Nanobiophotonics: Touching Our Daily Life
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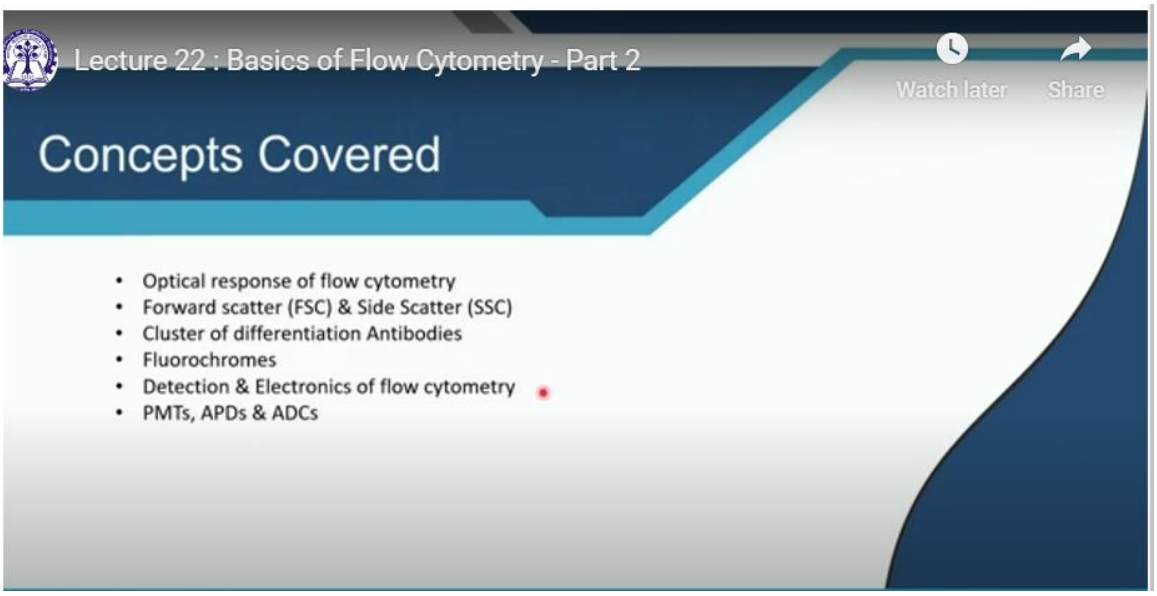
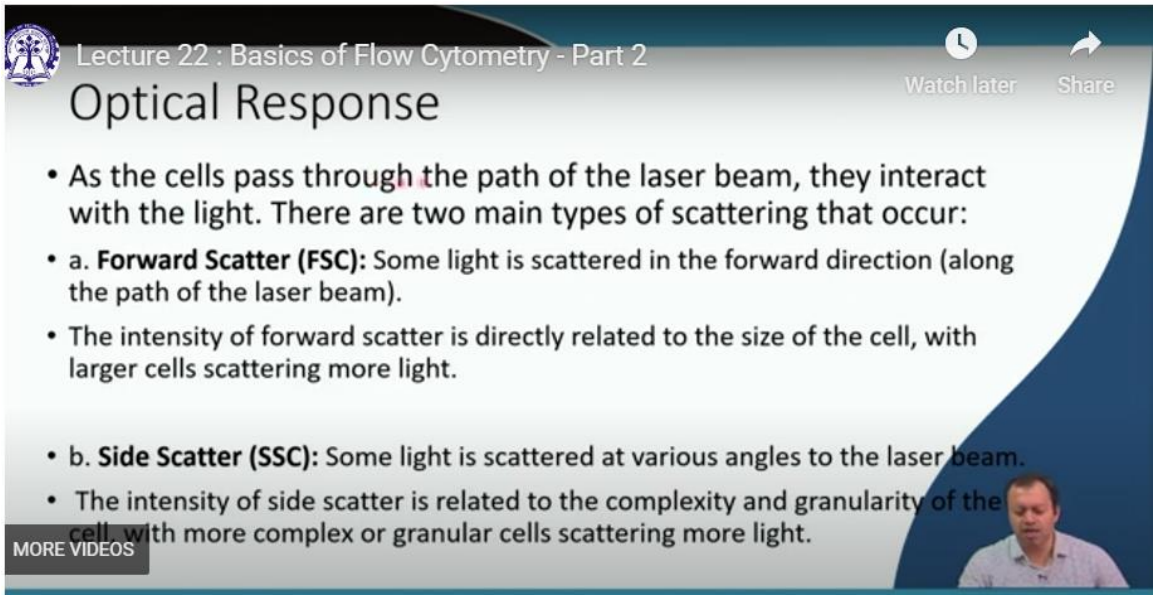
Lecture No. 22
Basics of Flow Cytometry - Part 2

Welcome back. We were in the previous class discussing Cell Cytometry. So, let us continue with our discussion.

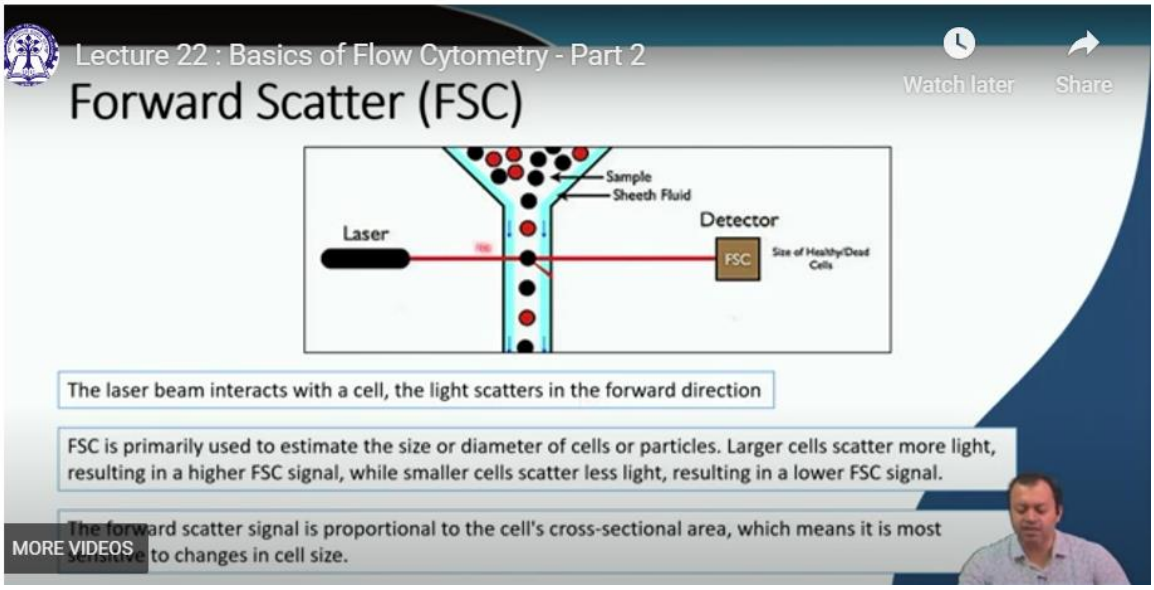


So, today I will be covering these topics on the Cell Cytometry. So, let us begin. So, in the previous class we have discussed the basic arrangement of the Cell Cytometer where we pass cells one at a time through a tubular structure and then it is getting illuminated by the laser light.



Now let us go little bit detail on the illumination part. So, what are the optical response we are looking through? As the cell pass through the path of the laser beam they interact with the light there are two main type of scattering that occurs, two main type of scattering that occurs a forward scatter and a side scatter. A forward scatter the light is scattered in the forward direction in whichever direction the light was going it simply scattered in the similarly along the path of the laser beam. The intensity of the forward scatter is directly related to the size of the cell with a larger cell scattering more light it is quite common sense.

Side scatter on the other hand the light is scattered at various angles. So, you have light, you have the material, you have the cell and the light is coming out scattered out of it. So, if the light goes just like this light is coming in the same path it is coming out. So, same path it is coming out it determines the overall the intensity overall determines the size of the cell. However, if the light is scattered in different direction that gives rise to the complexity and the granularity of the cell more complex or granular cells scattering more light.

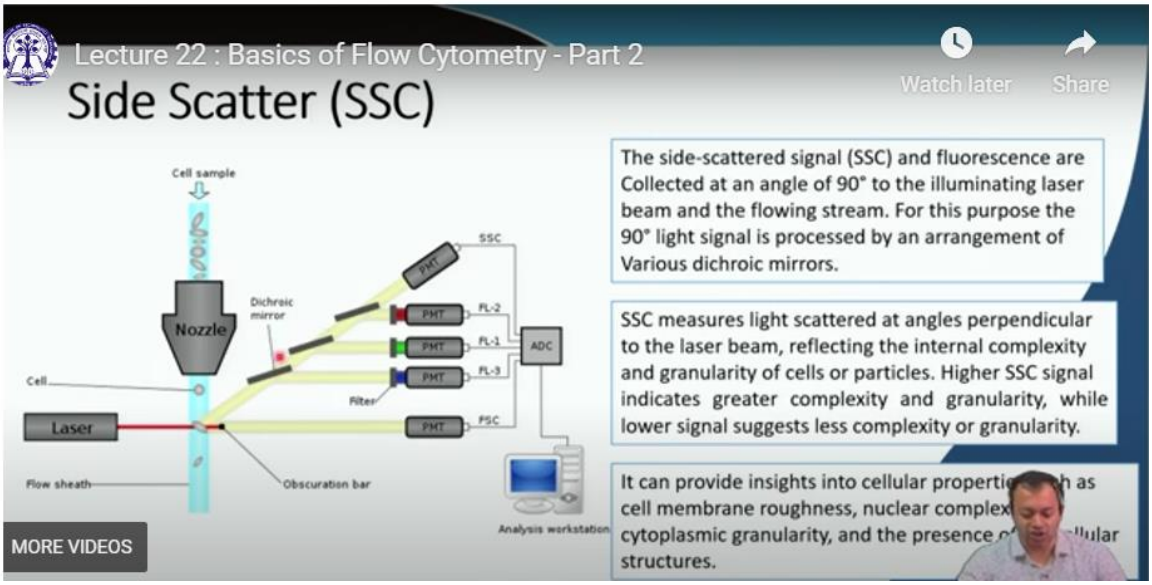


So, you are getting two major sorts of information from the type of cells that you are trying to classify first the size. Cancer cells of the same kind will have different size as compared to normal cell. Cancer blood cells will have different size, cancer brain cells brain tumours will have different size anything else will have different size. At the same time the granular complexity whether it becomes rough, it becomes strong, it becomes hardened, it becomes more flexible the cells contain some sort of an additional pathogen infected cells. The cells have been infected by bacteria, infected by virus their granularity, their shape, their structure, their size all of those things have changed.

You are able to get all that information simply by measuring the scattered profile. In this particular case you can say reflected profile the scattered profile through this optical measurement. So, see the laser is passing through you have the cell getting illuminated this is the straight line that gives you size of healthy or dead cells depending on what you have gone through and this side scatter this I think I have a better example of side scatter here, but let us not go ahead of ourselves. For the forward scattered FSC the laser beam interacts with the cell the light scatters in the forward direction. FSC is primarily used to estimate the size or diameter of the cells or particles larger scales scatter more light resulting in higher FSC signal while smaller cells scatter very less light resulting in lower FSC signal.

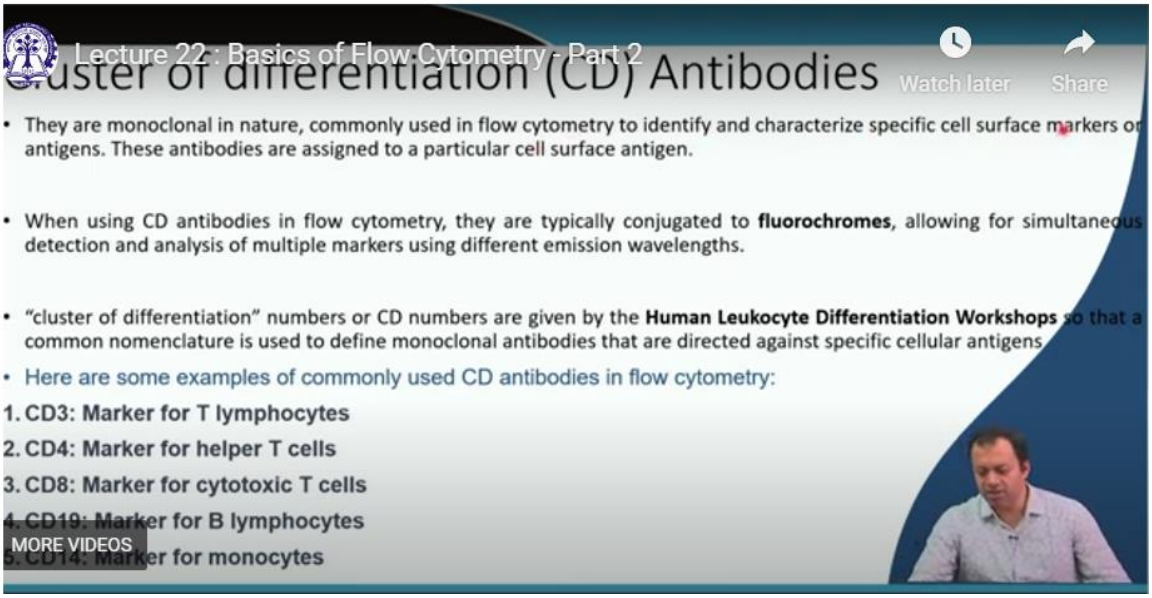
So, immediately we will understand the diameter of the cells that is passing through. The forward scatter signal is proportional to the cells cross sectional area which mean it is most sensitive to change in cell size. So, this light forward scattered determines the size of the cell. Cellular size changes in case of cancer it can change for other diseases as well, but one of the major things that we have absorbed for a substantial amount of a plethora of different type of cancer not all granted, but several types of cancers is first and foremost

the cellular size changing. So, if you have measured like a million cells and you are finding out that 50 to 70 percent of them have a different size than the normal one then it is something to be worried about then it is something to be you know checked about and then you can differentiate you can separate you can sort out the good cell the smaller cell versus the larger cell and then look into them under a microscope or further analysis spectrometer or anything such that you can you are able to understand whether it is actually cancerous or any other kind of fluid intake has happened and the cell has simply bloated up.



So, this is what forward scatter FSC does. Side scatter on the other hand you see the light is getting scattered in different direction those lights are being captured by dichroic mirror and put into PMT photomultiplier tube which is basically produce electrons when light comes into it when light gets detected they creates electron these electrons are then checked into ADC analog to digital converter into some kind of a digital signal and you analyze them using a workstation. So, side scattered signal the fluorescence are collected at an angle 90 degree to the illumination laser light and the flowing stream for this purpose the 90 degree light signal is processed by an arrangement of various dichroic mirror SSC measures light scattered at angle perpendicular to the laser beam reflecting the internal complexity and granularity of cells or particle higher SSC signal indicates greater complexity and granularity smooth or rough depending on presence of microbes presence of pathogens cancers what not while lower signal suggest less complexity or granularity. It can provide insights into cellular properties such as cell membrane roughness nuclear complexity cytoplasmic granularity and the presence of sub cellular structure whether it has been infected by some kind of virion some kind of virion particle it could be done. So, cells are passing through this nozzle this is the cell sample cells are passing through this nozzle you have the flow sheet and you have the internal if you can see it is a slightly lighter color than the surrounding blue I am partially color blind.

So, I think this is blue there is little bit whitish. So, this is the core liquid and the outside are the sheet liquid cylinder inside cylinder imagine it then laser light is illuminated one is the forward scatter and these are the side scatter this thing is available in this direction as well certain times it is also present in this direction and this direction. So, that the entire measurement could very well be done in a 360-degree rotation, but the more amount of these and detector photomultiplier tubes you do the more costly it becomes and more costly it becomes the more accurate it becomes. So, it depends on what you plan to do. So, the cluster of differentiation how do you do it they are monoclonal in nature commonly used in flow cytometry to identify and characterize specific cell surface markers or antigens these antibodies are assigned to particular cell surface antigens.

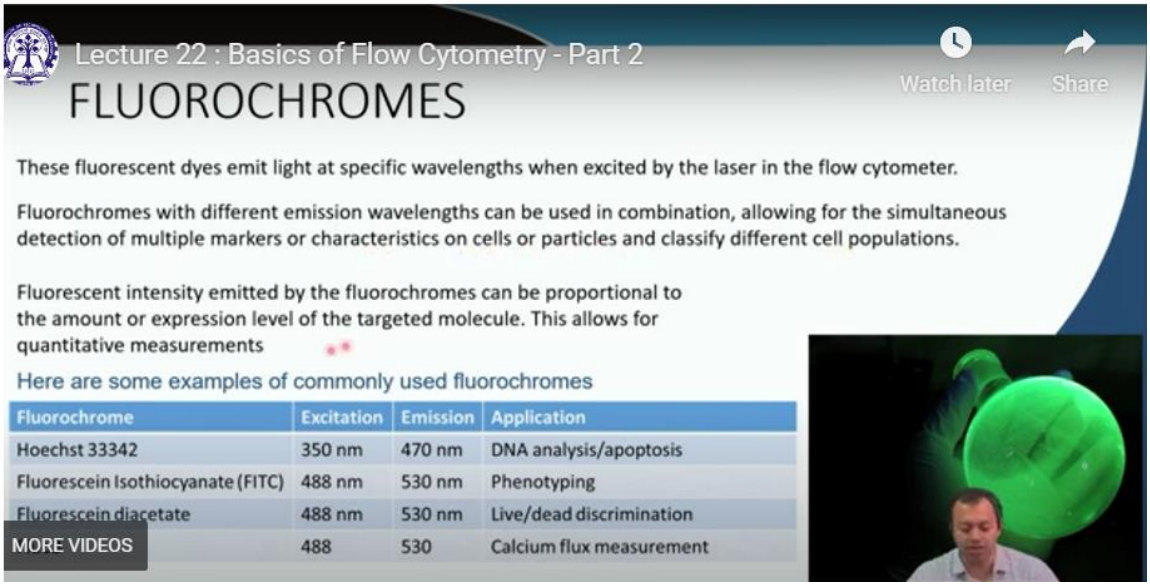


So, what are monoclonal in nature? Monoclonal means that you have taken out a particular antibody from a particular cell you have cloned the cell and you have produced the same antibody like a large number of times. So, you have artificially created this antibody by cloning by cloning you should know what a clone is the same copy it is the biological copy of the same cell or structure or organism clone by definition. So, identical twins are not clones identical twins are not clones clone have to be asexually reproduced right. So, a twin is not clone a clone is when you have you have probably heard of Dolly the first cloned ship in Kashmiri university in India they also managed to clone pashmina ship. So, clone is when a cell is taken and then from that the entire or either the cell is replicated the same cell is made copies it is like a photocopy it is a Xerox copy it is a photocopy machine.

So, the antibody is taken from a particular cell which has been cloned many times and you produce the antibody and that is inserted into all those cells that are passing through. While using CD antibodies in flow cytometry they are typically conjugated to fluorochromes fluorophores allowing for simulated detection and analysis of multiple markers cluster of differentiation number of series are given in human leukocyte

differential workshop I will describe this I will describe the marker for T lymphocyte at lecture number I think fourth lecture of this series where exactly example of cell sorting will be done. But for the time being understand that individual cells are attracted or attached with certain antigens they are connected with certain chromophore fluorophore and then they are made to pass through the laser light. Now, these antigen antibodies are very very specific these antibodies will not be attaching to a bad cell the cell whose structure has changed these are monoclonal. So, they are not variety of antibodies they are one single type of antibody attaching to one particular type of cell one particular variety of cell within the similar cell blood structure.

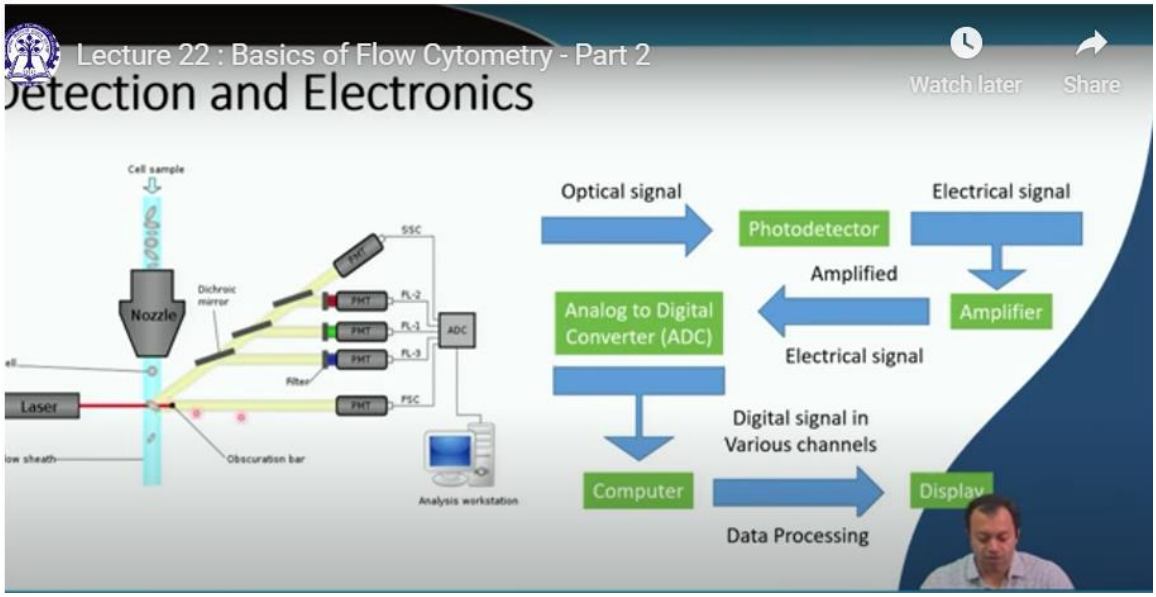
So, blood cell only it will attach with the proper normal blood cell or only it will attach with the cancerous blood cell and that will then attach the antibody will then attached with the fluorophore. Improper attachment will result in improper fluorescence and then you understand looking at the total number of proper versus improper arrangement improper fluorescence you classify you sort out you separate you differentiate that is it.



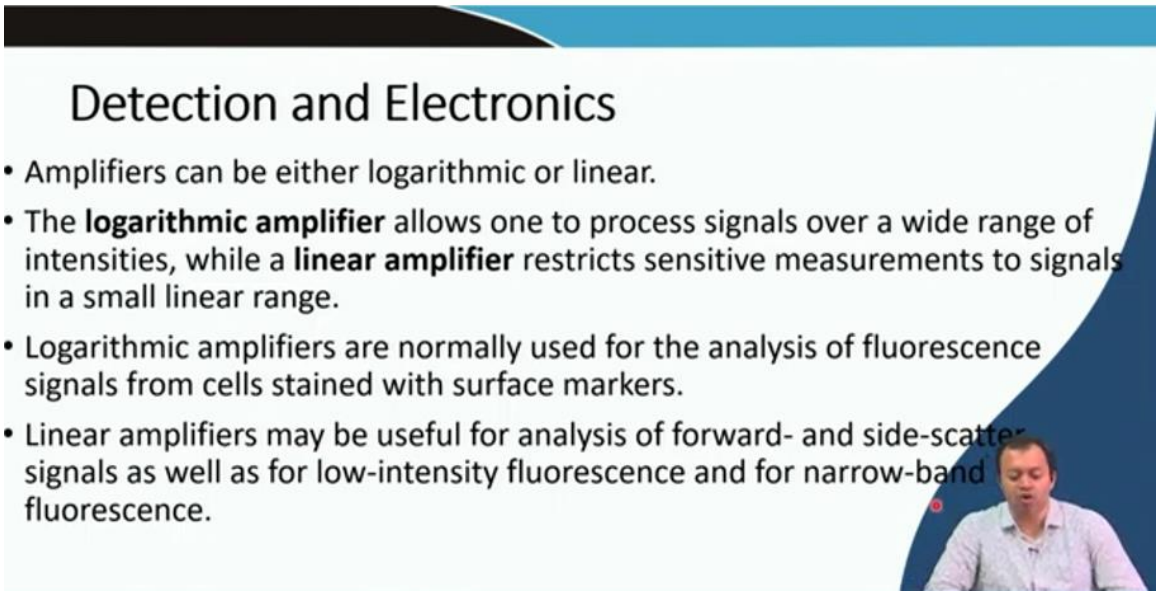
So, the fluorochromes this fluorescence dies emit light at specific wavelengths when excited by laser in flow cytometer. Fluorochromes with different emission wavelength can be used in combination allowing for the simultaneous detection of multiple markers or characteristics on cells and particles. Fluorescence intensity emitted by the fluorochrome can be proportional to the amount of expression level of the targeted molecule.

This fluorescence intensity determine how closely how much of a particular antigen expression means protein expression how much of a particular protein is present in the cellular structure the amount of protein can determine how good or bad the cell is a normal cell will have a specific amount of a specific protein. Protein has to be present in a specific

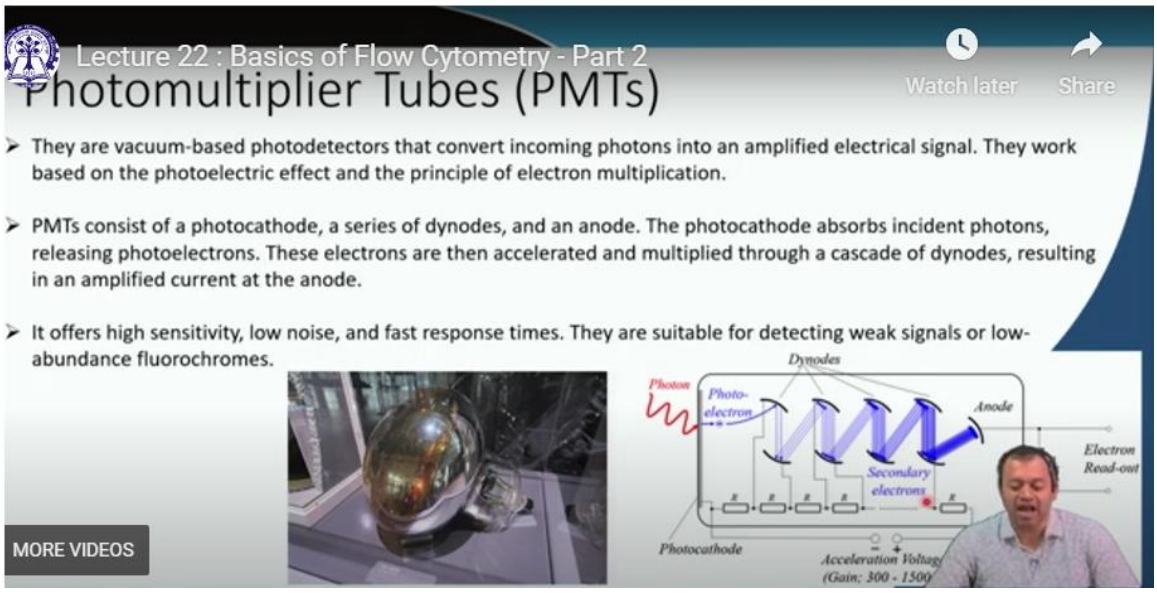
dose in a specific amount any change from that you are measuring with the intensity of the emitted light. So, these are the fluorochromes mostly used these are the excitation and emission and here is the analysis DNA analysis apoptosis we have discussed if not then I will discuss apoptosis. Apoptosis is basically cell death necrosis is murder apoptosis is suicide we will be discussing apoptosis and necrosis several times. Fluo 3 calcium flux measurement or live or dead cell discrimination phenotyping all of those things are available.



So, what exactly are you detecting by you know measuring the scattered light. So, forward scattering as well as side scattering the optical signal is passes through photo detector these kinds of PMTs this photo detector converts the optical light into electrical signal. These electrical signal light gets converted to electrical signal the electrical signal is passed made to pass through an amplifier the amplifier amplifies the electrical signal the electrical signal is then passed through an ADC analog to digital converter and you put it into the computer the computer analyzes the digital signals you can do Fourier transform and what not to find out individual dots and do the data processing and put it into a display.



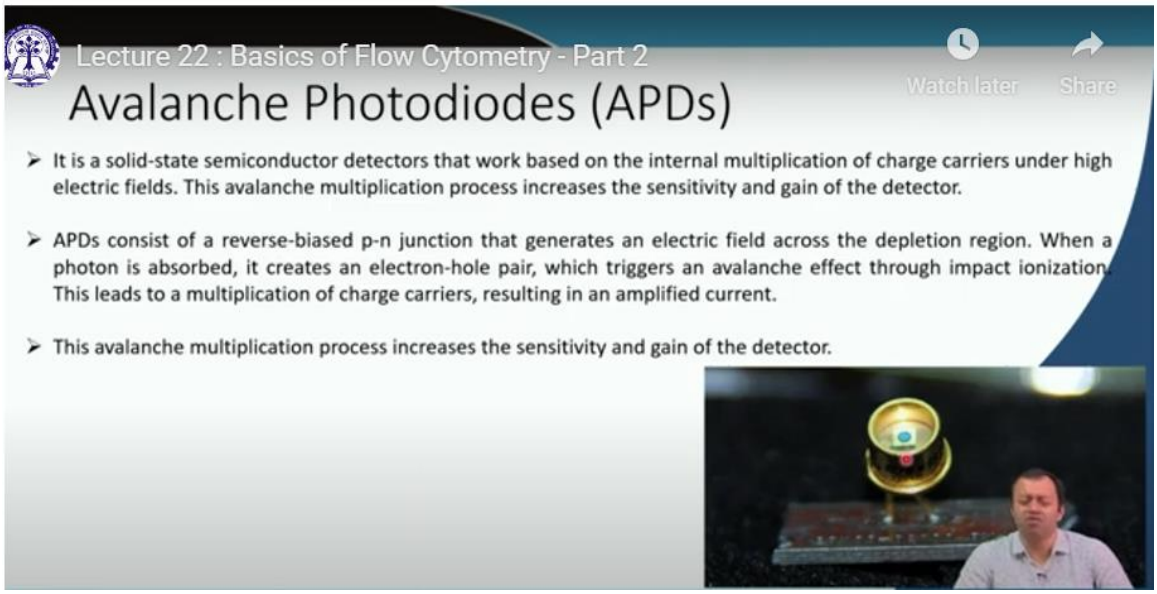
So, yeah that is basically its amplifiers can either be logarithmic or linear logarithmic amplifier allows one to process signal over a wide range of intensity including you have several amount of noise linear amplifiers restrict sensitive measurement to signal in small linear range logarithmic amplifiers are usually for analysis of fluorescence signals and linear amplifiers may be used for forward and side scattered signals.



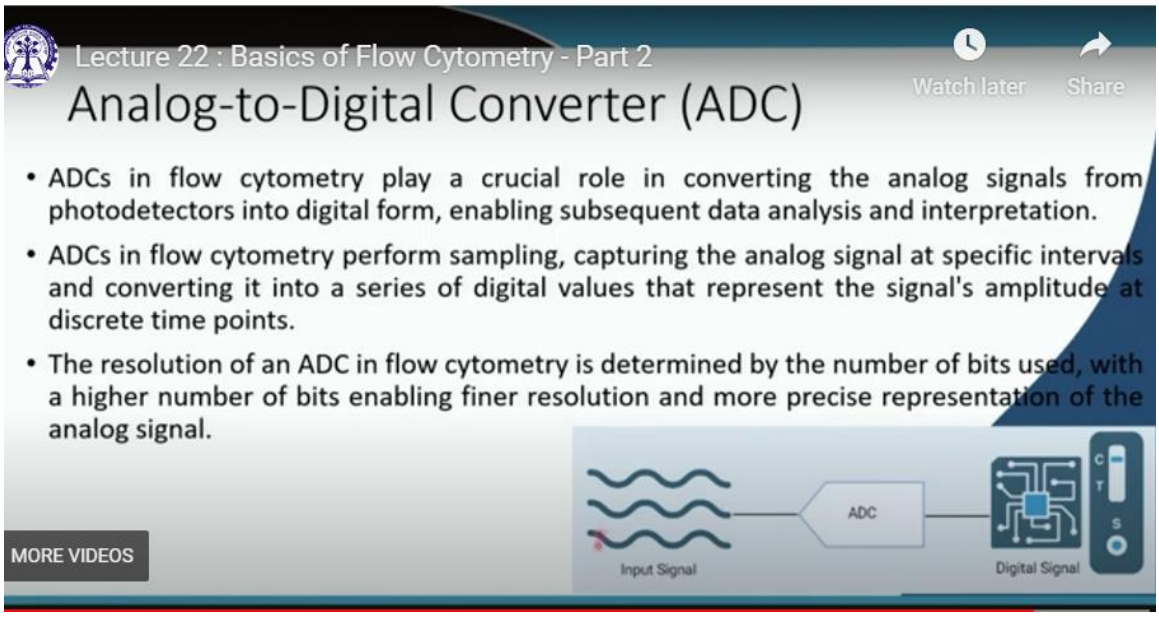
So, these are the PMT tubes the photo multiplier tubes they are vacuum based photo detectors that convert incoming photons into amplified electrical signals.

PMTs are still used, but they are old fashion. It consists of a photo cathode a series of dynodes they are the magnetic field they generate the magnetic field and an anode they

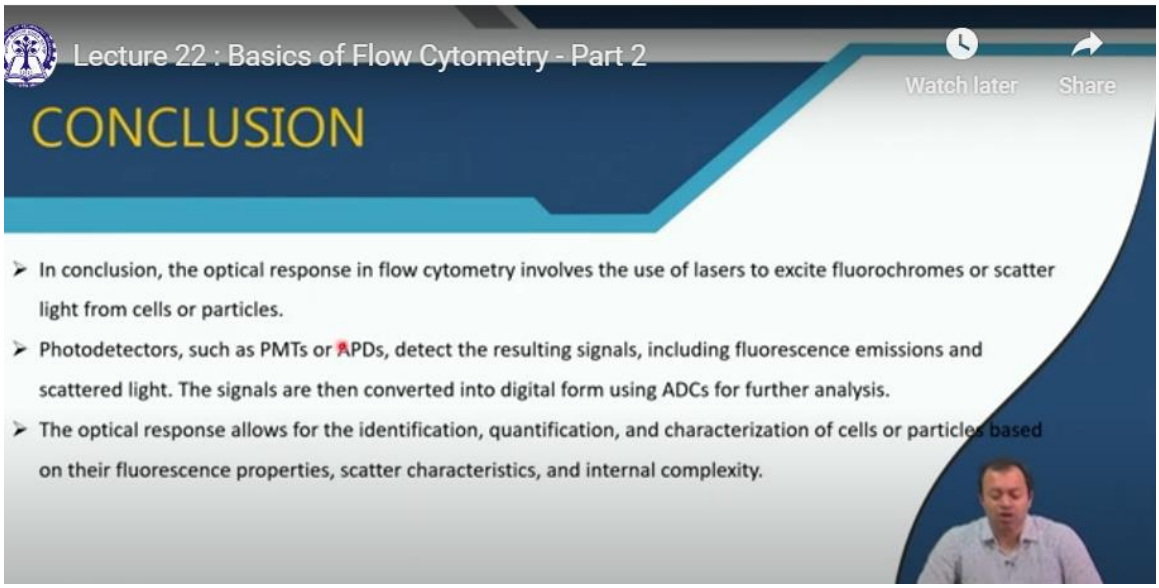
offer high sensitivity. So, basically, they PMTs creates electrons upon excited by photons they create electrons these electrons photo electrons with specific magnetic field are then made to pass through some kind of an electrical readout where you measure the total amount of current flow. Flow of electrons is electric current electricity current they are made to you know pass through several mirrors several magnetic field structures and you at the end of the day measure the overall amount of photon resulting from the total amount of electrons that you have detected.



We can also do photo diodes these are something that I am more comfortable with avalanche photo diode it is a solid state device it is simply a photo detector light falls electron hole pair is generated electron hole pair are generated is separated away. The so light basically cause the electron to go from lower level to upper level leaving a hole in the lower level you separate the electron and hole using anode and cathode and that flow of electron is electric current you measure the electric current and if you have measured the electric current with respect to the amount of photons you understand what exactly is the overall amount of photon that you have detected that gives you how much photon has been scattered how forward moving or side scattered. The avalanche multiplication process increases the sensitivity and gain of the detector presently to the best of my knowledge the one which I use are all APDs avalanche photo diodes.

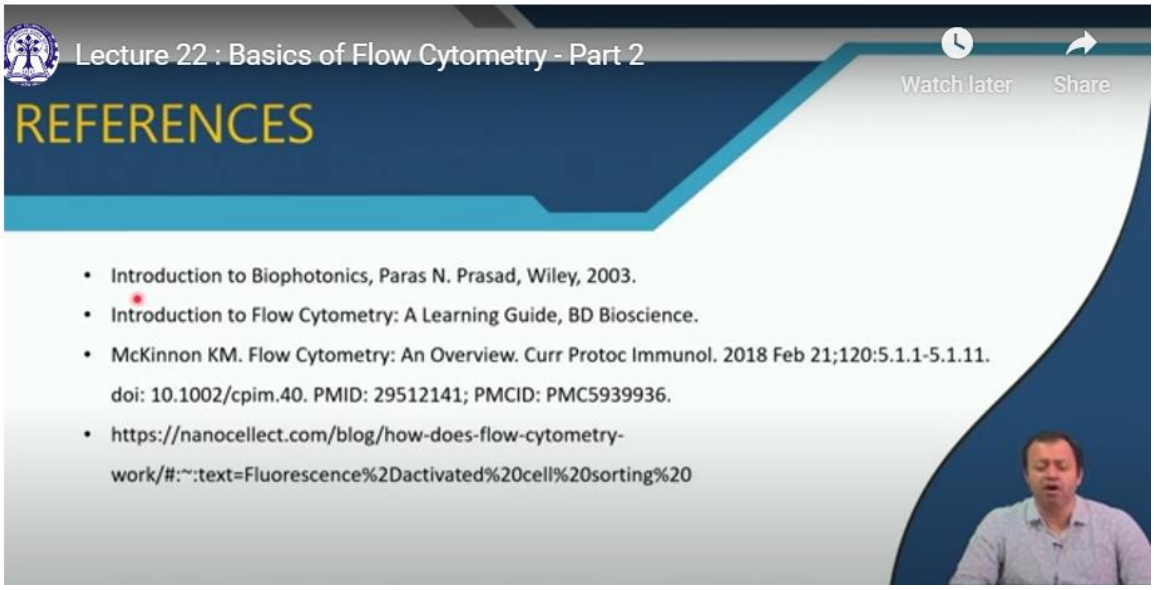


Analog to digital converter any electronics engineer student know this if you do not know biology students do not fret too much input signal is continuous passing through ADC this convert input signals break it down into individual points ADC's in flow cytometry convert the analog signals into digital form it does sampling you have as a child read about niquistrate and how to convert analog signal into digital I am not going to go into detail on to that because that is not part of bio photonics it is simply the continuous signal the waves are converted into individual dots. The resolution of ADC is determined by the number of bits used with a higher number of bits enabling finer resolution and that is it.



these are the conclusions of the optical response and the optical response allowed for the identification quantification and characterization of cells or particle based on their

fluorescence properties scatter characteristics and internal complexity. All you have to know in this chapter is forward scattering and side scattering forward scattering gives you the size of the cell side scattering gives you the granularity of the cell one gives you shape one determines the structure one determines the size one determines the internal properties of it. It can be big and smooth or it can be big and very rough similarly it can be small and smooth it can be small and rough. So big or small forward scattering smooth or rough side scattering that is it.



So, these are my references please go through some YouTube videos of cartoons and animations of how flow cytometry works and this concept will be completely clear to all of you. Thank you very much.