Basics of Biology
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Lecture - 34
Cell Death and Apoptosis

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and bioengineering IIT Guwahati and what we were discussing, we were discussing about the living organisms and so, far what we have discussed? We have discussed about the classifications, evolutions, we have discussed about the different types of biomolecules, their structure and functions, and then in the previous module, we have also discussed about the central dogma of life or the molecular biology.

And then, in the previous lecture, we were discussing about the some of the cellular processes and in that context, we have discussed about the immune responses, and how the antibodies can be generated within the host and how they are actually imparting the different types of immune responses.

So, now, in today's lecture, we are going to discuss about the some more cellular processes, but before getting into the detail of the cellular processes, we have to understand the techniques which are actually going to be used for the understanding the different types of cellular processes. So, that the cells are could be of different types.

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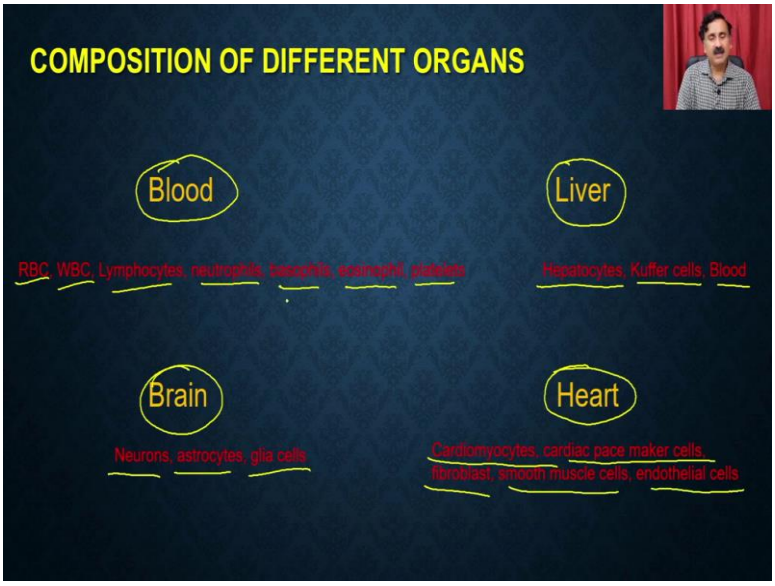

So, cells which are present in the blood. So, cells can be present in the blood, it can be present in a complex sample like the liver, it could be present in brain and it could be also

present in heart. So, you see, in the blood we have the RBCs, WBCs, lymphocytes, neutrophils, basophils, eosinophils, platelets.

Similarly, in the livers you have the hepatocytes, Kuffer cells, blood. Then you also in the brain, you have the neurons, astrocytes, glia cells, then in the heart, you have the cardiomyocytes, cardiac pace maker cells, fibroblasts, smooth muscle cells and endothelial cells. Now, all these cells are varying in the different types of properties.

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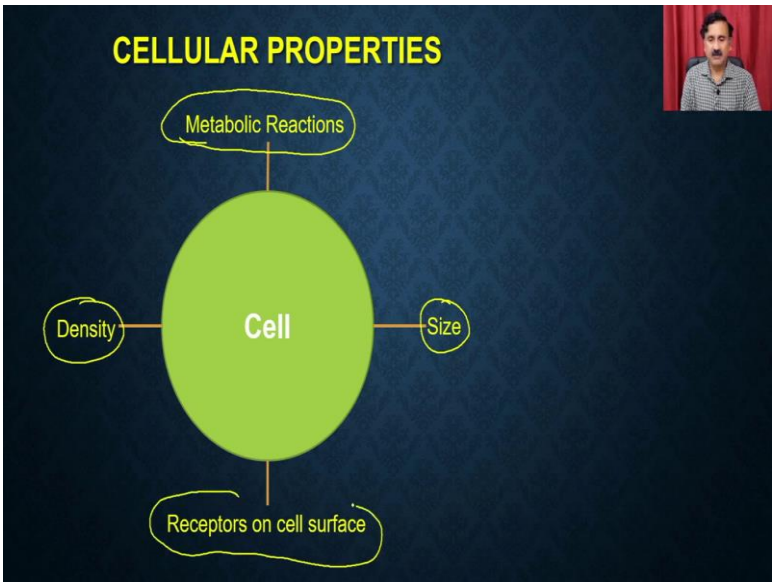

What are the different properties actually, these cells are varying they are varying in terms of the density of the cell lysate. So, every cell has the presence of the different types of organelles, different types of biomolecules, and that type that constitute for the density of that particular cell, then the cells can vary even in the size of the their shape.

So, they can also vary size like for the, they can be as small as the 10 micro meter or they can be as big as the 30 to 40 micro meter. Then the cells can also vary in terms of the metabolic reactions and then the cells can also vary in terms of the receptors that are present on their cell surface.

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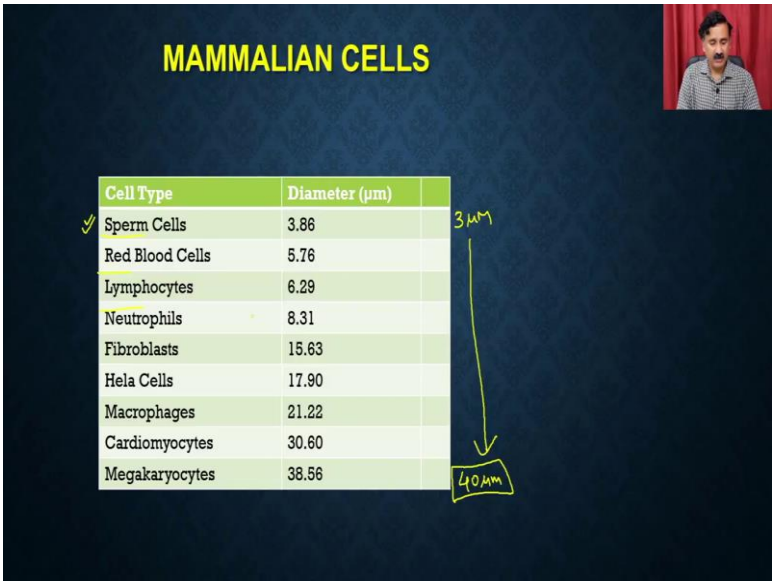

If you see the size, so, what you see here is I have given you an example of different types of cells like the sperm cells, red blood cells, lymphocytes, and what you see here is, they are varying somewhere around 3 micro meter to as big as the 40 micro meter. So, they are very big and they are very different. So, if you want to analyze the cells and if you try to understand some of the basic metabolic or the cellular processes, you require a machine which actually can be able to perform these particular tasks.

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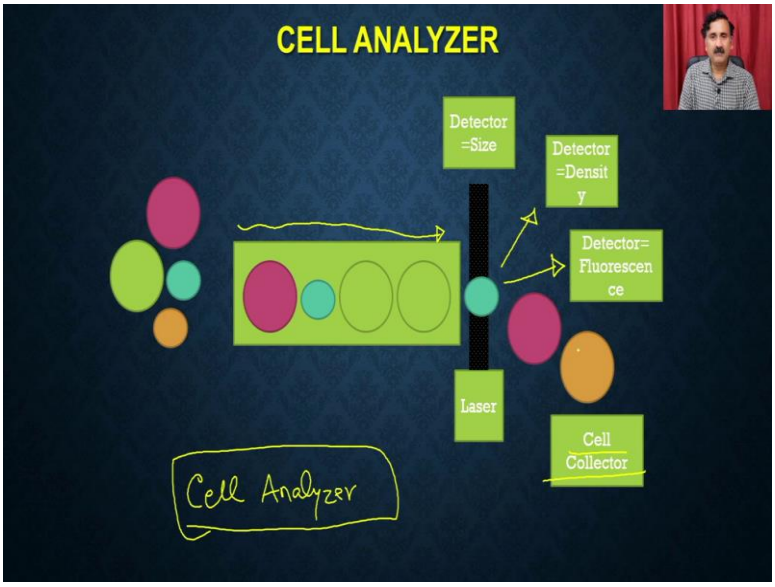

So, if you want to prepare this kind of machine, what you require is you require see you have cells of different size, you can also have the cells of different densities. So, I have given you the different colors. Now, what you require is you require a fluidic system, so, that it the cells

are going to be present individually and then when this individual cells are going to be illuminated by a laser beam, it can be detected by the different types of detectors. So, you can have a detector which can actually measure the size.

You can also have the detector which actually can measure the density, then you can also detect the different types of fluorescent signal. And ultimately if based on this if you require you can also collect these cells for further analysis. So, this particular type of instrumentation is being called as the cell sorter or the cell analyzers. And a typical cell analyzer is going to have all these components. You can have the fluidic components; you can have the illuminations or the excitation component and then you can also have the acquisition components.

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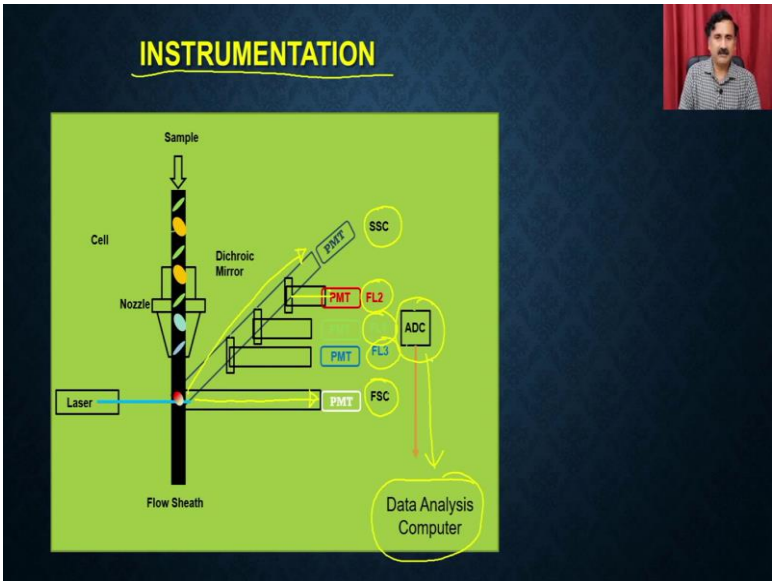

So, as far as the instrumentation is concerned, you are going to have a laser beam. So, you can first go into have a fluidic system, where a stream of fluid is going to run and that fluidic system is going to give the single cell suspension and once a single cell suspension is going to transfer through the nozzle, the single cell is going to be illuminated by the laser beam.

So, these laser beam then the cell is actually going to keep the signal, so, it can actually give the signal to the different types of detectors, so, it can actually give the signal to the PMTs. And that is actually going to be, give you the signal about the forward scattering, then it also can give you the signal to the other PMTs which is actually going to give you a signal about the side scattering.

And then it also can give you the signal for the different types of fluorescent signal like the you can have the FL2 can have the FL3 and you can also have a signal for FL1. So, all these signals are going to be collected by the ADC system. And that is actually going to be analyzed by a computer so that all that analysis can be done. And that is how the flow cytometry or this instrument which is going to be used for the analyzing the different types of cells as the 3 main components.

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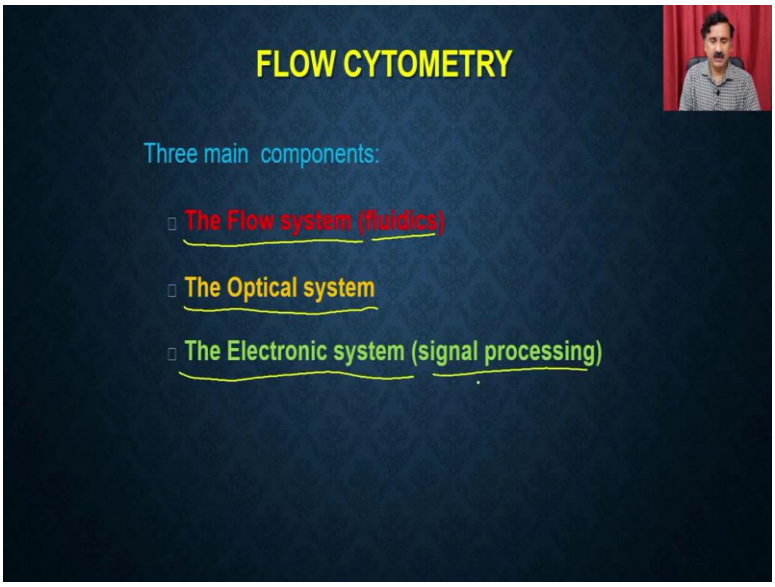

One, you are going to have the flow system, which is called as the fluidics then it is going to have the optical system which is actually going to use for illuminating the objects with a laser beam and then collecting the different types of signals. And then you are going to have the electronic system which is actually going to use for the signal processing.

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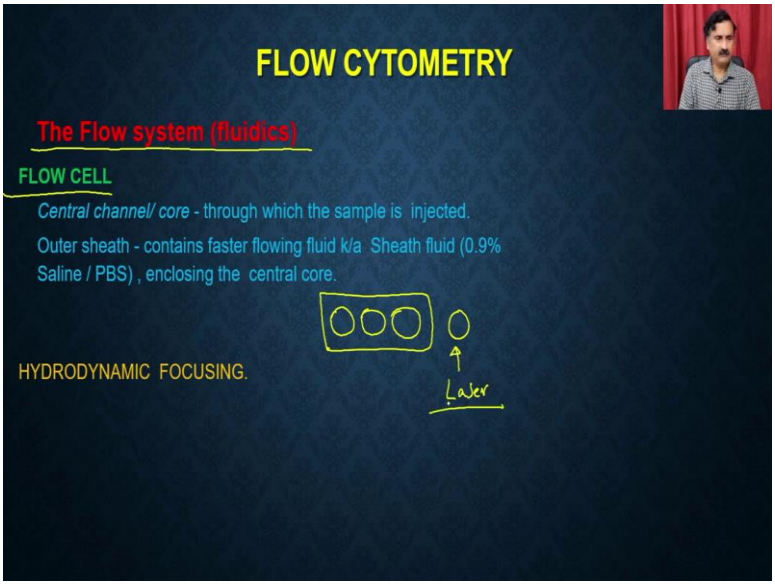

The fluidic system you are going to have the flow cells and these flow cells are actually going to streamline the cells so that it is actually going to going to give the individual cells for the optical system. So, it is once the cells are present in the single cell then the single cells are going to be illuminated by the laser beam.

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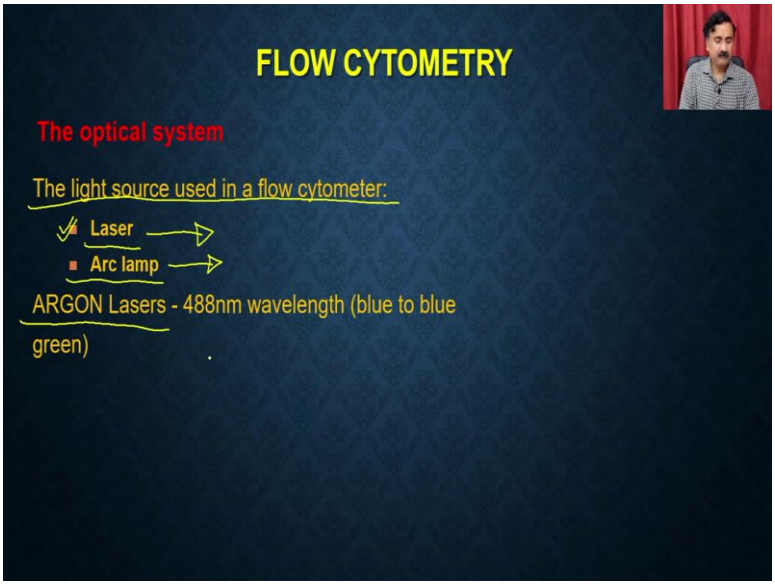

So, then in the optical system, you are going to have the laser beam, so, you are going to have the light source which actually going to be used for the exciting the laser the cells, so, you can have the 2 type different types of optical system, you can have the laser beams or you can also have the arc lamps. So, both of these sources can be used for illuminating the objects and

then some in some of the latest flow cytometer system you can also have the argon laser which actually can excite the samples.

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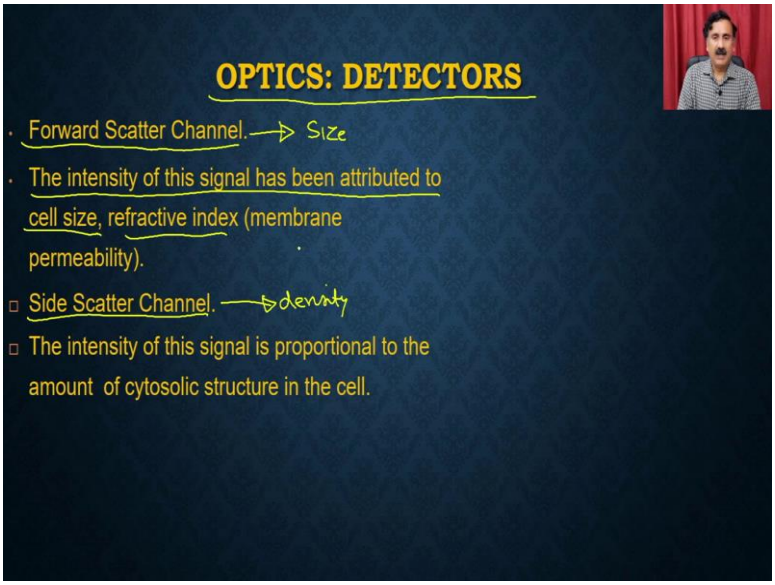

Then we have the optical system. So, optical system you can have the different types of detectors, you can have the forward scattering channel, you can have the side scattering channels and the forward scattering channel, the intensity of this signal has been attributed to the cell size and the refractive index. So, the forward scattering is going to give you the important information about the size of the cell whereas, the side scattering is actually going to give you the density of that particular cell.

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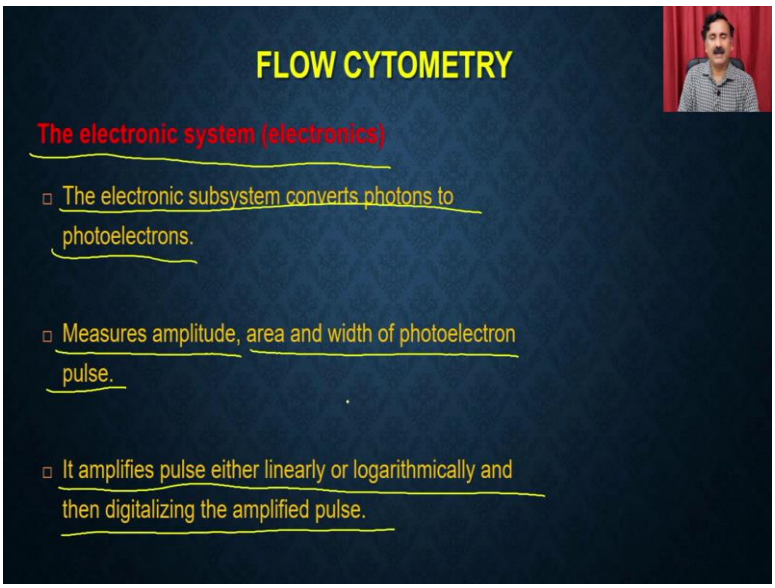

So, then we can have the electronic system, so electronic system the job of the electronic system is that it is actually going to acquire the signal from the optical system and then it is actually going to process. So, the electronic system is going to convert the optical system into the photoelectrons.

And then it is actually going to measure the amplitude area and the width of the photoelectron pulse. And it amplifies pulse either linearly or logarithmically and then digitizing does simplified pulse. You by utilizing the electronic system you can actually be able to plot the data in the 3 different formats.

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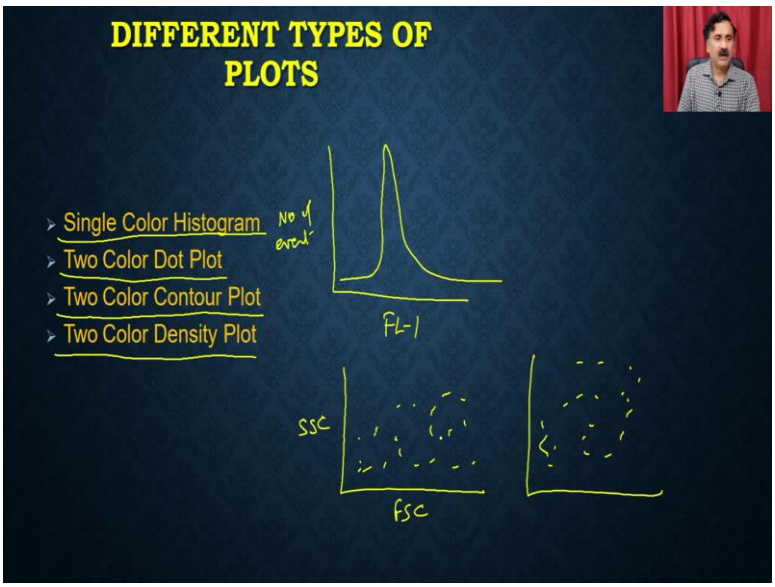

What you can do is you can actually plot the single-color histograms, you can color two color dot plots, you can color two color contour plot, or you can colored you can make that two color is density plots. Histogram plot, like the single-color histogram plot is something like this. So, it is going to give you a histogram.

So, you can have the single color like the FL1, so you can have the fluorescent signal, you can have the number of events on this side. And it is actually going to give you a histogram, it is going to give you a peak like this. So, this is called as the histogram plot, whereas it can also give the two color contour plots. So, in that case, you can have the, like, for example, side scattering, side scattering plot or the forward scattering plot.

And then it actually can give you a contour plot like this, so, it can give you the dot dot dot like this. So, that is a contour plot two color plot, and then it can also give you the control plot. So, this is a contour plot two color contour plot, so, it can give you a contour of the signal. And then the, it can also give you a two color density plot as well.

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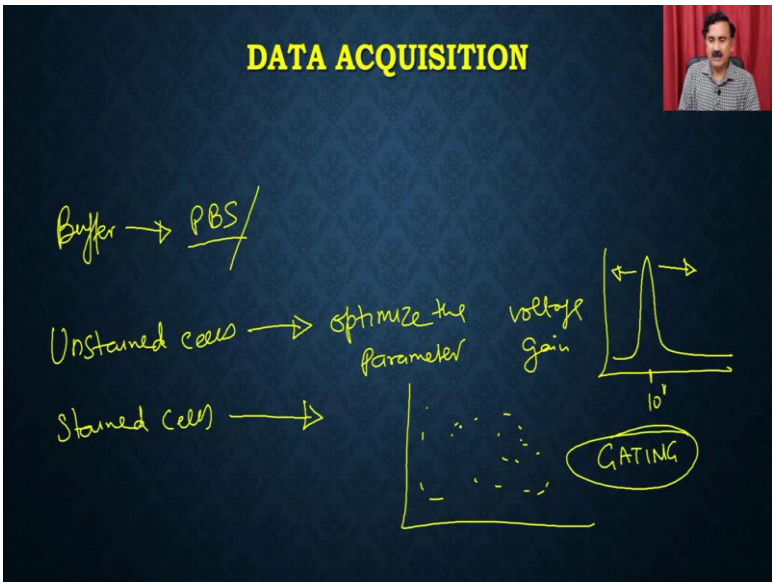

There are multiple steps in which you can actually have to do the data acquisition. So, first you have to bring the sample into the buffer. So, mostly the people are using the buffer, like the PBS, or any buffer, which is a smaller to the cells. So, you are going to have 2 different types of cells, or you are going to have the unstained cells and you are going to have these stained cells.

For whatever the reason I do come, so, with the unstained cells, you are actually going to optimize the parameters, you are going to optimize the different types of parameters like what will be the voltage, what will be the gain and all those kinds of parameters and that is how you are going to actually ensure that the peak what you are going to see is actually in the middle of the this particular graph site.

So, it should be at the level of 10 to the power 1. So, that if the peak will go either in the backward direction or in the forward direction, you should be able to monitor the moment. Then you can just keep the these settings constant and then you can start collecting the stained samples.

Apart from this, once you collect the sample you might be actually getting a lot of data, you might be getting a dot slides. So, in some cases, you might be only interested in looking at

some of the population within the sample site, so that can be done by process which is called as the gating. So, with the gating, you can be able to collect the some of the data.

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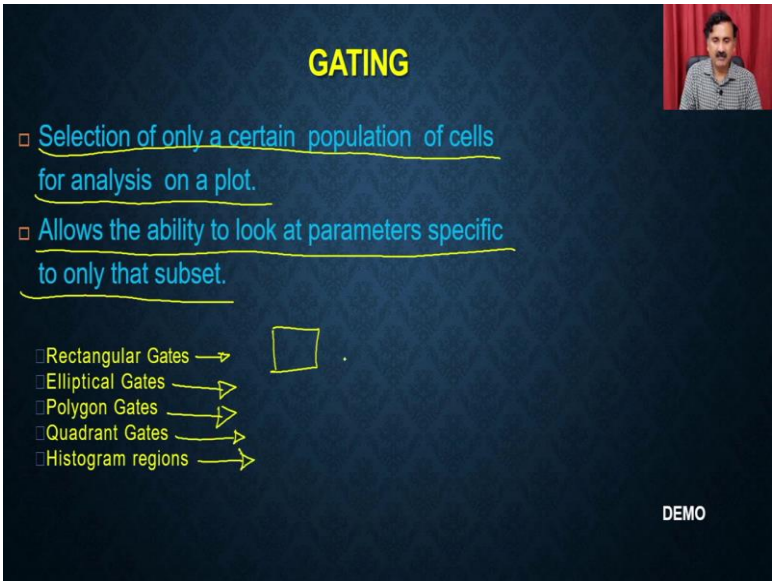

So, getting as the name suggests, you can select only a certain population of the cell for analysis on a plot, it allows the ability to look at the parameters specific to only that subset, you can have the different types of gates, you can have the rectangular gates, you can have the rectangular gate, you can have the elliptical gates, you can have the polygonal gates, you can have the quadrant gates and you can also have the histogram gate.

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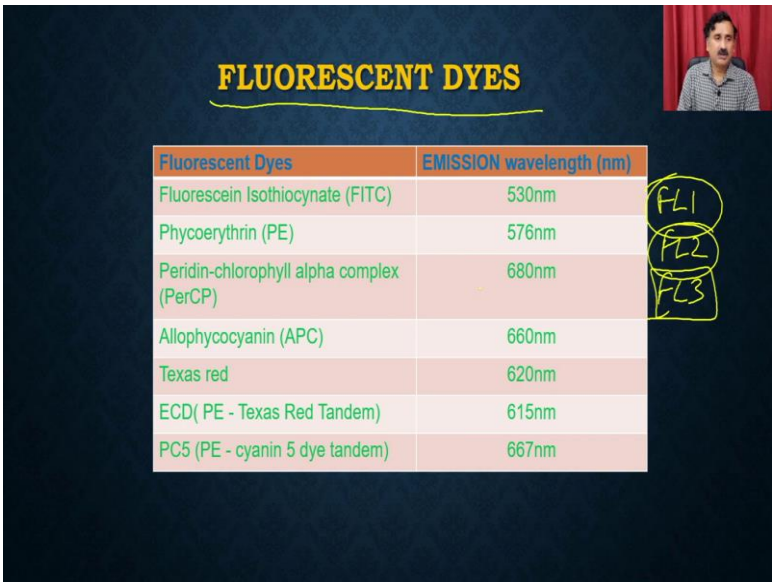

Fluorescent Dyes	EMISSION wavelength (nm)
Fluorescein Isothiocynate (FITC)	530nm
Phycoerythrin (PE)	576nm
Peridin-chlorophyll alpha complex (PerCP)	680nm
Allophycocyanin (APC)	660nm
Texas red	620nm
ECD(PE - Texas Red Tandem)	615nm
PC5 (PE - cyanin 5 dye tandem)	667nm

So, the dyes what you can also use in the flow cytometer you can actually use the FITC, Phycoerithyrin, you can use a PerCP, you can use the APC, you can use the Texas red and so on and all these are actually having the different types of wavelengths. So, according to wavelength, you can use the either FL1 or FL2 or FL3. So, these all this can be used according to the intensity. Now, what are the different types of biological processes?

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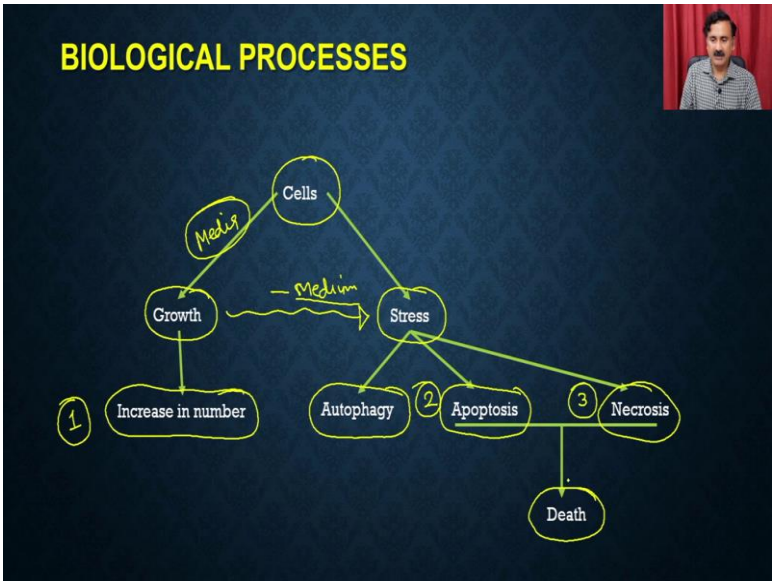

So, when you if you see a cell is actually going to and if you provide the nutrition, if you provide the media remember that when we are talking about the how to actually be able to prepare the media, it will actually going to grow for the growth. So, once it will grow for the growth, it is actually going to increase its number. So, that the first biological process is that how you can actually be able to monitor the increase in number the growth in terms of increasing number.

Apart from that, when the cell will actually be going to if there will be a loss of nutrition's then the cell is actually going to undergo under the stress. So, if there will be minus media like for example, if you do stop, feeding the cells with the fresh media, then they will be under the stress. And under the stress they can actually go through have the different pathways, you can take and also go with the pathway of autophagy. And they can also go with the and autophagy is a survival pathway.

So, autophagy will allow the cells to grow for some more time and going to stay for some more time. But if that time is also going to be over and the stress is going to be even beyond that, then the cell has 2 choices, either it will go for the apoptosis or it can go for the necrosis

and both of these events are eventually going to lead to the death of the cells. So, you can have the increasing number. So, that is the first event. The second is you can also study how the cells are going through the apoptosis or the necrosis or the death pathways.

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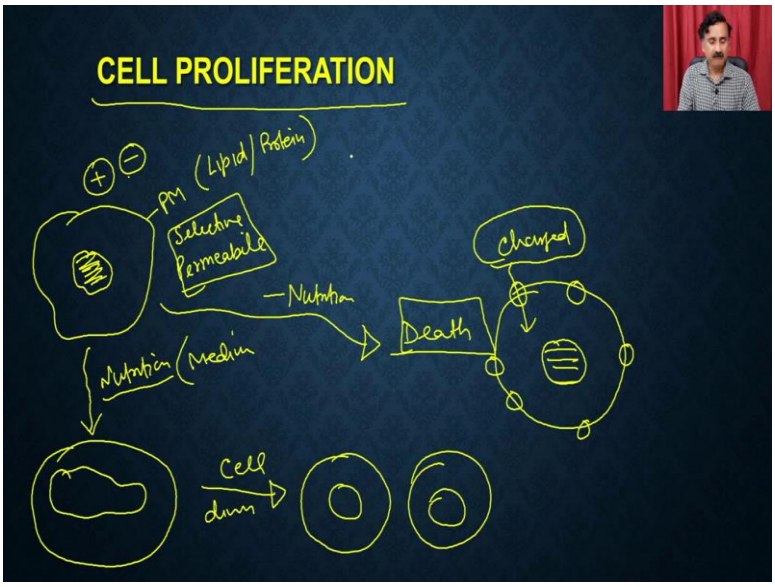

So, when we talk about the cell proliferation, the cell proliferation can be understood like this that you have a cell, and it has a nucleus. So, and I am sure you might have, so, this cell is surrounded by the plasma membrane and this plasma membrane is remember that when we were talking about this plasma membrane, it is selectively permeable. So, if it is a selectively permeable, it will not allow the entry of the molecule spontaneously, so it will not allow the entry of the molecule as per the concentration gradient.

Especially like the charged particles either negatively charged or positively charged will not going to pass the plasma membrane because the plasma membrane is made up of the lipid or the protein molecules and the these that is why the plasma membrane will not allow the charged particles, charged particle has to be go with a facilitated transport. And this is what is going to happen. So, when you are going to provide the nutrition, or you are going to provide the medium to the cell, it will actually going to start dividing the nucleus.

So, it is going to have the a growth in terms of nucleus, and then there will be a cell division. So, then there will be a cell division and that is why the single cell is going to be get converted into 2 different cells, but you can imagine that if there will be a loss of nutrition, so then the cell will, instead of going like this, like the if there will be a minus nutrition, the cell

will actually go for the death pathway. And once it will enter into the death pathway, it is actually going to form the holes on to its plasma membrane.

So, it is going to have to plasma membrane selectively permeability is going to compromise and if that will going to happen, it will going to start taking up the charged particles from the which are present in the environment. So, this is one of the way in which the people are studying of the cell proliferation, and they can actually be able to use that for counting the different types of cells.

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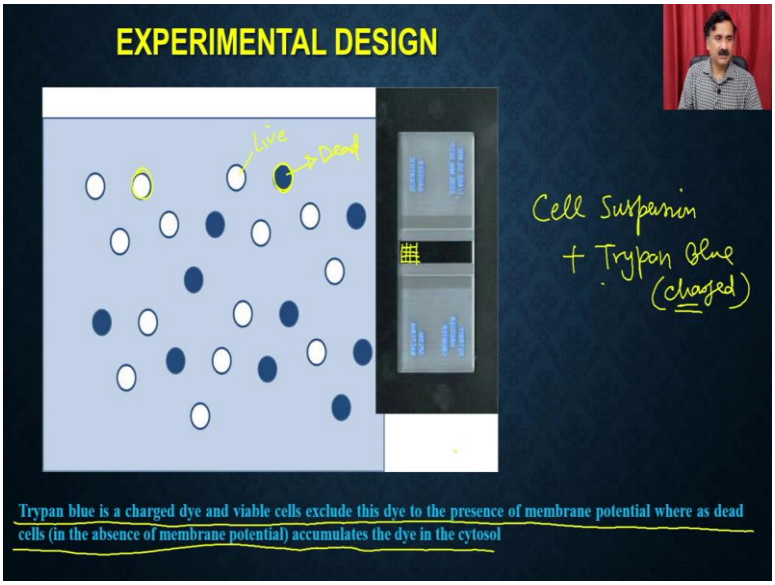

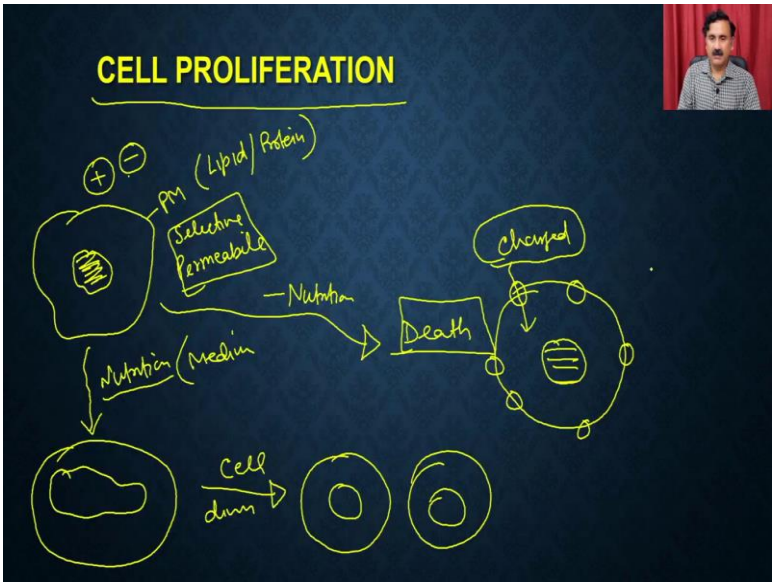

What you can do is you are actually going to mix the cells so you take the cell suspension, where you are actually be interested to see how many cells are live or dead. And then what you can do is you can just add the small amount of trypan blue, so trypan blue is actually a

charged dye. So, as I will say, in the here is sample like if the cell is live, so this is a live cell, and this is a dead cell. So, if a live cell will have a plasma membrane integrity maintain, then it will not allow the charge particles to go inside.

And as a result, this cell is not going to take up the stain. Whereas in the case of the dead cells, because the plasma membrane is lost, it is selectively permeability and it is lost its ability to stop the injury of the charged particle, the charged particle will enter and as a result, it is actually going to be it will take up that molecule.

So, because of that, if you stain this cell suspension, that trypan blue, which is a charge dye, it is actually going to give you the cells with the appearance like this. So, this is a cell without any dye and this is a cell which is actually having a dye. So, this is actually a dead cell and this is a live cell. So, that you can actually put into a hemocytometer.

So, hemocytometer is having a chamber on to this and these chambers, you can actually be able to visualize under the inverted microscope and then you can be able to count TT cells. So, trypan blue is a charge dye and viable cell exclude this dye to the presence of plasma membrane potential, whereas the dead cells in the presence of plasma absence of plasma membrane potential accumulates the dye in the cytosol.

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So, what you are going to do, you are going to take the glass slides, cover slips, you require the hemocytometer and then you also require the hemocytometer and inverted microscope.

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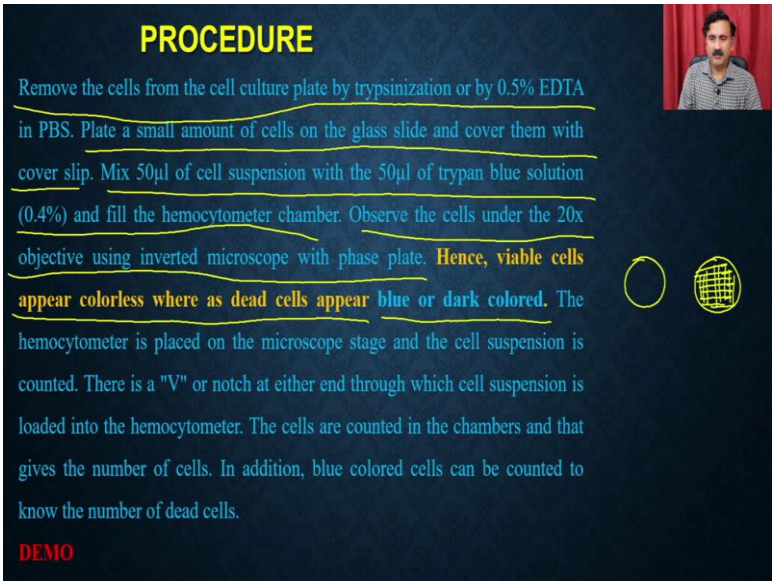

You will remove the cells from the cell plate by trypsinization or the EDTA treatment. Then you plate a small amount of cells onto the cover slide and covered them with the cover slip. You mix the 50 microliters of cell suspension with the 50 microliters of trypan blue and you fill that into the hemocytometer chamber. Observe the cells under the 20x objective using inverted microscope with the phase plate.

And the viable cells are actually going to appear colorless whereas the dead cells are actually going to appear as the blue or the dark colored. So, the viable cells are actually going to appear as the colorless and the dead cells are actually going to be appeared as that they will take up the dye. So, this is going to appear as a blue color.

And there is a discrete procedure through which you can actually have to load the hemocytometer with the stain cells and all that. So, that we have discussed in this small mini demo, where the student has discussed how you can actually be able to stay in the hemocytometer, how you can be able to stain the cells with the trypan blue, how you can load the hemocytometer with the into the cell, and then how you can be able to count under the hemocytometer microscope.

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We have to remove the remaining media then trips in a distance and we will count the distance and so on. After sensor data detergent we have taken into plain Falcon then we have to send it to the cells. As the cells are very delicate, we have to centrifuge at 1500 RPM for 2 minutes. Now, we have to remove the supinated and re-suspension cells in fresh media. After re-suspension, we have to count the cells, so, I am going to take 40 microliters of the cell suspension and mix with the 20 microliters of Trypan blue and counter the new (())(21:53).

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Before counting we have to see how a counting chamber hemocytometer (())(22:11). This is a typical hemocytometer are so called as new bar chamber which contents this square in the upper side and lower side. With each squares having the tub find 1 DMM and area of 0.0025 millimeter square. Now, I am going to put a cover slip on this chamber then I will add slowly cell suspension through capillary action it will spread all over the squares.

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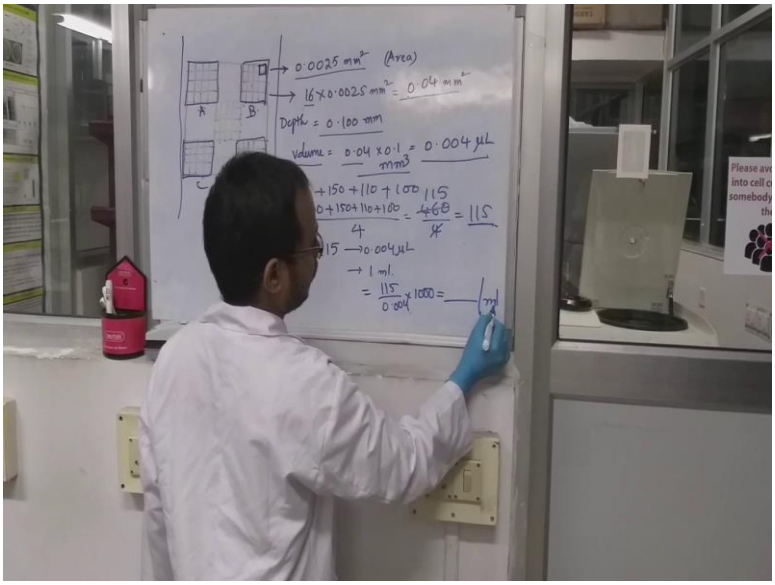

So, we checked the how many cells are there in all the squares. Now, we how to count, how to count the cells. So, here a typical new bar chamber which contains squares 5 squares. So, we have to count sensing these squares. So, each square is an area of 0.0025 millimeters square and total small squares 16. So, total area of this whole square is 0.04 millimeters square. So, that depth of the this each square is 0.1 millimeter. So, what is the value? 0.04 into 0.1, so that is total 0.004 millimeter cube or 004 micro liter.

So, say we have combined these cells in each way. Say this is A, B, C, D here we have 100, here we have 150, here we have 110, here we have 100. Again so, the total cells we have to take average. That means 100 plus 150 plus 110 plus 100 divided by 4 total 4 square counting. The average is 102. So, 115 cells in 0.004 microliter volume. So, how many cells per 1 ml so, that we can calculate simply 5, 0.004 into 1000 that will give the value cells per kelvin.

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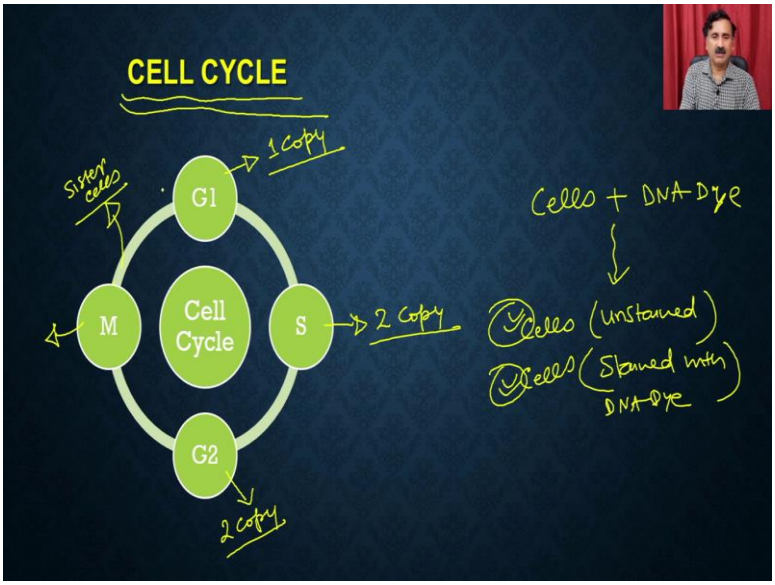

Now, next, let us move on to the next biological event. So, next biological event is so, where we can also be able to study the cell cycle. So, remember that we will have already discussed that a cell will go through the different phases within the cell cycle, where you can have the G1 phase, the S phase, G2 phase and the M phase. Now, what you have to remember is that the amount of DNA is going to vary among these phases from the amount of DNA is going to be a one copy of DNA in the case of the G1 phase, and 2 copies of DNA is going to be in the S phase.

And that 2 copy will continue as the 2 copies in the case of the G2 phase and as well as in the M phase it is going to be divided. So, you see that the DNA content is varying, when you are going from the G1 to S and G2 and M phase and at this stage, there will be a cell division and because of that, they will be a sister cell is going to be formed and main cell is again going through with the cell cycle.

So, what you can do is you can take the cell and you can stain it with the DNA dye. So, if you take the stain it with the DNA dye, it is actually going to DNA dye is actually going to bind the nucleus, bind the DNA within the nucleus, and therefore it is actually going to give you the staining for the DNA. And that can be used as a measure. So, what you can do is you are going to have the 2 different types of cells, you can have the cells, which are unstained and you can also have the cells which are stained with the DNA, DNA dye.

For example, you can use the property of iodide or some other type, so stained with DNA dye. And what you can do is you can just analyze these 2 cells under the flow cytometry and

that is actually going to distinguish between the amount of signal what is coming from the different types of cells. And that is how you can be able to study the cell cycle events.

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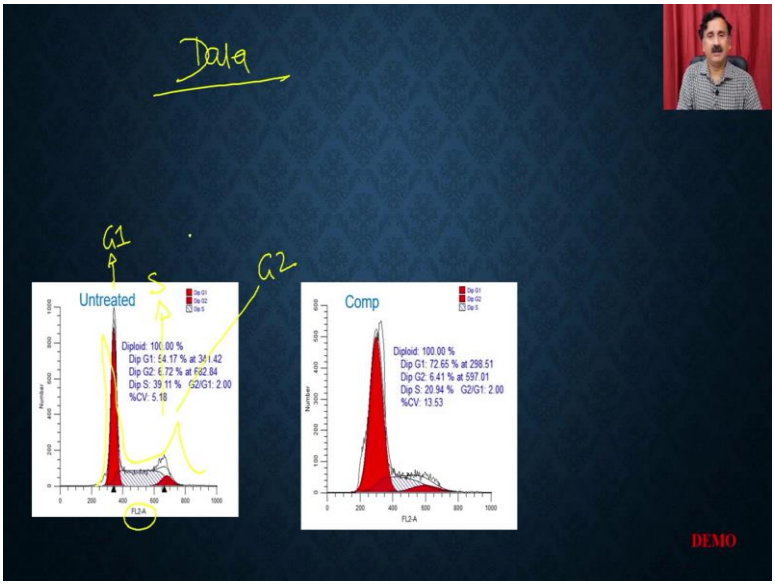

So, this is what you are going to happen when you see the data. So, data of this cell cycle is like going to be like this is going to give you this so this is the G1 phase. So, you see the amount of DNA is less. And then this is going to be called so as you see, it is going to give you a pattern like this, it is going to give you a pattern like these, then it goes like this and that. So, this is actually the G2 this is the S.

So, because you see the different phases, it is going to give you the proportion of the cells under the G1 phase G2 phase in the S phase, and you can be able to use that to calculate or you can be able to use that to study if there are the condition which are compromising the cell cycle events or if there are compounds, which you are trying to develop, so, that they will interfere with the cell cycle. So, that is also we have prepared a very small demo to show you how you can be able to stain the cells, how you can be able to process the samples and how you can be able to analyze the data.

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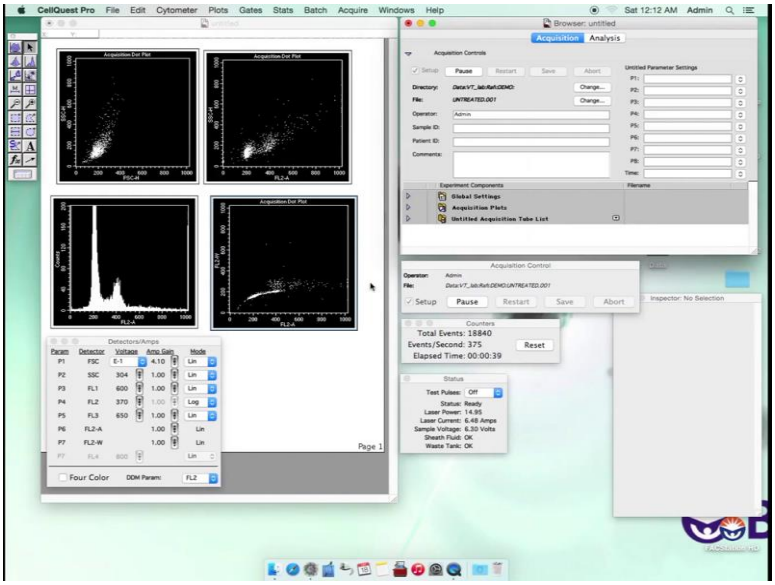

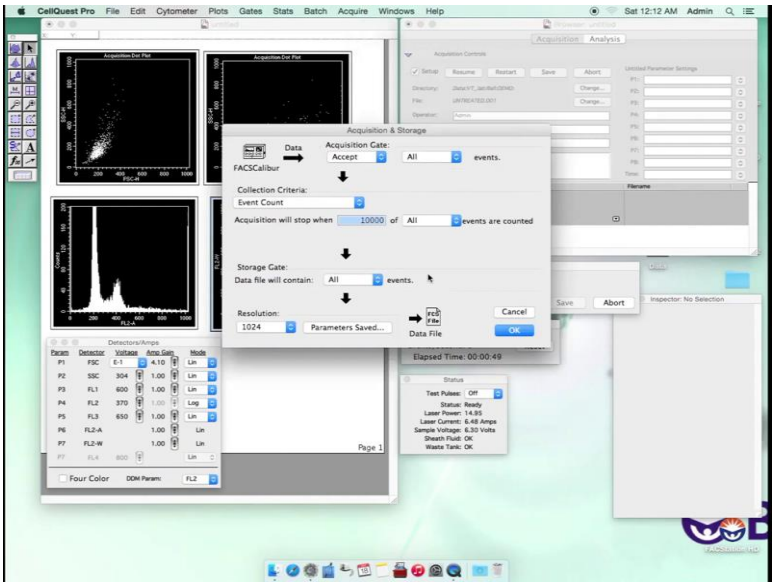

For checking the data, we have kept it on the setup so, that we can see whether the data is coming properly or not. And then we can remove it from the setup and then acquire the data. So, now we are going to load the sample onto the sample injection port and then press acquire. As we can see in that the number of events has started recording in the FSC and the SSE plot we can see that most of the population is seen between the 200 and the 200 mark.

The population which is coming away from the is that of doublets in FL2-A and SSE we can see that there are 3 different popular populations and G1 as an G2 and in the FL2-A and the accounts in which you can scan the longest way it is the G1 the in the between one is the S and the smallest way is the G2. So, before acquiring the data, we have to set the number of events so we go to acquisition and storage and press record 10,000 events.

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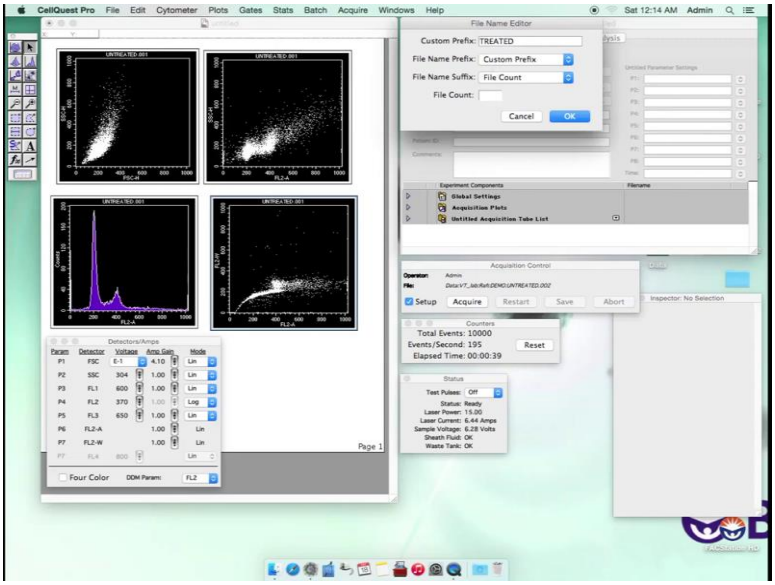

So, in order to record the data, we have to remove it from the setup and then set up and then press Acquire. In the FL2-And the FL2 channel we can see that we can see there are 2 different populations one is a thin line and other is the presence of some doublet or clumps of cells. It is (()) (30:10) population might be presence of the doublet cells or clumps of cells. So, while acquiring the data, we can see this but in the FCS Express software, we can remove that.

So, now that we have acquired the data, now, we can take the data from the an arthritic sample. We have to remember that we do not have to change the parameter conditions in order to check the compound the untreated then the treated samples.

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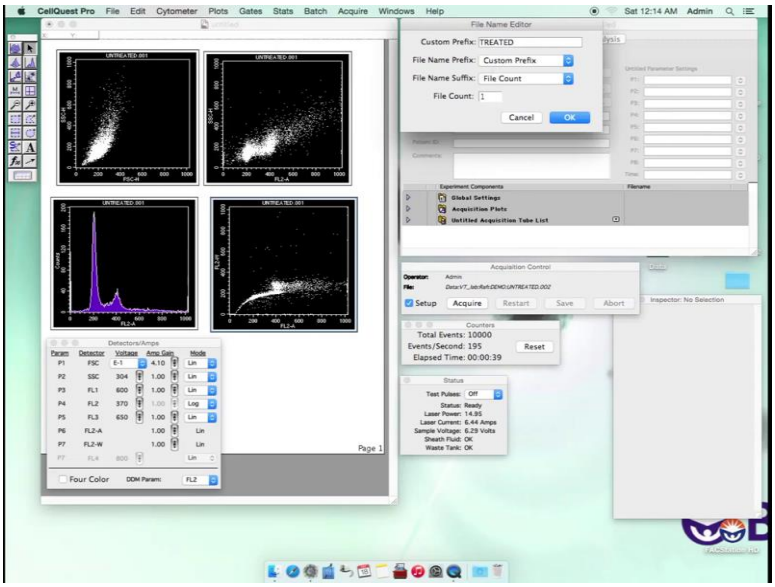

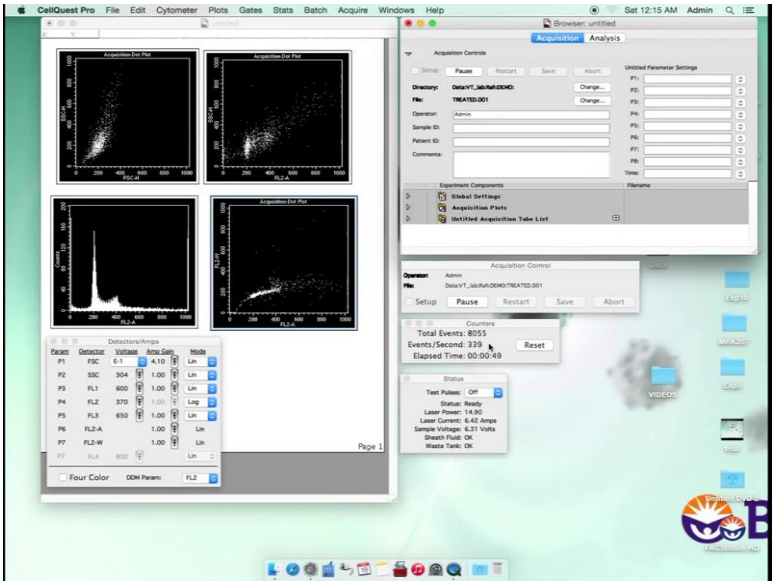

Now, we have to change the sample name from untreated to treated and also change the file count to 1. So, it way and then acquire we can see that there is a little shift in the S and the G2 phase but we can say for sure that there is a change in the data. So, for that we have to acquire, we have to process the data on the FCS Express software in order to see what is the difference between the untreated and the treated sample. So, now, we will remove it from the setup and then acquire the data for the treated sample.

Sometimes we can see that events per second will be low. So, in order to increase the events per second, we can pause the recording and then tap the sample once or twice in a while to shape the sample and then the flow will be continuous again from the sample injection port and the events per second will increase. Now, we can see that events per second has increased because we have time to sample and data acquisition will be a little bit faster.

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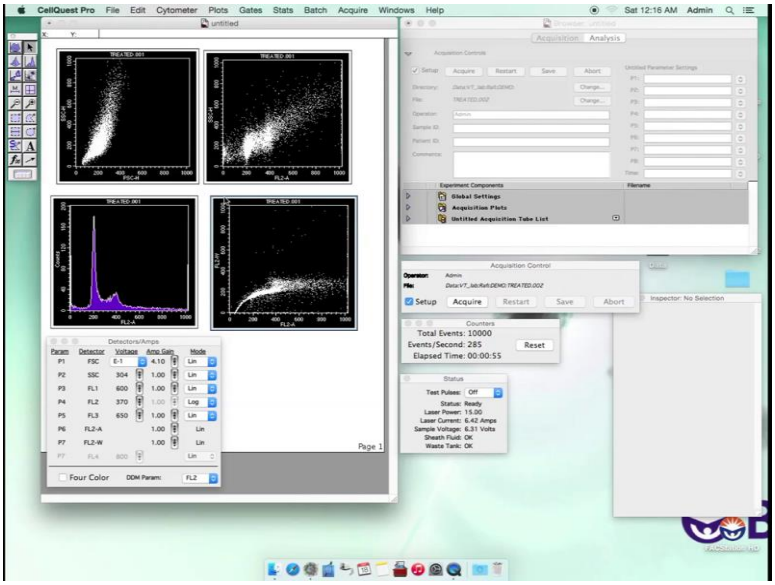

So, now we have acquired the data for the untreated and that we did samples. In order to see in which cell cycle phase of the cell cycle the virus has take place. So, we have to process that data in the FCS Express scribe software.

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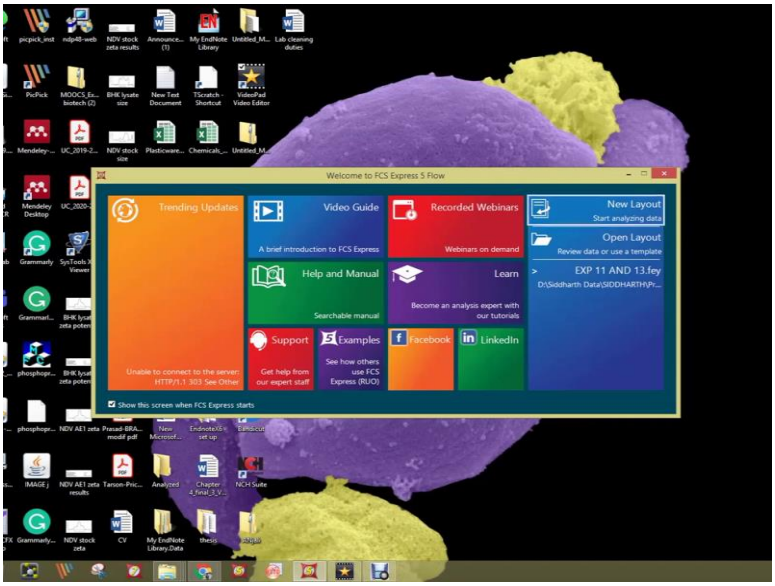

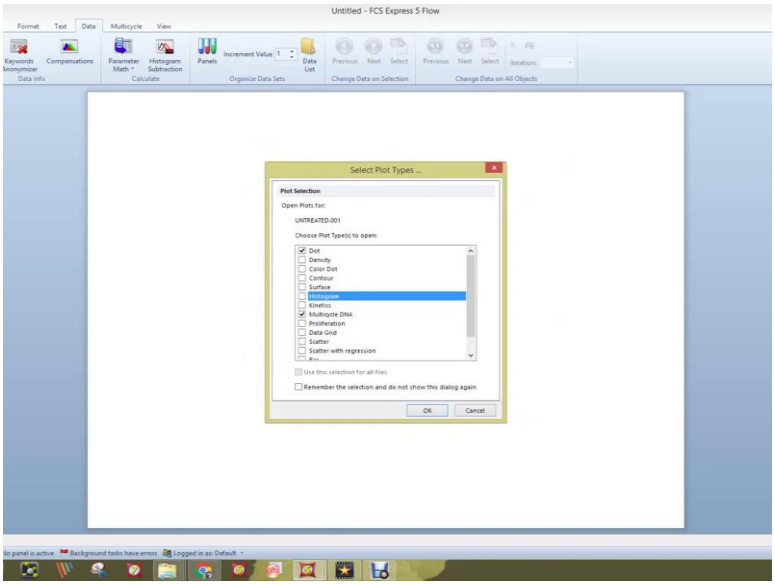

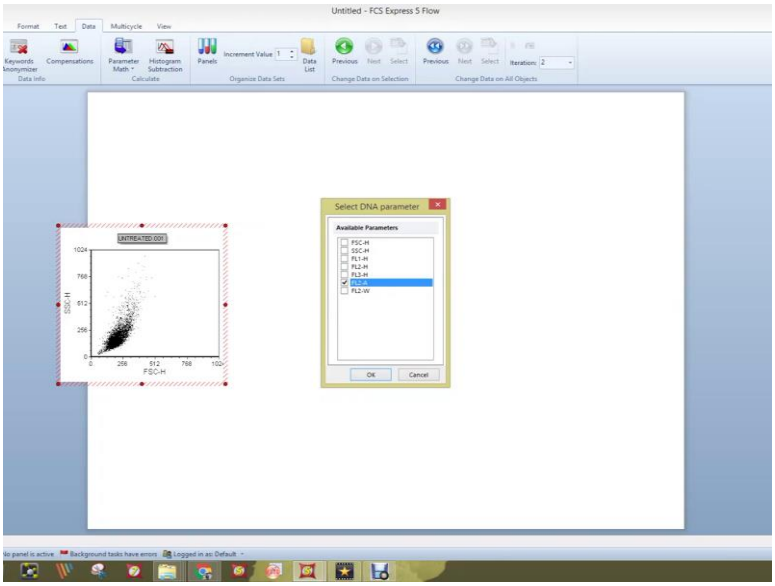

We have to now process the data so for that purpose we use the new layout. As we can see that there are multiple options available and the dot, density, color dot, contour, surface, histogram and multi-cycle DNA and kinetics. For this we a cell cycle DNA we only need multi cycle DNA plot and the dot plot. So, we are going to open these 2 because we have recorded our data on the FL2-A channel and also because the propidium iodide is only shown in the FL2-A channel we are going to open the FL2-A channel.

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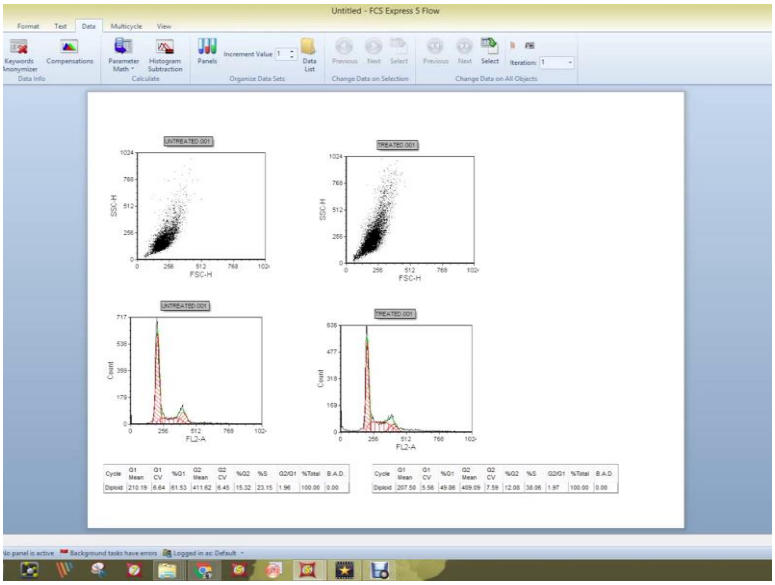

So, we have open the dot plot and the cell cycle DNA plot we can see the means how much population of cells is represented by the G1 S and G2 by right clicking on the plot and then selecting statistics and then show DNA cycle statistics. So, a small window will appear in which it will show that the percentage G1 the percentage G2 and the percentage S, so this is called the untreated sample and let us see for the treated sample.

As we can see that there is some change in the untreated and the treated sample both in the both plots that are plot and as well as the cell cycle plot. So, in order to see how much percentage of the cell cycle phases have changed, we right click on the plot, then show DNA cycle statistics and then place it right beside the untreated one in order to compare. So, we can see that there has been a reduction in the G1 phase from 61 to 49 and from G2 also from 15 to 12.

But there has been an increase in the percentage S phase which has increased from 23 to 38. So, we can say that there is a significant change in the phases of the cell cycle, but we can only be sure after doing the experiment in triplicate so that we get the proper standard deviation and also the standard error. So, this is one way of processing the data.

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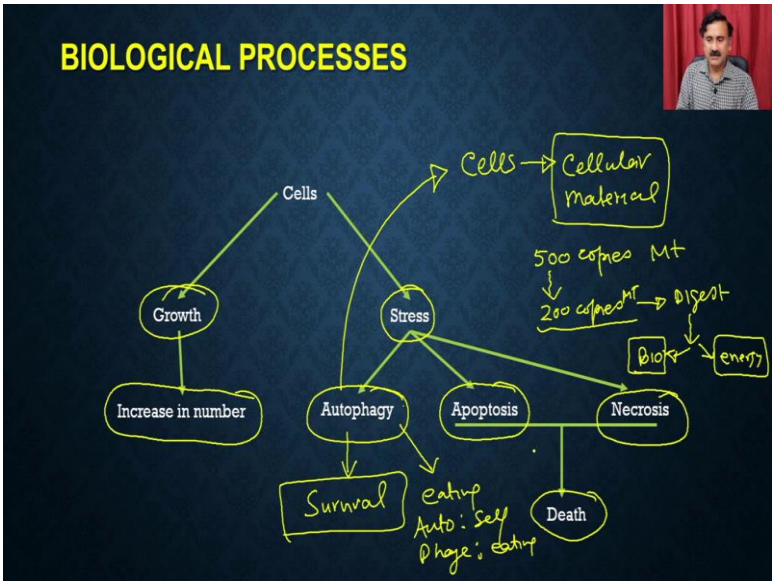

Now, what we have discussed? We have discussed about the growth and we have discussed about how you can be able to monitor the increase in number, but under the stress conditions it the cells has the 2 options one is the cell survival pathway which is also called as the autophagy, so that is going to be a survival pathway.

And what is mean by the autophagy is that autophagy is a eating yourself. Auto means self and Phage means eating, so if you eat yourself it is called as autophagy which means under the autophagy conditions. So, under the autophagy conditions the cell is going to start utilizing the cellular material for its survival, which means it going to start recycling the cellular material.

For example, you can have the different amount of mitochondria, you can have the different amount of chloroplast, you can have the different amount of other organelles. So, what you can do is and cell probably say for example, if you have a 500 copies of the mitochondria, so, if you have a 500 copies of mitochondria, what you can do is you can or what the cell is going to do is it can actually, digest the, for example 200 copies. So, if we digest the 200 copies, so, if it digest of like 200 copies, and if we digest utilizing the lysosomal enzyme.

Then it is actually going to produce 2 things, it is going to produce the biomolecules and it is also going to produce the energy and both of these things. So, the biomolecule as well as the energy is going to utilize by the cell to run its basic metabolic reactions or it is going to utilize that route pass the time because these cells are under stress, they are not getting any

kind of nutrition. So, they have to wait until the nutrition is going to appear. So, during that phase, they can start utilizing the cellular content.

For example, they start eating up the different types of organelles, different types of bio molecules and so on so that they can be able to produce the biomolecule which are essential for the survival and they can also be able to produce energy and that all these events are coming under the process which is called as of autophagy. Once the autophagy events are going to be failed to provide the sufficient energy and sufficient bio molecules, then the cell will actually going to go for the death pathway.

And within the death pathway, you can have the 2 pathways one is called as the apoptotic pathway the other one is called as the necrotic pathway and within the apoptotic pathway, you have the 2 different types of pathway. So, that we are going to discuss when we are and how you can be able to study the apoptosis as well.

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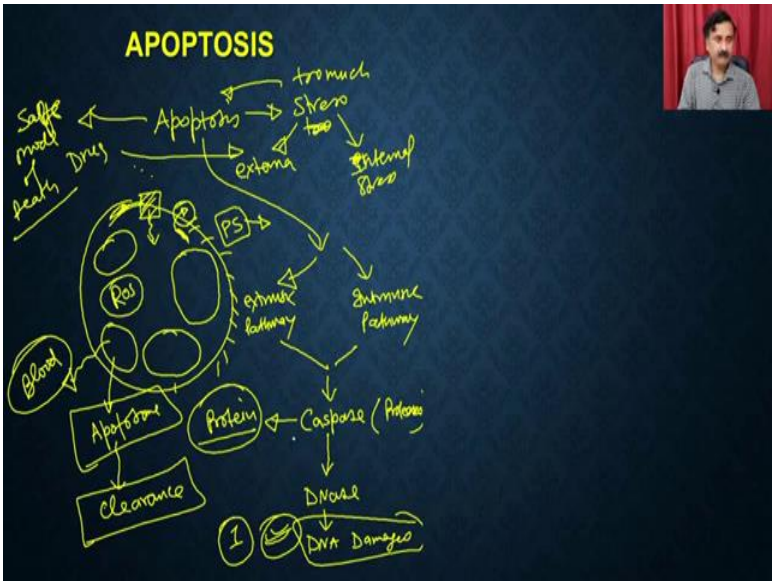

So, apoptosis is going to utilize the, so apoptosis is going to utilize the. So, you can imagine a cell. So, cell can have the is happening because you have too much stress. So, if you have a too much stress, it is going to induce the apoptosis, but this stress could be external stress or it could be internal stress.

So, now, for example, if you add some drug molecules, this is going to be an external stress. So, these external stress are actually going to be received by the receptors what are present onto the cell surface, whereas the internal stress there would be an internal stress also. Like

for example, there will be a lack of nutrition, the production of Ros, that all those kinds of things are going to be the internal stress.

So, because of that, it is actually going to activate the two different parts of it one is called as the extrinsic pathway. So, it is going to be called as extrinsic pathway. And the other one is called as the intrinsic pathway. Irrespective of the, so there are different events what is going to happen when you are going to activate the cell surface receptors and then it is actually going to activate the extrinsic pathway or when it is actually going to activate the extrinsic receptor.

Ultimately, both of these pathways are actually going to activate the caspases. And these caspases are eventually going to activate the, so caspases are proteases. So, these caspases are proteases. So, they will actually going to activate, they are going to start degrading the proteins. And the caspases are eventually going to activate the DNase. And these DNase are they actually going to start damaging the DNA.

And so, there will be a damage of proteins, there will be a damage of DNA and because of that, the cell will eventually going to go for the apoptosis. Then what will happen the cell is actually going to engulf the material and it is actually going to trap these degraded material into the membrane bound structures and these membrane bound structures are called as the apoptosome.

And these apoptosome are then going to be released into the blood or into the tissue and then they are actually going to be taken up by the other cells for the clearance. So, they are actually going to be cleared from the circulation. So, that is why the apoptosis is considered as the safe mode of death, because it is actually going to secrete the, it is going to release the apoptosome.

And these apoptosome are going to be cleared from the circulation without getting the too much inflammations and other kinds of immune responses. Now, if you want to study the apoptosis, what you can do if you can actually be able to first study what will be the DNA, how, whether the DNA is damaged or intact. So, you can actually be able to study the DNA damages or you can also be able to study the alterations, what is going to happen onto the plasma membrane.

So, one of the major alterations what is happening onto the membrane is that it is actually going to have the reversal of the lipid composition. So, mostly the phosphodal saving which

is actually lipids is always present onto the inner surface of these plasma membrane, but under the apoptotic phase it is actually going to flip and then it is going to be present on to the external surface of the plasma membrane.

So, that also can be detected. So, there will be a limit symmetry which is actually going to be disturbed during the apoptosis and that can be detected with the help of the different types of analytical tools or you can actually be able to study whether the DNA is intact or the damaged.

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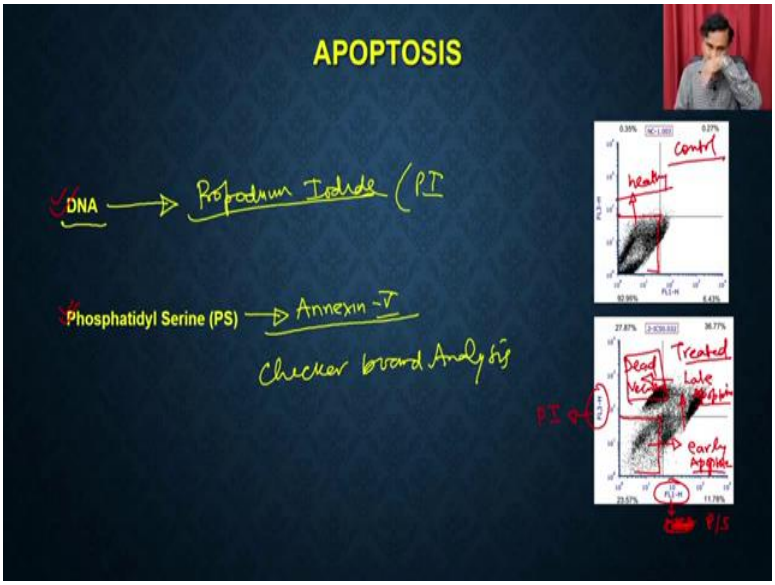

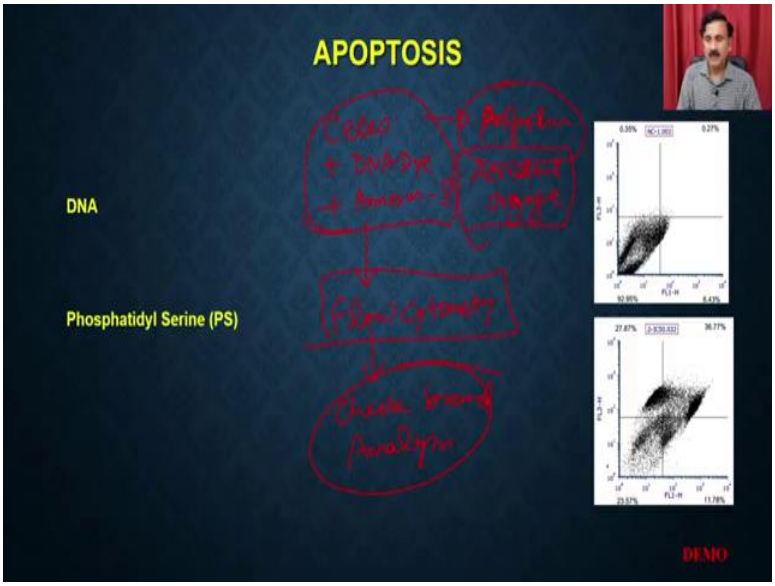

DNA can be studied by the different types of DNA binding dye, for example, you can use the propodium iodide or the PI and phosphatidyl serine can be studied by a probe which is called as annexin 5. So, if you stain the cells with the and then you can do a checkerboard analysis,

so, you can do checkerboard analysis and within the facts, so, what you can do is you can stain the cells with the propodium iodide and annexin 5 and it is actually going to give you a pattern like this where the inner quadrant or the first quadrant is actually going to give you the cells which are healthy.

And they are going to be, so, these, if you do a checkerboard analysis all these cells are going to be considered as the healthy cells. Now, what you see here is this is the control cells or the untreated cells, whereas these are actually the treated cells. So, when you treat the cells with some agents, which is actually going to activate the extrinsic apoptotic pathway, what will happen is, you see that the number of cells under the first quadrant is going to be reduced and the cells are now moving into the all-other quadrant.

So, it is they were moving into the quadrant number 2, 3, and they are moving into the quadrant number 4 also. This quadrant number 2 is actually having a high signal for FL 1, but they are actually going to having the lower signal for FL 3. So, these cells are called as early apoptotic cells because they are actually having the low or very low signal of the FL 1. FL 1 is going to be give you a signal about the PS and FL 3 is actually going to give you the signal about PI.

So, they are actually having the DNA damage, but it is not going to have the very high quantity or high signal for the PI. And then you can have this quadrant. So, in this quadrant you have the high propidium iodide signal and high Annexin. So, this is actually a late apoptosis. So, because it is actually showing the PS and as well as their DNA signal, whereas in this one you have the low PS signal and the high DNA damage signals, that is actually a dead cells or the necrotic cells.

So, this is the way you can actually be able to identify the different types of species of the cells what is present into the apoptosis. How you can do that? You can actually take the cell and you can be able to stain it with the DNA dye. And you can also use and stain it with an annexin 5. So, when you do that, it is actually going to stain the cells and then you can actually be able to study or analyze the cells under the flow cytometry.

And so, flow cytometry and then you can do a checkerboard analysis. So, when you do the checkerboard analysis, it is going to give you the cells of the different species. Apart from this, you can also use the another method where we can also use the two different combination of the DNA dye like the propidium iodide.

And we can use the acridine orange, and that is also a very common, or the popular method of detecting the apoptosis. So, we have prepared a small demo, and it is actually going to give you a clearer idea how you can be able to study the apoptosis within the cells.

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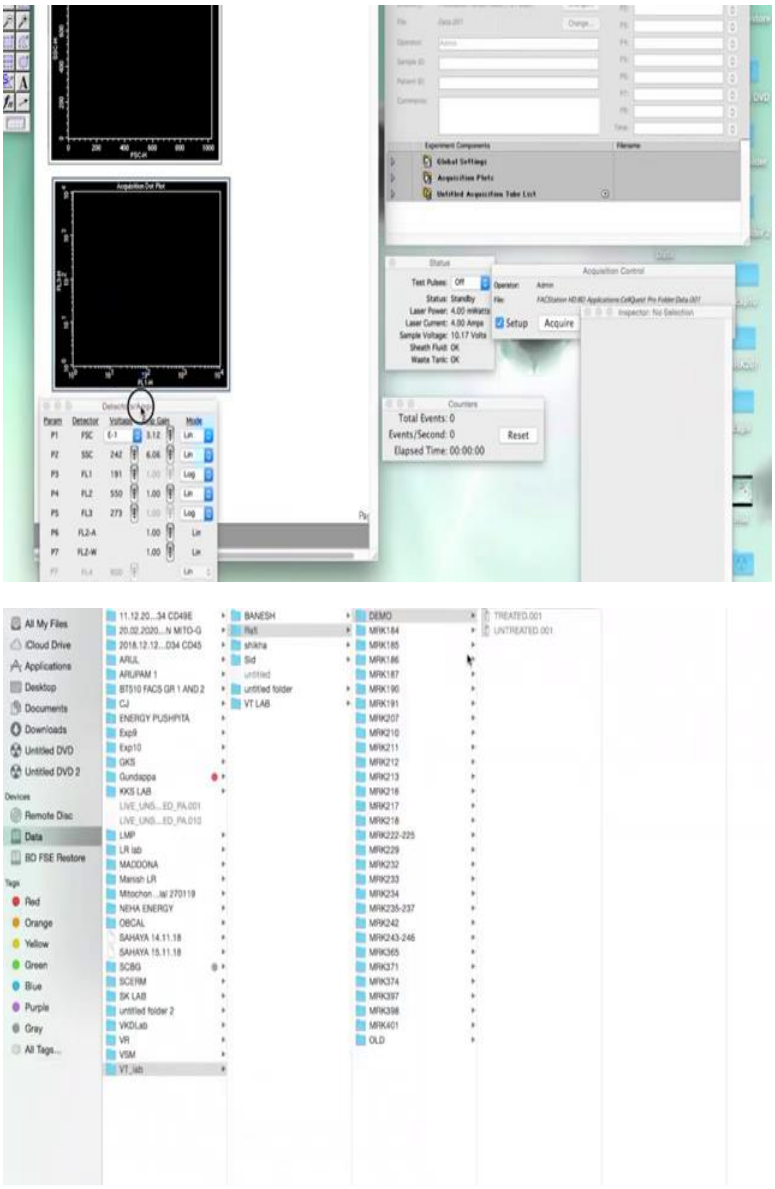

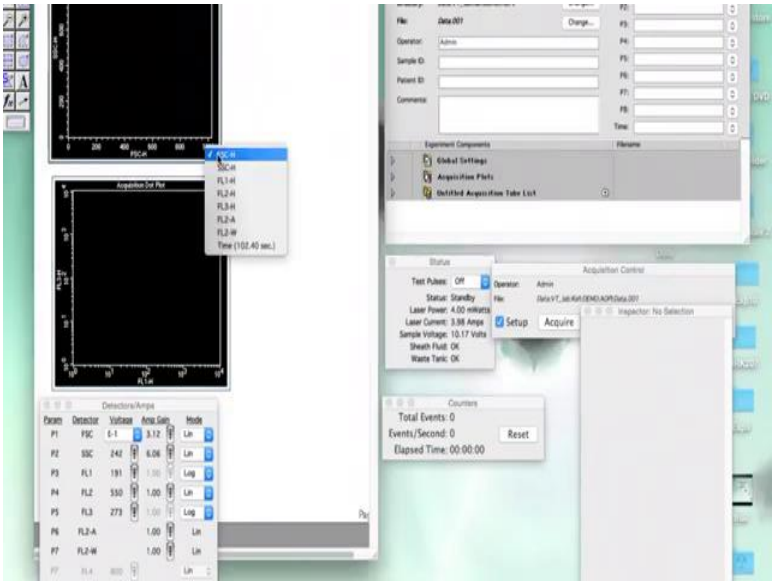

So, after adding the acridine orange and propidium iodide to the cells, we have to acquire the data on the CellQuest Pro software. So, the first thing we do is open the CellQuest Pro and connected to cytometer. And then we need the counters, the detector and amps and the status. For the acridine orange propidium iodide staining we need two dot plots, so one is for the FSC SSE for the forward scattering and the side scattering, and the other one is for the FL 1 and FL 3. The FL 1 plot is on the x-axis, where as the FL 3 plot is on the y-axis. The FL 1 plot is for the acridine orange and FL 3 plot is for the propidium iodide. After taking the plots, we have to set the directory and save the data in our required location.

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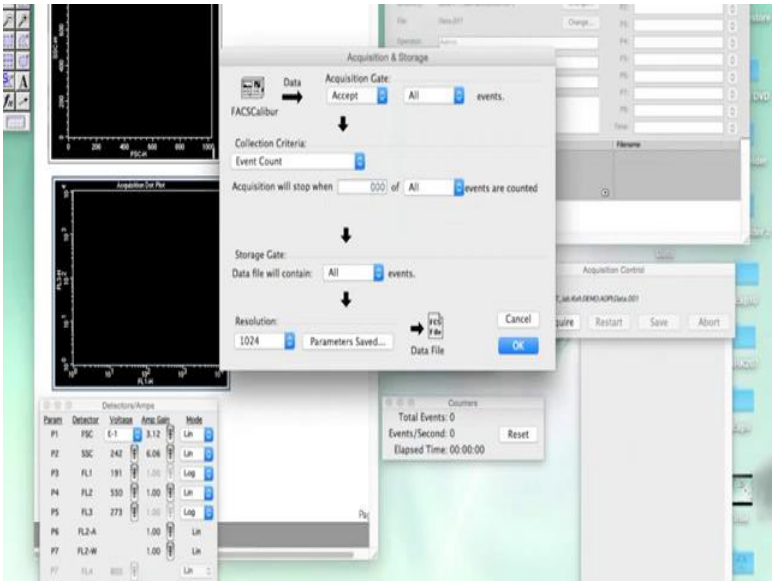

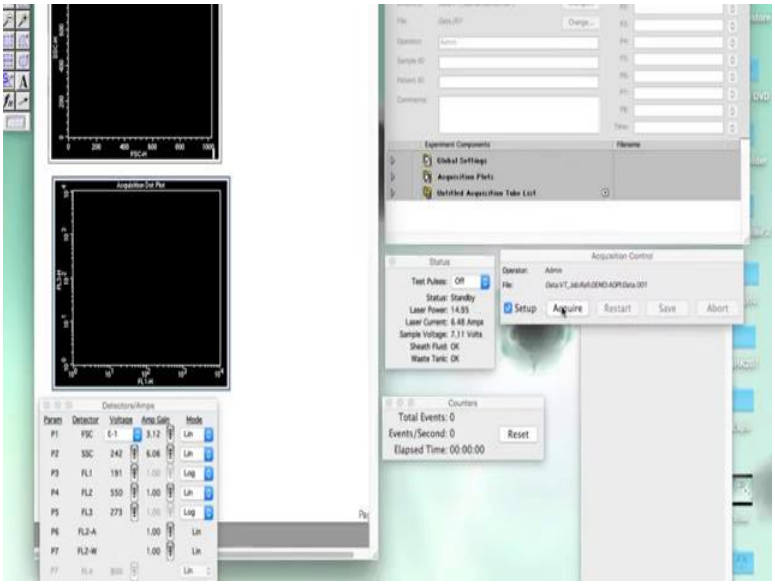
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Setup
Acquire
Restart
Save
Abort
Counters
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Events/Second: 0
Elapsed Time: 00:00:00
Reset
Detectors/Amps
Param Detector Voltage Amp Gain Mode
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P2 SSC 242 6.06 Lin
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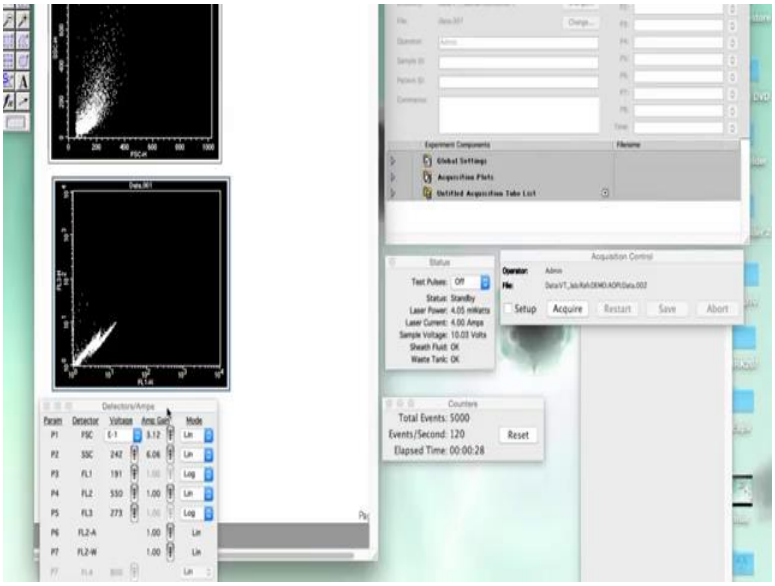
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Acquire
Restart
Save
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Param Detector Voltage Amp Gain Mode
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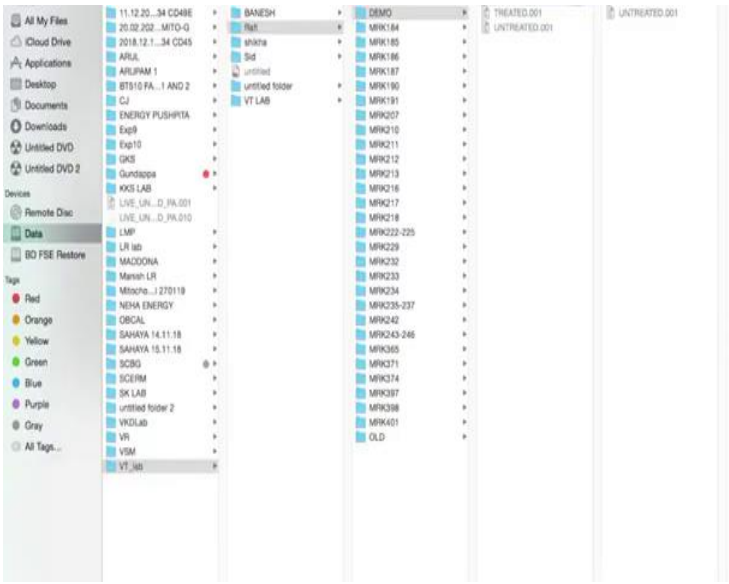
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iCloud Drive
Applications
Desktop
Documents
Downloads
Untitled DVD
Untitled DVD 2
Devices
Remote Disc
Data
BD FSE Restore
Tags
Red
Orange
Yellow
Green
Blue
Purple
Gray
All Tags...
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Rafi
shikha
Sid
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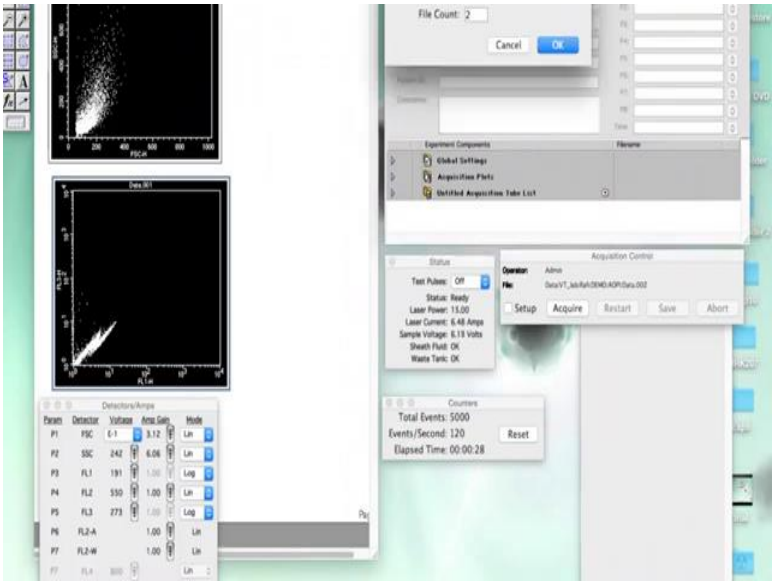

In the detector and AMS we have to remember that we have to set the population of the healthy cells in the first quadrant that is 10 to the power of 1 and 10 to the power of 1. So, after we set the untreated cells in the first quadrant, and then we analyze the treated cells. And then we can say whether there is any shift in fluorescence in the untreated and treated cells. For the treated cells in the third and the fourth quadrant that represents the apoptotic and the necrotic cells.

So, now we will be a taking the sample. But before analyzing the data, we have to set the number of events to the 5000 and then keep it on setup and first we will see whether the events are coming properly or not. Now, we press Acquire. As we can see that in the FSC and SSE plot, we can see the events coming near 00, that represents the healthy population. As well as in the FL 1 and FL 3 we have set the healthy population between the 10 to the power of 1 and 10 to the power of 1.

So, this is this represents the first quadrant. We will show in detail how to do the quadrant analysis in the FCS verify software. Now that we have set the population in the first quadrant, we will move remove it is from setup and acquire the data. After the untreated samples, we have to take the treated samples on the same parameter description which we have set for the untreated cells.

Now, we change the sample in the sample injection port to the treated sample. In the, we have to remember that we do not have to change the parameters or else we will never be able to say whether there is any shift in the untreated or the treated cells if we change the parameters.

After changing the sample, now we have to choose the directory for the treated cells and then change the name also to treated and also the file count to one then press OK.

And then now we acquire the data on the same parameters. As we can see that there is some shift in the population of the cells. The population is having a little bit more fluorescence than untreated cells, which represents the apoptotic and necrotic cells in the third and the fourth quadrant. In the fourth quadrant mostly the necrotic and the dead cells are present, whereas in the third quadrant, the late apoptotic cells are present.

After we did the untreated under treated samples after we acquired the data for the untreated under treated samples, we have to analyze the data in the FCS Express Pro software using quadrant analysis. In the quadrant analysis we can see how much populations of cells are present in which quadrant and therefore we can identify the number of healthy populations and the apoptotic and necrotic cells.

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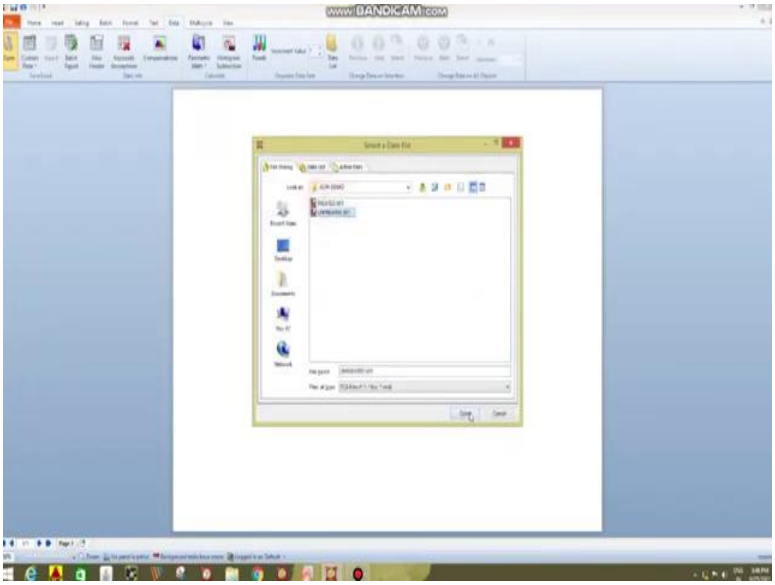

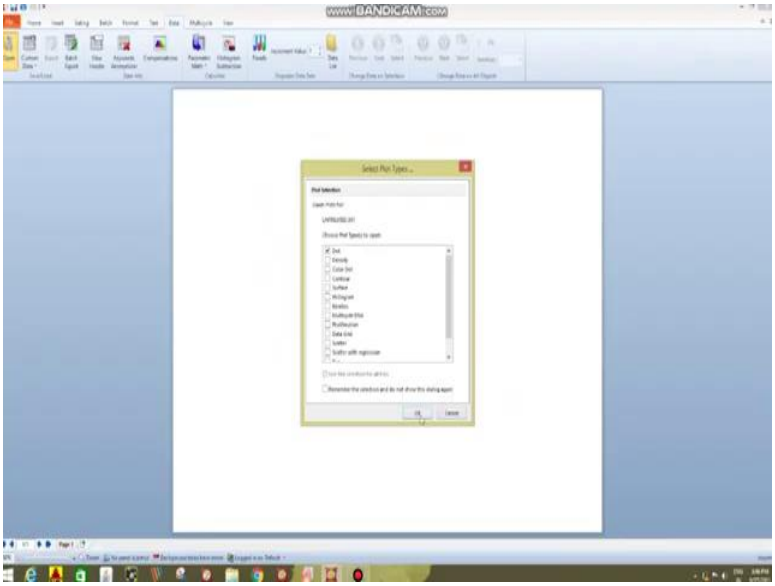

So, after acquiring the data in the fax equipment, we have to now analyze the data in the FCS Express software. So, the first thing we do is we open the new layout and change the orientation to landscape. And then now we input the data. First thing we do is we take the untreated file and then OPEN the dot plot.

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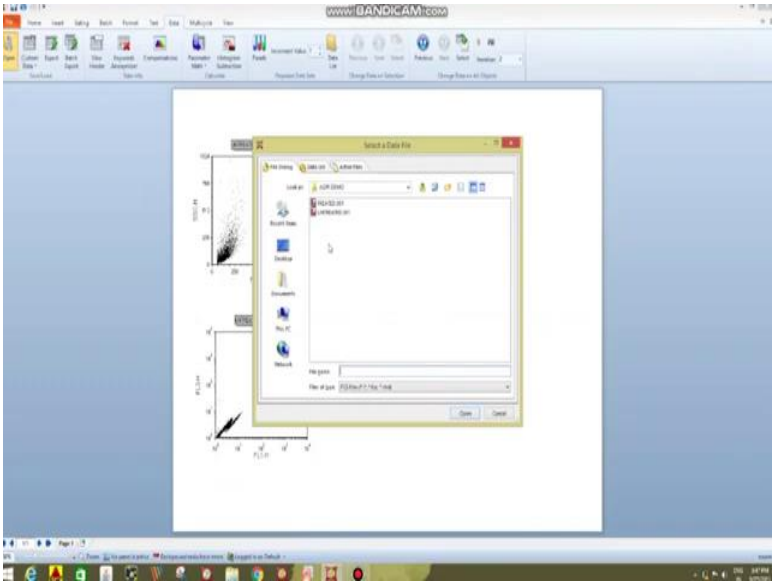

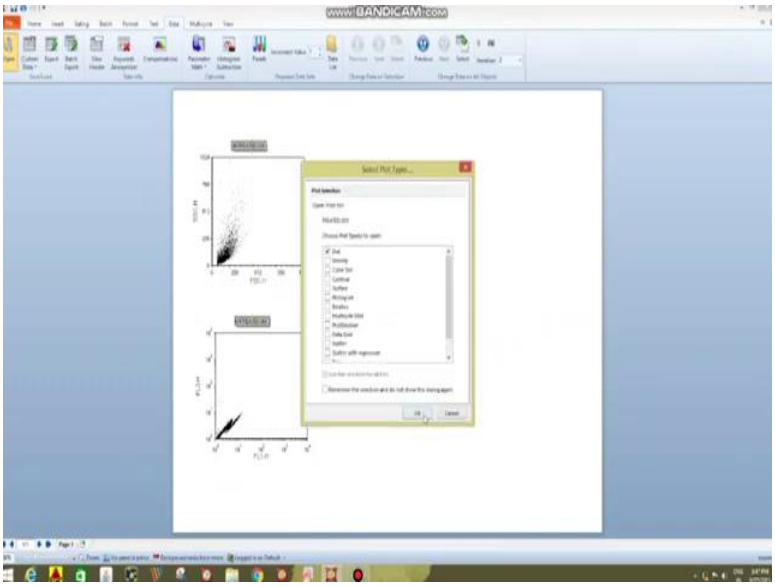

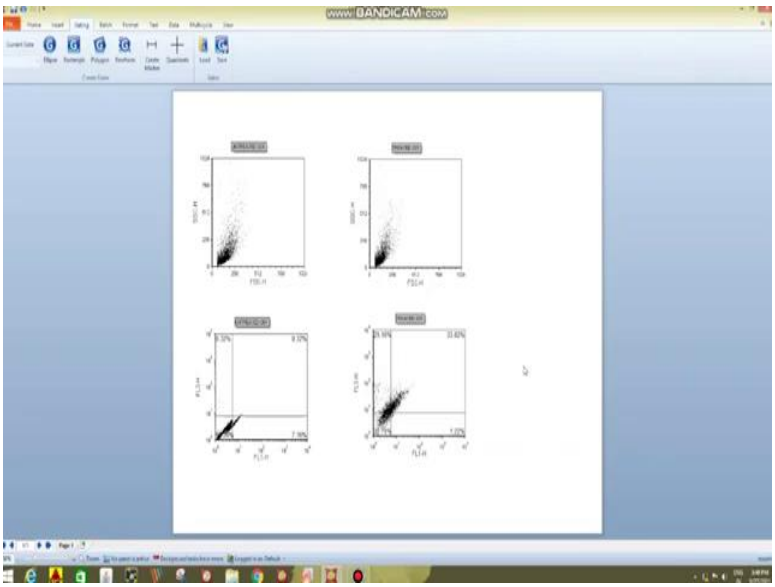

We need two dot plots, the one is the FSC SSE and the other one is the FL 1, FL 3. The FL 1 FL 3 dot plot shows the live and dead cell staining. The FL 1 is responsible for the acridine orange whereas FL 3 is responsible for the propidium iodide. Now, we take the treated file. And again we select the dot plot. In dot plot as well, we need the FSC and the FL 1 FL 3 plot. As we can see that there is a difference between the untreated and treated sample.

Now we have to find out how much percentage of the cells have gone actually the apoptotic or necrotic. Like we have to divide the population of cells into four quadrants using the quadrants option. So, we go to the gating and then take the quadrant, and then apply it on the FL 1 FL 3 plot, we have to apply it in such a way that we cover all the cells in the untreated plot.

So, let us say that, in the untreated plot, we are having 93, 92 percent live cells and 7 percent are in the early apoptotic phase. And then after applying the quadrant to the untreated, we have to apply the same quadrant to the treated one in order to find out the difference between the two. So, just we click on the quadrant, and then we copy and paste on the treated one.

So, in this way, we can say that in the first quadrant, in the untreated sample, we have approximately 93 percent of live cells, whereas in the treated one, we have, like in the treated one we have only 35.7 percent healthy, whereas the 33 percent have gone are in the late apoptotic and 29 percent are necrotic cells.

So, in this way, we can use acridine orange and propidium iodide to determine the healthy the apoptotic and necrotic cells in different treatments. And also we can establish a relation between different concentrations of treatment and the number of live and dead cells in any experiment. So, this is the way we analyze and process the data on the fax equipment in order to do the alive and dead cell staining. Hopefully, this video was helpful.

So, I am sure you might have understood the different processes what is happening with when you want to study the apoptosis of the cells utilizing the flow cytometry. And with this, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects of the cellular processes.

And we have, what we have discussed so far, we have discussed about the basics of the flow cytometry and how this technique can be used to study the cells of the different sizes as well as the different densities. And this is a very robust technique that we can use for studying the different types of cellular processes and then utilizing the flow cytometry, we have studied

about the cell cycle, we discuss about the cell proliferations and then ultimately, we have also discussed about the apoptosis. So, with this, I would like to conclude my lecture here. Thank you.