready with these two the next thing comes is the sample collection and the target or the sample preparation which then can be hybridized to the preplanned chip which now is ready to go for the scanning in the form of image scanners.

Prof. Sanjeeva Srivastava: right.

Mr Pankaj Khanna: So, the fourth steps involves actually the hardware beginning up where we as a vendor from the molecular devices come in place and this data once scanned in the form of raw data now can be made data building, filtering as well as the normalization followed by the last steps by bioinformatics and biostatics which help in the data analysis. So, in these fashion basics these six steps are important for any microarray experiment.

Prof. Sanjeeva Srivastava: So, just to clarify summarize here. I guess it is the content which is just different whether its DNA or protein but once we have the chips in hand then entire assay and the whole procedure is very similar. So, manufacturing of the microarray will be different for each of the content type. Then experimental design one has to choose depending upon the content and the biological question what they ask.

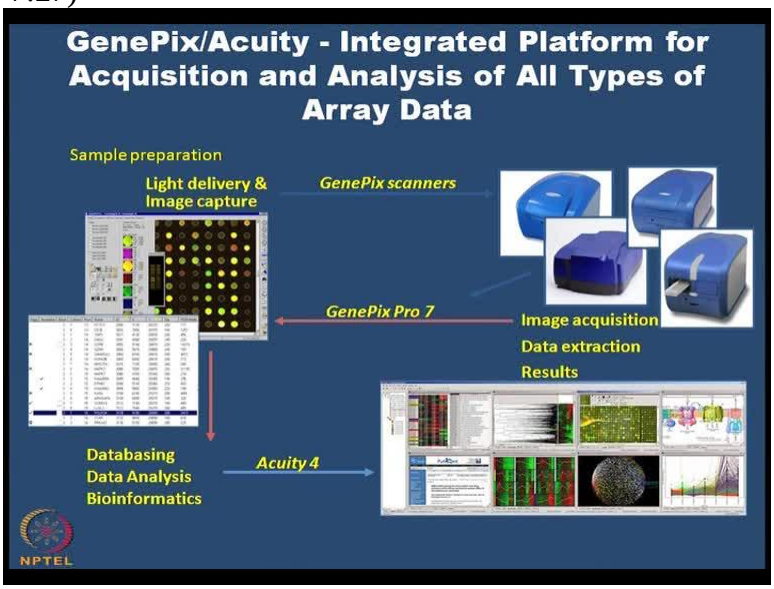
And once these things are in placethen one has to choose what type of biological samples or clinical sample they want to probe and then followed by perform the hybridization steps and then

once the experiment is performed then one need to acquire the images and that is where these hardware and scanners come in to the play.

Followed by we need to do the data analysis for thes and then the statistical part comes into the play where we need to identify the meaningful information from these experiments. So, these scanners are user friendly and the sort of a even the cost wise and overall performance wise they are quite good. They are used worldwide. I had been using it in my research earlier in different places and also I am continuous user of this scanner. So, I am quite familiar with the overall configuration in the hardware part for GenePixscanners.

Mr Pankaj Khanna: Okay so, as you are using 4000B which is actually the as you said world best soft the hardware in the form of si3 and si5 types being used. Apart from that people use for different applications as well.

(Refer Slide Time: 7:27)



Prof. Sanjeeva Srivastava: Right. So, can you talk little bit about the platforms which are used for acquisition and analysis of these microarray data?

Mr Pankaj Khanna: Sure. So, in form of hardware as we have discussed there are number of different hardware possibilities in form of GenePix right from 4000 B to 4300, 4400. These are actually doing the hardware part which allows the laser to scan the chipso, 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 and 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 the data acquiring software called GenePix pro and these GenePix pro software is now give the results to Acuity which can be further analyze for statistical analysis.

Prof. Sanjeeva Srivastava: Yes obviously you need one software which can help in the performing scanning process and then once the images are acquired then you need to obtain some meaningful information from those images and that is where I think software like Acuity can help.

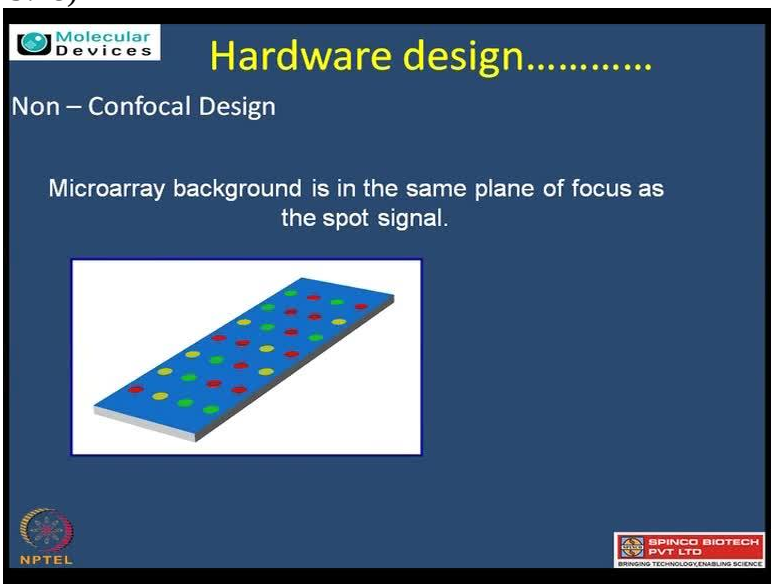
Mr Pankaj Khanna: Yes.

Prof. Sanjeeva Srivastava: So which technology is better for microarray and what do you think is the more unique features of using this hardware?

Mr Pankaj Khanna: So, GenePix microarray as said is based on two special designs one of them is non confocal design of hardware and second is inverted chemistry.

Prof. Sanjeeva Srivastava: So what is this non-confocal design?

(Refer Slide Time: 9:18)

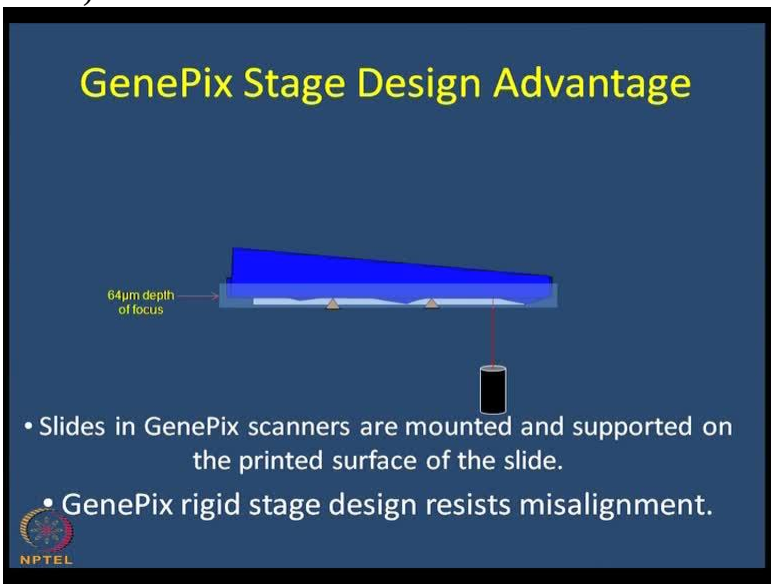


Mr Pankaj Khanna: So non-confocal design is like where you have a spots in the same plane where the scanning is done is called as a 2d dimensional. Say when you go for a tissue arrays or analysis where it is very very higher so, in the form of spot size specially at the level of imaging of scanning images 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 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 form of signal to noise ratio.

Prof. Sanjeeva Srivastava: So what are some of the advantages of this design of the GenePixstage?

(Refer Slide Time: 10:29)



Mr Pankaj Khanna : Right. So as we discussed non confocalcoupled with the inverted inverted scanning how does it help is usually we see in the glass slides there are lot of small deformities which our eyescannotsee. 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.

Prof. Sanjeeva Srivastava: okay.

Mr Pankaj Khanna: So, what it helps is it helps in looking at the deformities and directly allows the scanning to happen on the phase 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 validate for any analysis.

Prof. Sanjeeva Srivastava : So, this process can actually resist the any misalignment which may happen due to the design of the slide and overall scanning procedure.

Mr Pankaj Khanna: Very true sir, very true.

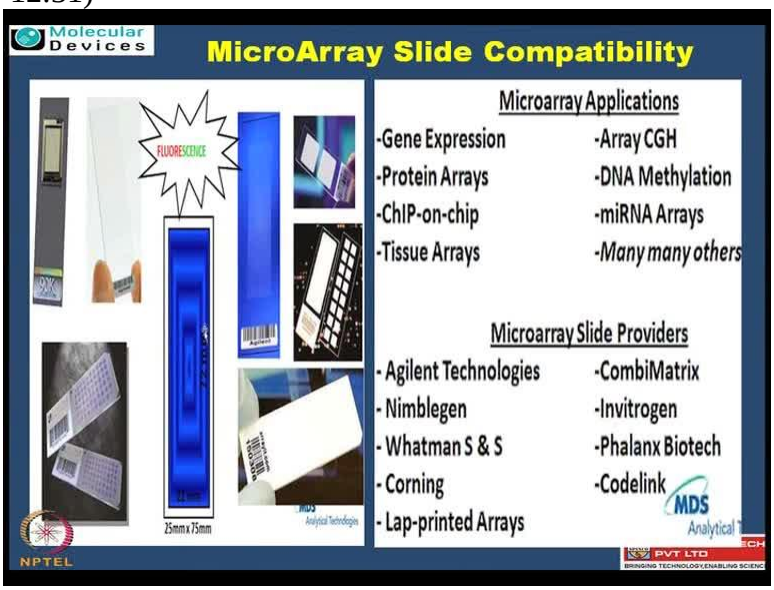
Prof. Sanjeeva Srivastava: So the different type of slides which are compatible for this type of experiments.

Mr Pankaj Khanna: So as you have already said these systems are used worldwide and in different different academic institutes peoples use at the various academic as well as commercial vendors. So, ranging from different applications to different vendors these support to name few say for example agilent, nimblegen, cornings these slides are all compatible with them which usually supplies in the form either the RNA or the DNA chip.

Invitrogen which supply the proteins chip is also compatible with GenePix pro. So, apart from lot of toronto all chips are also being supported and incoming tissue array which are there they are all supported with this particular technology as well.

Prof. Sanjeeva Srivastava: So, this is very useful to have a platform where one can use actually the slides from different commercial resources. Otherwise for example if one wants to use a same scanner for doing DNA arrays as well as protein arrays may it has become difficult but as you mentioned like we can also use a protoarrays invitrogen as well as DNA arrays from various type of commercial places. So, I think it just show that this platform is quite robust for wide applications.

(Refer Slide Time: 12:31)

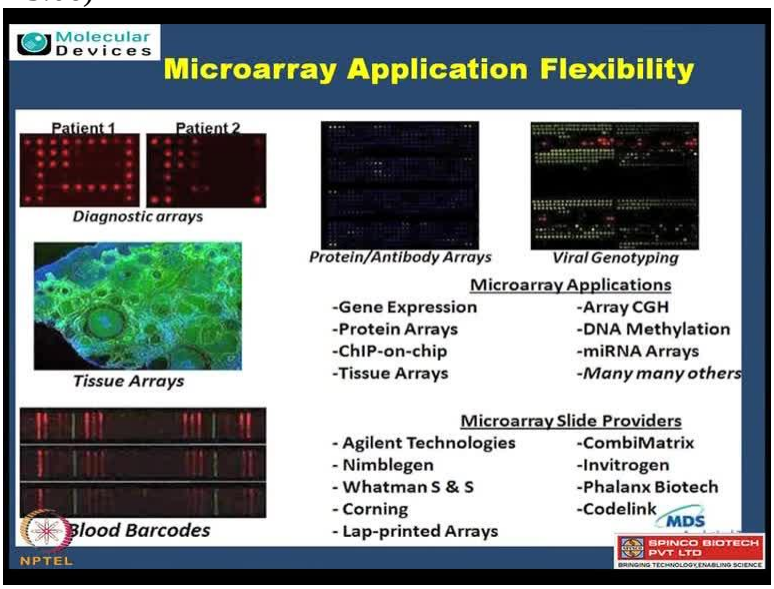


Mr Pankaj Khanna: Yes it is. Indeed the latest all applications possible. They are all compatible with GenePix because of their pixilation at 2. 5 also which is now available.

Prof. Sanjeeva Srivastava: Now can you just brief some of the applications of the microarrays which people usually perform by using these type of scanners and this software.

Mr Pankaj Khanna: Yes so, classically microarrays are being used for the differential gene expression which usually people categorise in the gene expression format.

(Refer Slide Time: 13:06)



So, later as the development has happened people went for SNP arrays as well as a CGH which is called as comparative genome hybridizations. 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 functions what many scientist believe…

Prof. Sanjeeva Srivastava: right.

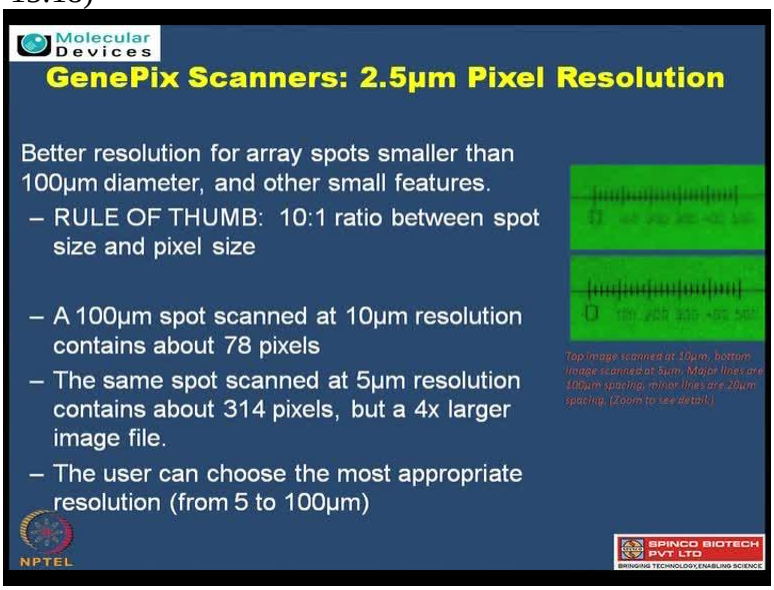
Mr Pankaj Khanna: … and I am also a strong believer. So, now people are moving from the DNA to real functional part how they are directly affected. The with the major bottleneck was looking at the antigen antibody reactions. Plus the proteins are all being seen there is no general formula but now the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

Prof. Sanjeeva Srivastava: So although like whenever we talk about microarray people get feel about its like probably (())(13:56) or DNA based microarray thing which we are talking but slowly now this overall process from DNA microarrays to protein microarray now field is shifting in that direction but only major limitation here is that how to generate that content protein content in the similar high throughput format what people used to do for the DNA arrays.

So, major success for DNA arrays was the easy availability of DNA and the stability of DNA and that is why the high density arrays are possible from the DNA technology but applying the same at the protein becomes very challenging because purifying that many protein keeping them stable and functional it becomes very challenging and thats why like in my previous lecture in the class I have discussed some of the latest methods which people can use for making protein arrays and we have talked about different type of technologies including how one can use the cell free expression based system for generating the protein contents which could be used for the high throughput microarrays for the proteins.

So, ya its good to hear that there are different type of applications which one can use here starting from DNA to proteins. So, now these scanners, what type of pixel resolution they can scan and what is suitability for scanning?

(Refer Slide Time: 15:18)



Mr Pankaj Khanna: Right so, our scanners is 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. 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 say 10 is a resolution and 1 is the spot size. So that is the major rule. So, just to give you brief out say for example if you have spot of protein is 100 microns in size so, what best if you do a10 microns basically you are getting 78 pixels so this is actually 1 by 10. If you do by 5 micron resolution although the pixilation will increase to 314 but the size of the image actually also increases.

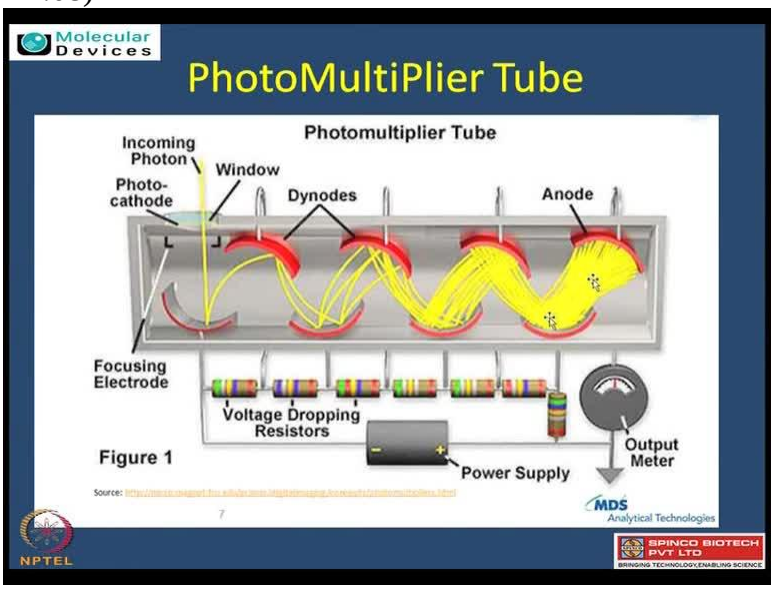
So, apart from making more than 10 time the size spot it does not really help at the level of resolution if you increased. Only thing is you are increasing the size but the data quality is just increased by few very very minor changes. So, that is why the best roll is 10 is to 1 which 5 to 2.5 micron usually is most suitable where the people are making the slides.

Prof. Sanjeeva Srivastava: So, there are different scanners which aim for wide variety of scanning pixilation and resolution. But I think what some of thumb of rule which you have mentioned is very widely applicable for any scanning.

Mr Pankaj Khanna: Yes.

Prof. Sanjeeva Srivastava: So the microarray data could vary in wide range. So, how to accommodate that variation while performing the scanning?

(Refer Slide Time: 17:08)



Mr Pankaj Khanna: Yes, essentially because there all not present so, the data could be right from the 0 intensity which will be include 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 a very low fluorescence intensity wheras the high will give very high.

Prof. Sanjeeva Srivastava: 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.

Mr Pankaj Khanna: Yes.

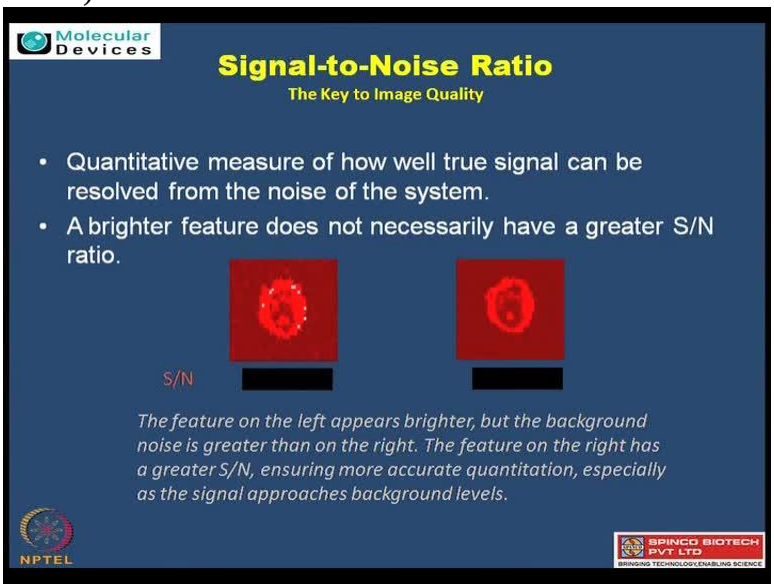
Prof. Sanjeeva Srivastava: So, just maybe you can clarify here like how one can adjust that parameter of PMT gain.

Mr Pankaj Khanna: So, the basic thumb rule there is in any scanning. You do not want to see a white spot. The white spot essentially means the saturation and to avoid saturation exceptional cases for few controls people avoid that happening. So, the best way is to look at photomultiplier 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 been 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.

Prof. Sanjeeva Srivastava: So, how to scan slide for best possible results? you obviously like to change various parameter to obtain the best result right? So, what are the things which one need to look for?

Mr Pankaj Khanna: Yes, so the major variations actually come going to different sources one could be technical one could be biological and another one is going to the assay or the chemistry. So, the technical ones in the form of hardware can be control at the level of PMTs.

(Refer Slide Time: 19:05)



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 as well as the non-confocal chemistry we are able to achieve the best.

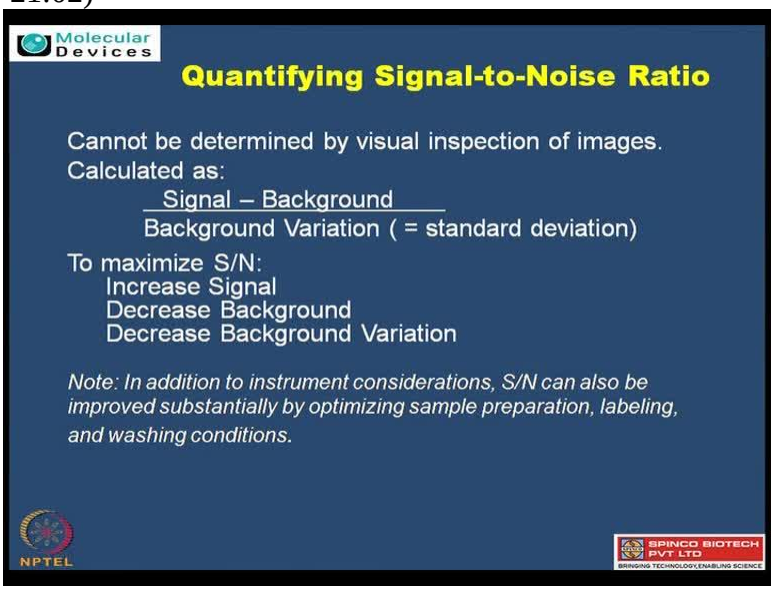
So, in short if by visibility you may not be able to look like what is happening to the images? So in the firm there here in the image which is being shown the which which looks brighter to the eye may not be a true sense. So, when you look at even the dull there the background is very low, without looking at numbers if I see them I say this is dull, that is bright but that is not true always because when you calculate reality based on what you have controlled the level of PMTs and others you quickly come to 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 you can always look at that.

Prof. Sanjeeva Srivastava: So, one need to differently adjust the signal to noise ratio to achieve the very good image quality and obviously simultaneously need to perform PMT adjustment as well as the other parameters.

Mr Pankaj Khanna: True, so as as you go for the signal corrections so, basically what you are trying to see same thumb rules of not looking saturations and then you expect the images going to be with least background to the valid information which is coming in the form of intensities become a true signal.

Prof. Sanjeeva Srivastava: So, how to quantify the signal to noise ratio?

(Refer Slide Time: 21:02)

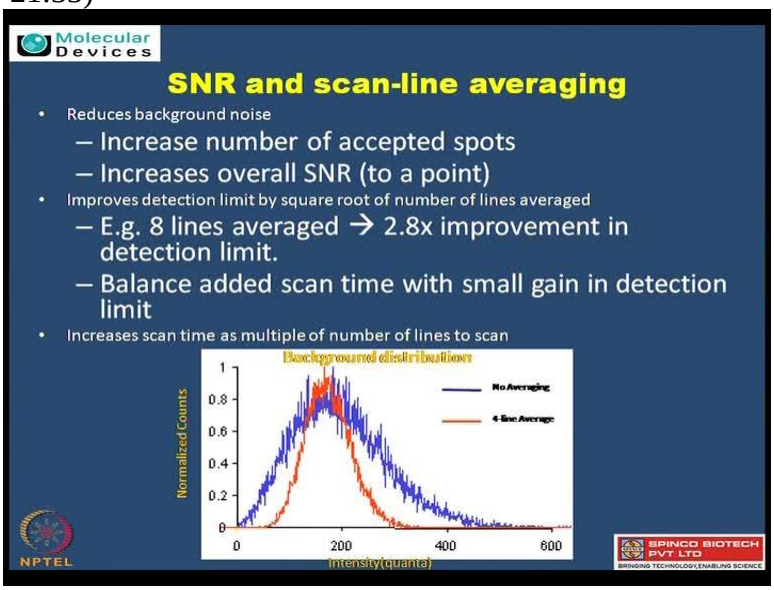


Mr Pankaj Khanna: It is very easy to calculate. Once you have a signal and then when you have a background which is already calculated so basically signal is the intensity which is coming from the expected spot and where there is no spot that particular intensity which is coming is naïve and there is another way of calculating local background which is just nearby surrounding area of the spot that is called as background.

So, we calculate signal minus background so, we divide that with overall background deviation that is standard deviation of the background that will help calculating asignal to noise ratio.

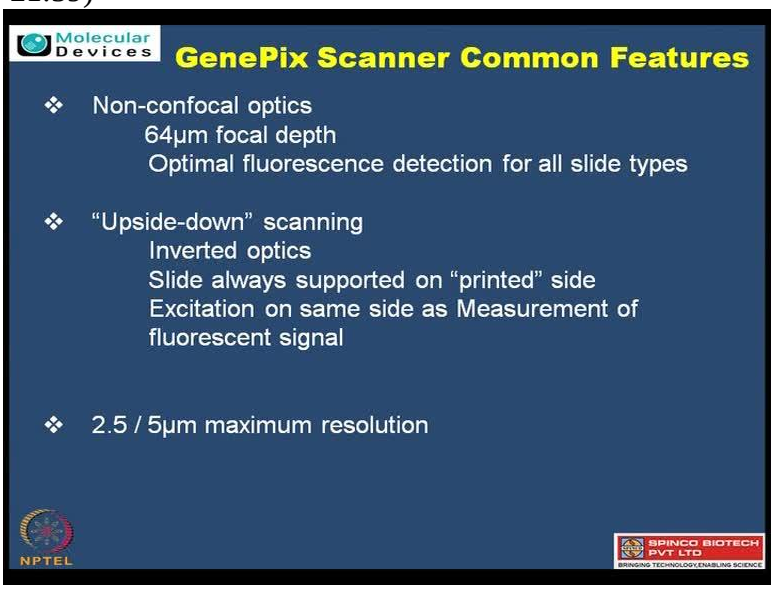
Prof. Sanjeeva Srivastava: Now one parameter which we see for the background correction is basically looking for the scan line averaging. So, can you say elaborate on that.

(Refer Slide Time: 21:33)



Mr Pankaj Khanna: Sure. So basically if you scan once so, image is being collected. So if you want to have multiple scannings being done so that that all intensity can be average 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 as in average them so, these helps in reducing the signal to noise ratio in a fashion that you get a real good intensity coming up.

(Refer Slide Time: 21:59)

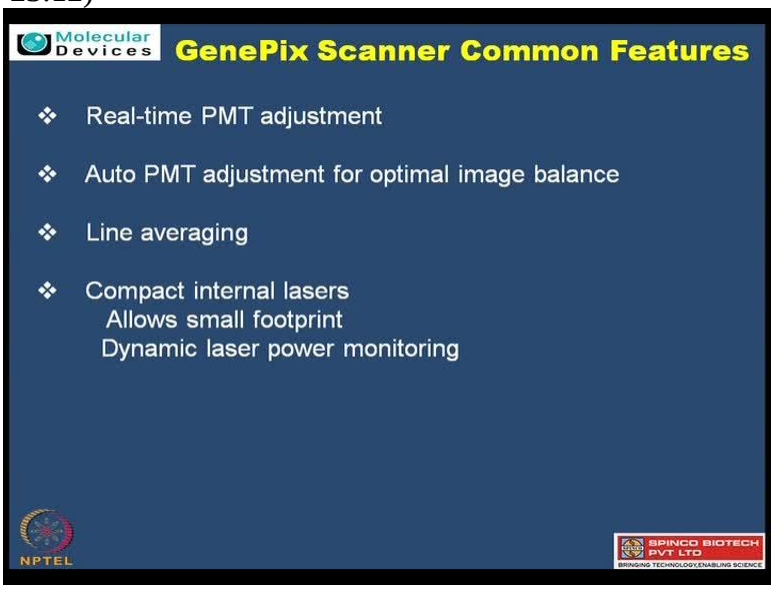


Prof. Sanjeeva Srivastava: So, just if you can brief some of the very common features of this scanner because then only we can learn more when we talk about the software interface.

Mr Pankaj Khanna: Sure. So, the basic concept 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 firm where it can get or acquire the data and the depth is 64 microns which allows a wide variety of applications possible. So, if you want to use some slide based slides that is having a coverslip so, that the depth is little bit you know kind of lower so, you want to a focus at the different range.

So, in this fashion there are 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 sides being scanned directly and controlled for the variability as discussed earlier and the resolution at 2.5or 5 microns based on the system which is available can be also used.

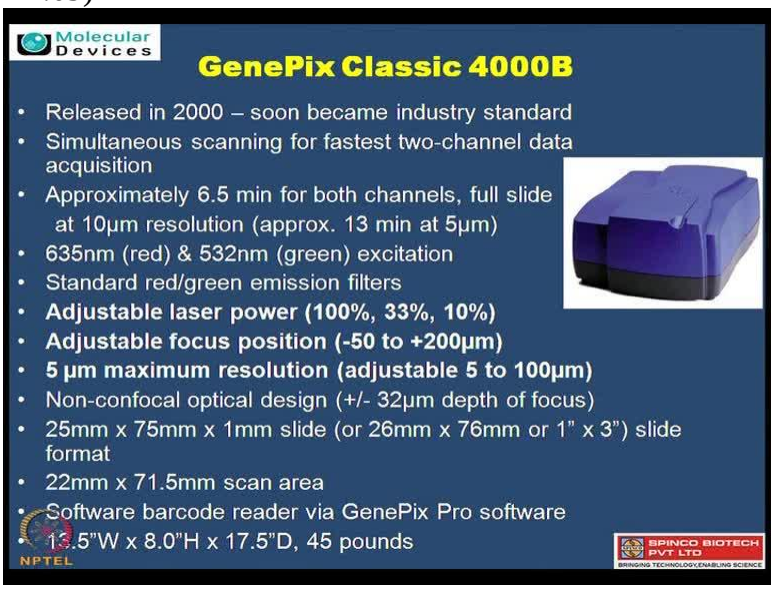
(Refer Slide Time: 23:12)



And real time PMT adjustment PMT we have already elaborated the photo multiplier tubes and the real time is at 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 bus of looking at how to control them we have given auto PMT option. This auto PMT option allows Acuity to get the best output but GenePix pro allows to multiple scan by itself. So, the best PMT suitable for one application can then we see immediately.

Prof. Sanjeeva Srivastava: Now I am user of 4000 B so I would like to know little bit more about some of the features for this 4000B which is more classical scanner.

(Refer Slide Time: 24:09)



Mr Pankaj Khanna: So, GenePix classic 4000 B is called classic because itslike first introduced in 2000 but after that everybody started loving this machine going to the fact that it is having 6.5 minutes of simultaneous scan.

The meaning of simultaneous scan is both laser scan at the same time so, you have very less time for scanning. Apart from that it has got 2 lasers actually 635and 532 nanometers which is classically used for the si3 and si5 and there on compatible dice.

Prof. Sanjeeva Srivastava: right.

Mr Pankaj Khanna: So, in in view of this we have standard green and standard red ambition filters to accommodate all si3 and si5 application. The laser power can also be adjusted in the form of 100 percent to 33 percent to 10 percent. So based on what application and what intensity you want based the laser power can be adjusted as well.

So adjusted focal position is from - fi 50 to 200 microns so, this allows you to focus in 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 to100 microns of resolution.

So, non-confocal again it is using the non-confocal optical design and any standards like can be used for the scan. So, it gives wide application possible in that.

Prof. Sanjeeva Srivastava: So data acquisition is always very important aspect because once we acquire a good image and good data then only one can analyze to obtain any meaningful information. So, can you elaborate how to acquire the image?

Mr Pankaj Khanna: As we discussed 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.

Prof. Sanjeeva Srivastava: Right.

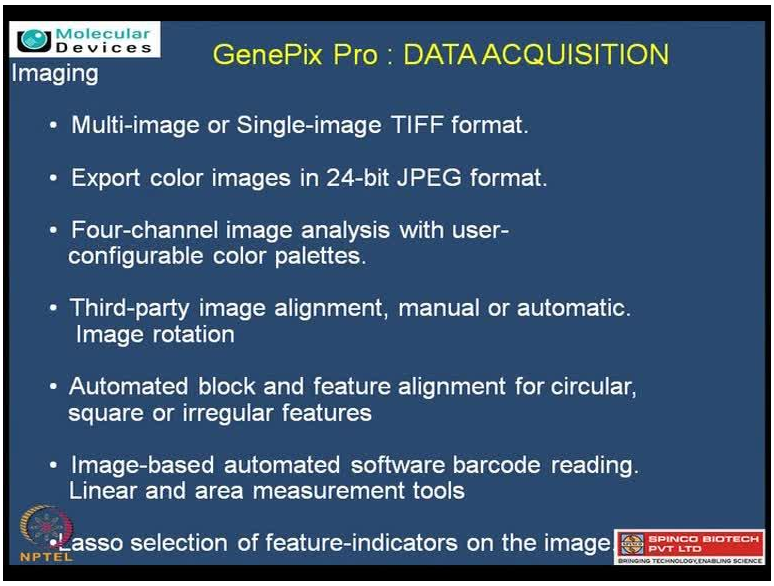
Mr Pankaj Khanna: So imaging is being done in the form of multi-imageor single image TIFF formats. So we use TIFF format to create the images form and exposed these colour images in 24 bit JPEG formats. So, this JPEG format allows one to only see but the basic data processing will be done on the TIFF format.

So so once GenePix pro is beingallowed in different sense of hardware control then image acquisition can be done using the number of laser availability. It ranges from 2 to 4 and many times few application use 1 laser as well. 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 intern contents the features. So, these features are actual genes or the proteins or the representative of the biological material what we 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 form circular, square to irregular features which can be handled at the level of the image alignments.

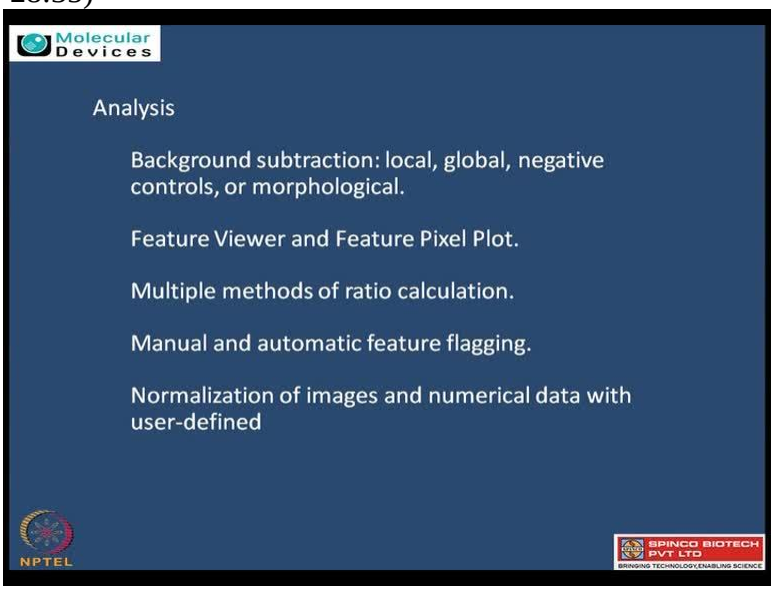
(Refer Slide Time: 27:41)



Prof. Sanjeeva Srivastava: So, I think you have mentionthe good point about data acquisition because one need to look at various type of features and different type of analysis later on which one need to perform. So, the data acquisition has to be very flexible in terms of the sometime the spot could be regular and there could be a different type of background, there could be different type of chemistries being used. So I think by looking at some of these parameters one can acquire good image and then the data acquisition can be further used for further analysis. Ya, so maybe you can talk little bit on the analysis aspects.

Mr Pankaj Khanna: Sure. 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 that is a very important part to cover.

(Refer Slide Time: 28:35)



And apart from that you have a background subtraction so that you get a true signal coming in. So GenePix pro thus help in the background subtraction in a different format and also in 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 which would not bind to anything and leave blank and whereas the local and global can be calculated in 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 plot 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 aremultiple ways of calculating in the form of ratio calculations after normalization or during the normalization of the data. So analysis immediately after those involved few of the normalization process which genepix pro can very well handle

and other important feature is the flagging of the spot. In biology we see some spots are really not good or because of some artifact they are not supposed to be taken for analysis.

Prof. Sanjeeva Srivastava: Ya.

Mr Pankaj Khanna: So essentially we need to make that spot as flag as could bad or absent. So, this can also be done with GenePixpro software. Lastly normalization of the images and the different formats is also allowed to happen in GenePixpro.

Prof. Sanjeeva Srivastava: I think you rightly mentioned the need and the importance of the control features because many times like whole experiment is the quality of the experiment depends on how well your controls are performed that applies to both negative and the positive control and when you talk about the DNA microarray technology obviously we are talking about very high density arrays here where lot of spot and lot of controls are already inbuilt in place.

Mr Pankaj Khanna: Right.

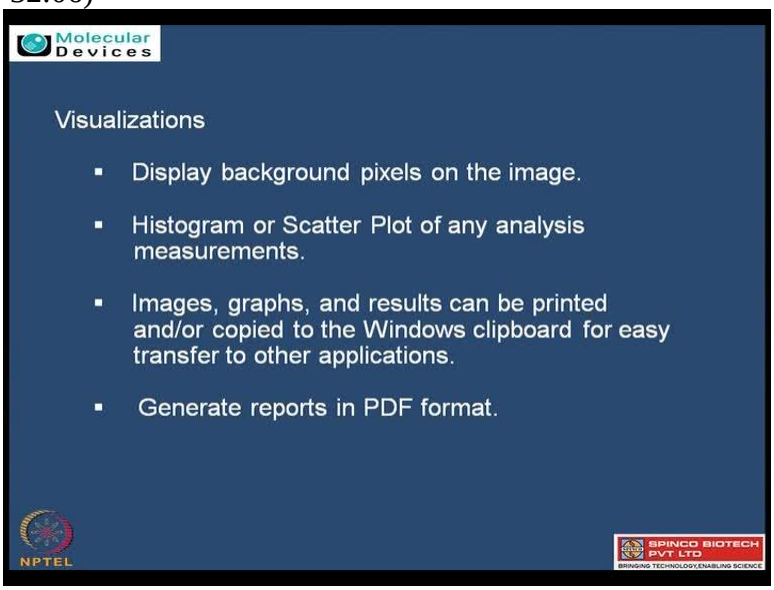
Prof. Sanjeeva Srivastava: Many times when I talked about protein microarrays and speciallywhen we talk about functional type of arrays so, we actually put different type of controls just based on that one particular experiment. For example I am looking for some biomarker response I need to have certain positive control some biomarker which need to light up on the array which will guide me as a positive control. Then if I am looking immune response I need to have some sort of igG and some of those type of control features which will guide me this s just how nonspecific the response could be.

So, again some empty spots we need, some type of spot where there is no DNA or no protein is printed there so, no biological material is there. So, lot of different type of control features are usually part of this array design and that helps further for background subtraction as well as looking at how good quality data we are obtaining.

Mr Pankaj Khanna: True. So, that is the major conclusion for the background.

Prof. Sanjeeva Srivastava: Right. So, let us talk about visualization like how one can really generate some good image and which can be further used for analysis.

(Refer Slide Time: 32:06)

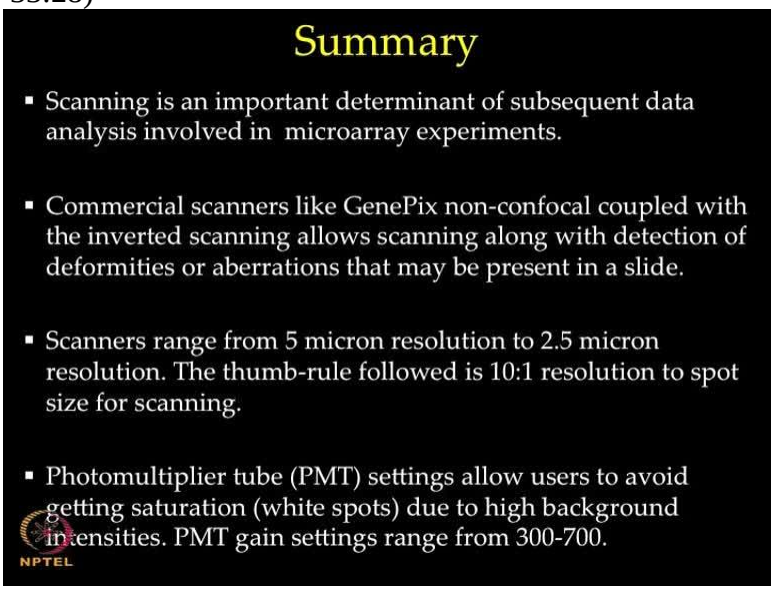


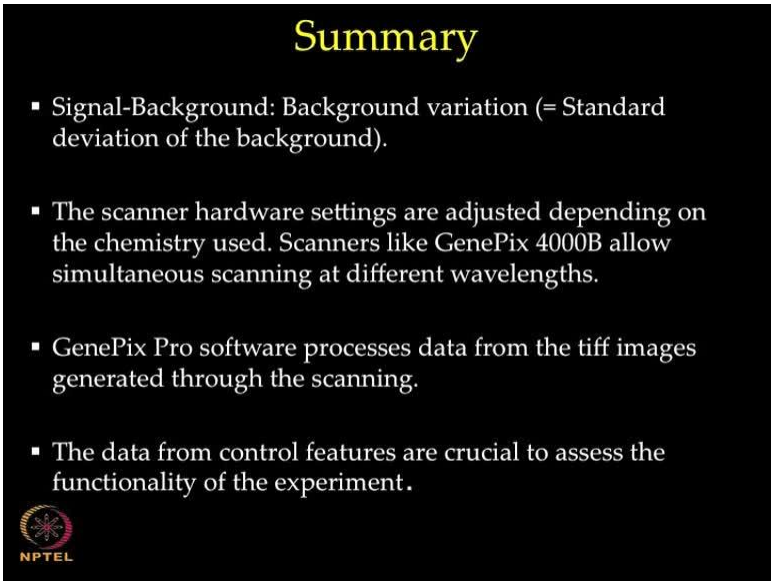
Mr Pankaj Khanna: So, once the image is being acquired so, immediately scientist wants how my data has performed. A s you have given a very beautiful talk on how the controls works and specially few people use even the bacterial controls which is non-related to the biology as such totally they they want to see just the assay control.

So, first they say whether my assay has worked or not? So, best way to do for them is looking at the graphs which are being given differentiation in the level of backgrounds and those also can be done at the level of different histograms and the scatter plots possible and thses scatter plot can be plotted once against the channel types say laser 1 versus laser 2 or wavelength 1 versus wavelength 2. In classical say si3 versus si5. So, how these two things are behaved for me. So, again different kinds of graphs and so also the images can also be exported to PDF as well as being visualized in GenePixpro for your further screening for different QC applications.

Prof. Sanjeeva Srivastava: Thank you for this interesting discussion on the basics of scanning parameters and image generation. I think our students are now stimulated for our next lecture which would have the live demo of the GUI surrounding the specialized software used for image scanning, processing and analysis. Thank you.

(Refer Slide Time: 33:28)





References

- Anna Pursiheimo, L.T.A. (2015). Statistical Analysis of Protein Microarray Data: A Case Study in Type 1 Diabetes Research. J. Proteomics Bioinform. 8.
- Bengtsson, A., and Bengtsson, H. (2006). Microarray image analysis: background estimation using quantile and morphological filters. BMC Bioinformatics 7, 96.
- Chandra, H., Reddy, P.J., and Srivastava, S. (2011). Protein microarrays and novel detection platforms. Expert Rev. Proteomics 8, 61–79.

References

- DeLuca, D.S., Marina, O., Ray, S., Zhang, G.L., Wu, C.J., and Brusic, V. (2011). Data processing and analysis for protein microarrays. Methods Mol. Biol. Clifton NJ 723, 337–347.
- Gupta, S., Manubhai, K.P., Kulkarni, V., and Srivastava, S. (2016). An overview of innovations and industrial solutions in Protein Microarray Technology. Proteomics 16, 1297–1308.
- Rydén, P., Andersson, H., Landfors, M., Näslund, L., Hartmanová, B., Noppa, L., and Sjöstedt, A. (2006). Evaluation of microarray data normalization procedures using spike-in experiments. BMC Bioinformatics 7, 300.

References

- Syed, P., Gupta, S., Choudhary, S., Pandala, N.G., Atak, A., Richharia, A., K P, M., Zhu, H., Epari, S., Noronha, S.B., et al. (2015). Autoantibody Profiling of Glioma Serum Samples to Identify Biomarkers Using Human Proteome Arrays. Sci. Rep. 5, 13895.

- Wellhausen, R., Seitz, H., Wellhausen, R., and Seitz, H. (2012). Facing Current Quantification Challenges in Protein Microarrays, Facing Current Quantification Challenges in Protein Microarrays. BioMed Res. Int. BioMed Res. Int. 2012, 2012, e831347.