Nanobiophotonics: Touching Our Daily Life

Professor. Basudev Lahiri
Department of Electronics and Electrical Communication Engineering
Indian Institute of Technology, Kharagpur

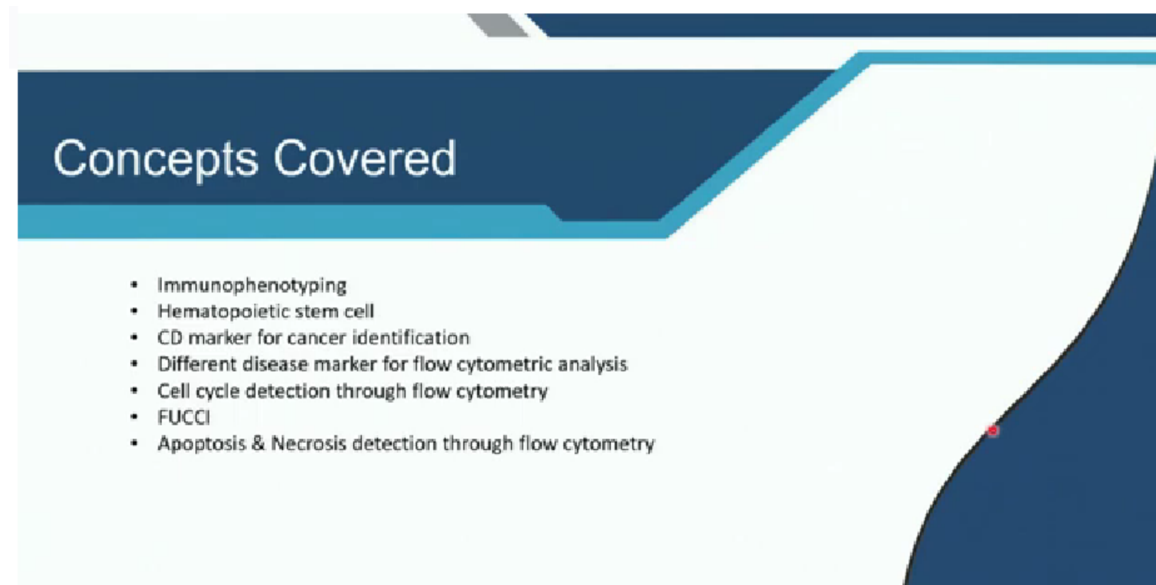
Lecture No. 24

Application of Flow cytometry in Biology

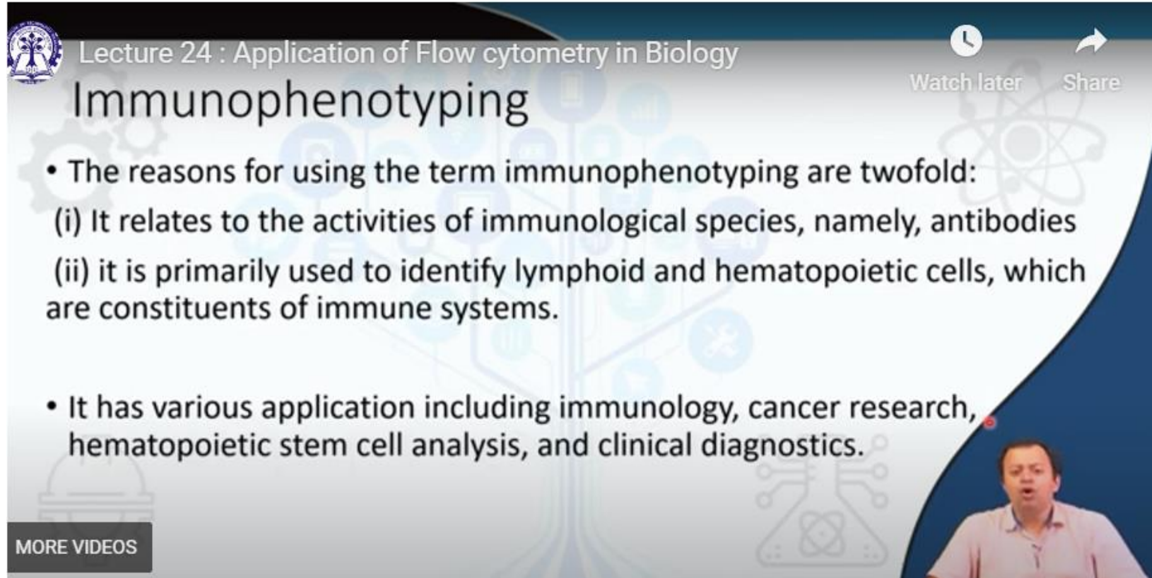
Hello and welcome. We are at module 5 of our topic of flow cytometry. And in the previous 3 slides, we have discussed about what exactly is flow cytometry and what are its various manifestations and how it could be utilized for disease detection. Thus far, I have given you the principles and you know how to interpret the data and what exactly takes place in flow cytometry. In today's lecture, the penultimate lecture, I am going to talk about two major real life applications where flow cytometry can be directly utilized for disease detection. And I have two very specific diseases for you to discuss.

One happens to be blood cancer, another happen to be HIV, AIDS that is. Obviously, there are several other diseases that could be detected using the methodology applied in flow cytometry as we will see. But for the time being, I will be discussing these two major diseases completely separate from one another. One is cancer, non-communicable and another is AIDS which is caused by the virion, caused by the virus and is very much communicable.

So, these two separate diseases both could be detected, both could be diagnosed using biophotonic technology of flow cytometry as such.



So, these will be the concepts that I am going to discuss. For the first time, I will be discussing immunophenotyping. So, let us understand what phenotyping actually means. Of course, I know every single biology student from their first year onwards know what phenotyping is.



But for those of us who are from electronics or physics background, they might have heard this term, but they do not know the exact meaning of it. So, there are two basic term by which an organism can be described. One is genotype and one is phenotype. I will give you couple of examples and couple of analogies so that for the people who are from the non-medical background clearly understand phenotype versus genotype. You will be hearing these terms more and more often in newspapers, in scientific articles etc.

But you need to now fully comprehend and fully differentiate what these are. Genotype basically is the genetic makeup of an organism. So, for example, a human being what sort of genes I have, what sort of genetic makeup I have as I was born. I have inherited certain genes from my parents both from my mother's side as well as from my father's side. And based on those genes, it is determined or I am predisposed to have to be very tall, short or medium height.

The color of my eye will be black, brown, blue. I will be susceptible to diabetes or heart disease. I might be lactose intolerant. I might be hemophilic. So, these are the genetic makeup.

This is the genetic makeup of a person or for an organism. It could be a plant, it could be an animal, anything that contains gene, any basically any living material. These are the genes that determines several traits, several characteristics. That is genotype. So what is phenotype? Genotype is genetic makeup plus the environmental causes or the nature where

the overall area, overall environmental, overall external factors that influences the behavior trait of the organism.

Genotype is internal. Phenotype takes care of the internal and the external. For example, both my parents are very tall. So it is predisposed that I am also going to be tall, but from a childhood, I have decided to eat only junk food, not go to any exercise, not do any exercise, abuse substances like alcohol, like tobacco, etc. Do you think I will still be tall? I have the potential, I am predisposed, but the external environmental factors are making me not tall, not show the particular trait.

So it is nature plus nurture, internal factors plus as well as external factors and that is phenotyping. Phenotyping takes care of the environment in which the organism is born, is interacting, is getting stimulus or stimuli from. So you can, no analogy is perfect, but I would like to give an analogy to the sports lover of you among you. Genotype is talent. Genotype is hard work, talent plus hard work.

You require both to excel. I have not a single iota of talent to play football. I love to watch football. So from today onwards, if I practice 10 hours a day for 10 years, football, do you think I will ever be able to play like Messi? I am a Messi fan. Do you think I will ever be able to play like Messi? Yes, I have done huge amount of hard work, but no, you require some amount of talent.

You require some amount of talent to become great. At the same time, having just talent and doing no practice whatsoever, just simply thinking that since I am predisposed, I have this genetic makeup, that is it, no more hard work is required. So will I succeed? You need both. So phenotype takes care of both. In this particular case, we are talking about immunophenotype, i.

e. how exactly are my immunity? I have inherited my immunity. How strong it is? You remember because of immunity, several people were asymptomatic to COVID. They contracted COVID, but they were fine. This displayed no symptom except one or two mild symptoms like cough and mild fever and then the fever went away and they were cured just by themselves without any kind of vaccine during the first wave, at least when the vaccine were not developed. At the same time, several people having the similar environment, staying in the same house, people from the same family got severely ill by COVID, the same virus.

So immunity depends on two basic factors. First, the genetic predisposition, what kind of genetic material you have inherited plus what is your local condition, what kind of food habits, how much exercise you have done, whether you have any other disease, you are

immunocompromised or not. So the overall idea, the overall behavioural trait that takes care of your genetic predisposition as well as the environmental factors, what is your lifestyle basically determines phenotyping and here we are going to talk about immunophenotyping and we can utilise or immunological species or immunophenotyping basically determines the type of antibodies your body is able to produce when it is attacked by an external pathogen. None of our body had previously encountered COVID type virus. It is a completely new virus.

It is a completely new virus that came up and your body was absolutely not able to recognise it at first. How fast your body, your immune cells can adapt, create some sort of an antibody from scratch new antibody which will be able to prevent COVID virus from destroying your cells is basically your immunophenotyping. Some people had wonderful experience, no effect of COVID, some people were severely ill, some people actually lost their lives. We primarily want to identify lymphoid white blood cells and hematopoietic cells which are constituent of the immune system. It has various applications meaning basically what we are trying to do using flow cytometry is trying to detect the type of WBCs white blood cells, lymphoid, lymphocytes, leukocytes etc.

and different types of white blood cells and hematopoietic cells and try to understand the immune profile of the patient. We have extracted some amount of blood from a patient, we pass it through our flow cytometer and we try to measure the amount of WBCs, RBCs, red blood cells, white blood cells, platelets and hematopoietic cells. What are hematopoietic cells you ask?

Hematopoietic Stem Cell (HSCs)

- HSCs are multipotent stem cells that give rise to all blood cell types, including red blood cells, white blood cells, and platelets.
- HSCs have the unique ability to self-renew, maintaining their population, while also differentiating into more specialized progenitor cells that are committed to specific blood cell lineages.
- HSCs primarily reside in the bone marrow, where they are found in specialized microenvironments called niches, also be found in smaller quantities in other tissues, such as cord blood and peripheral bl

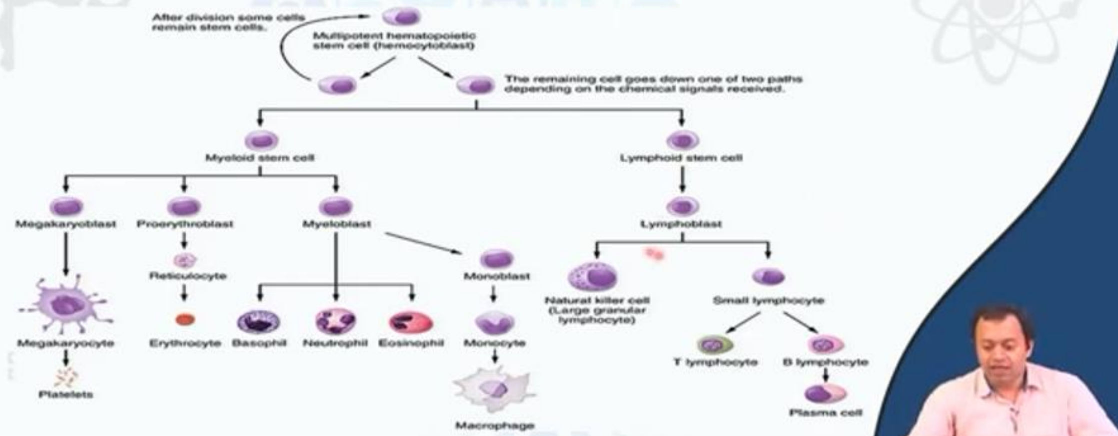
These are stem cells, they are multipotent stem cells. These are the progenitor or the pre disposed cells that differentiate into different types of blood cells. You have red blood

cells, white blood cells, platelets etc.

So they all could originate from HSCs, hematopoietic stem cells. I will call them HSCs because they are much easier to pronounce. HSCs are multipotent stem cells that give rise to all blood cell types including red blood cells, white blood cells and platelets. HSCs have unique ability to self renew, maintain their population while also differentiating into more specialized progenitor cell that are committed to specific blood cell lineage. You should know by this time irrespective of your background that RBC, WBC, platelets all have their different functions.

One carry oxygen, one is for immunity, one is for something completely different plus they could be divided break, break on down into several different varieties like WBC, white blood cells can be broken down into T cells and B cells and what not. You may not need to go too much detail on to it, but there are several different types of blood cells. All of them, all of them could be created from HSCs. They are the stem cells, they are the blood based stem cells that can give rise to all type of blood cells. And HSCs primarily resides in bone marrow where they are found in a specialized micro environment called niches and their characteristics HSCs characteristics which has the capacity to create all type of blood cells to create any type of blood cells reside inside our bone marrow in specialized micro environments.

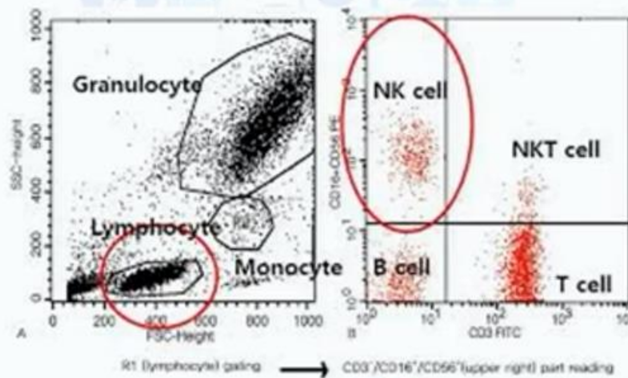
What does that mean? That mean that HSCs will give rise to WBCs, antibodies all that depending on two things. The genetic factor, what kind of gene the organism has inherited and what kind of environment it resides in. So, depending on your food habit, depending on your lifestyle choices, if your bone marrow is pulverized, if your bone marrow is not sufficiently good enough, your HSCs will be compromised. If your HSCs will be compromised, your red blood cells, platelets, white blood cells, all will be compromised resulting in bad immunity. Take care of your body, take care of your health, do not eat junk food, though I eat pizza twice every two days.



So, HSCs can divide itself into several different types of blood cells as I said. Lymphoblasts, WBCs they can be into T cells, plasma cells, B type cells, natural killer cells, these are cytotoxic cells. They can attack T lymphocyte can be a bacteriophage, they can attack a bacteria, these natural killer cells NK or NK T cells, natural killer T cells. What does T stands for? T stands for thymus. These cells go to thymus, the endocrine gland and create their own properties, become more how to say, I want to use the term virulent, but more prone to violence, let us call it.

So, these are the type of cells that provides you with immunity. Natural killer cells, the name is funny. So, these types of cells, they all come from hematopoietic cells and they help you in your building your immunity. There are other type of cells, monoblast, monocyte, macrophages, these are the one they actually go on, these cells physically go on and attack a bacteria that has entered your blood. They will physically go, there are beautiful YouTube videos where you can see this cell like you used to play this video game called Pac-Man, you know, it goes and eats up those things.

Monocytic cells goes up and attack a bacteria and you know, pulverizes its membrane and basically eats it up. So, these are the type of cells and there is erythrocyte, the red blood cells, all of those things, all of those things are created by HSCs.



They have different shapes and these cells have different granularity. WBCs are very granular, white blood cells have different sorts of granularity, WBCs, white blood cells can be further divided into as I said T cells, B cells etcetera, they have different granularity and red blood cells have different shapes. Remember sickle cell anemia, sickle cell anemia, you have had learnt about this genetic disease in childhood where the red blood cells have a different shape than normal.

We can understand, we can understand all of that from our cell cytology, cell flow cytometry, flow cytometry because remember flow cytometry, those fluorescence based detection gives rise to, gives you two separate information. First is the size of the cell and second the granularity of the cell. So, depending on the amount of hits you are getting, this NK stands for natural killer, natural killer T cells, depending on the granularity you can understand how much of your blood contains what type of granular cells, I hope white blood cells, natural killer T cells and the other type of monocytes, bacteria killer cells, antibody based cells and if your erythrocyte red blood cells, if your erythrocyte red blood cells is of the required shape or not, right. If you have the required shape or not that could be passed as normal blood. So, when you have profiled the blood, a full blood profile has been done cell by cell in a cellular level to find out the total amount per centimeter cube of amount of WBCs and RBCs per centimeter cube and then compare it with a reference value, then compare it with a reference value that how much a blood normal persons, human beings blood should look like, you can easily compare it and can understand if everything is right, all right with you or not.

Because the amount, the presence of both foreign particles as well as some substantial deviation from the amount and the characteristics of WBCs and red blood cells per centimeter cube of a human blood could very well be determinant factor to see if the person

is suffering from blood cancer or any other type of blood based genetic disease like hemophilia, sickle cell anemia so on and so forth. You are aware of hemophilia, right, blood does not clot, platelets are for that purpose.

Marker	Present in	Related Disease
CD34	primitive hematopoietic stem and progenitor cells	myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL).
CD38	progenitor cells	multiple myeloma and some forms of CLL.
CD45	expressed on all hematopoietic cells.	various hematological malignancies, such as lymphomas and leukemias
CD90 (Thy-1)	found on HSCs and other stem cell populations.	hematopoietic malignancies, including acute lymphoblastic leukemia and some cases of AML
CD133 (Prominin-1)	CD133: is a marker expressed on hematopoietic stem and progenitor cells.	acute leukemias and certain non-Hodgkin lymphomas

At the same time, there are these several markers, biomarkers. What are these? These are protein molecules, some specific, specific protein molecules which are present in different types of cells. At this present moment, I am mostly focusing on HSCs because HSC is the precursors and the presence or absence or the amount fluctuation of these types of biomarker protein molecules give rise to, can be utilized to detect these several, several types of disease, myeloma, hematological malignancies, hematopoietic malignancies, Hodgkin's lymphomas.

These are different types of blood based diseases, acute myeloid leukemia as you know that is cancer, acute lymphoblastic leukemia, blood cancer is of various different types. So, these are the two major types. So, they are specific and so let us recapitulate. There are certain protein molecules which are present in HSCs. The presence or absence or the amount of these protein molecules could directly be corroborated with the presence and absence of a particular disease.

So, these are the protein molecules present in these are the specific places HSCs and their presence or absence or how much of they are can be considered as a marker, as a point to determine these types of diseases. So, what do you do? You create an antibody, another protein molecule that chemically bind with CD34 only. You create an antibody, another protein molecule that bind with CD38 only, bind using covalent bond, bind using covalent bond, very strong bond, not Van der Waal bond or hydrogen bond or anything. These antibodies, this artificially designed antibodies, monoclonal antibodies, etcetera is then

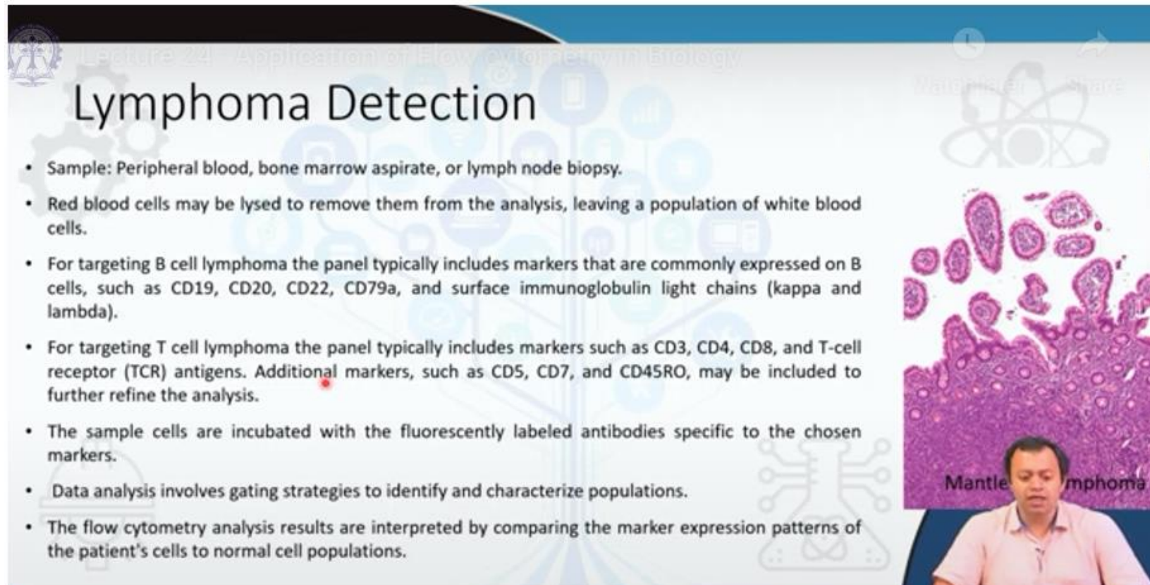
connected with some sort of a fluorophore, is then connected with some sort of a fluorophore which will emit say probably red light when excited by blue light and they have attached themselves with CD34 presence in HSCs, not CD38, CD45, CD90, CD13, only with CD34.

Now you are doing flow cytometry. The amount of intensity of red light you will get when each blood cell one at a time, each RBC, WBC, platelets, etcetera one at a time flows through and you are exciting a laser light from the sideways and trying to get it, trying to get some kind of a emission, red emission. The amount of redness will be directly proportional to the amount of antibodies containing the red fluorophore being attached with CD34. You then compare it with a normal blood, how much the intensity of a normal blood should be which is been attached with the same way. Any deviation you can thereby comment on the presence or absence of these kinds of diseases such as blood cancer. You get the concept, you get the concept now applied to all of these, now apply the same thing, you prepare another type of antibody for CD38, another type of antibody for CD133.

What are these? These are protein macromolecules. These are markers like we have identified presence of this marker, presence of smoke is a marker that there is fire, presence of this in this particular cell is a marker for this particular disease. You detect it and it will go through. Remember if the antibodies matching then there is a covalent bond. You made a particular antibody another protein molecule, the counterpart of this protein molecule.

This will attach with CD34 in covalent bond. Any other thing will not attached or very weakly attached. If you then take an entire blood sample, mix it, I do not think mix is the proper term, centrifuge it or mix it using antibody of CD34 only. If CD34 is present it will attach, if CD34 is not present it will loosely attach or not attach at all. You then filter or wash, again those are not biological terms but I am giving an example, vigorously wash the entire thing. If it is loosely attached it will be filtered out but if it is covalent bond your filtration process will not work.

It has been attached to it. So, it is a compound, it is a molecule. So, it will not pass through those pores that you have decided and it will not work. Then subject it to flow cytometry and then see how much red dots you are getting. Compare it with a normal blood which you have previously done the same process you know somebody have normal blood, reference blood that is already present and then compare it and that is it, you understand the disease.



Similarly, we go for lymphoma detection, blood cancer type, peripheral blood, bone marrow, aspite or lymph nodes biopsy for targeting B cell lymphoma.

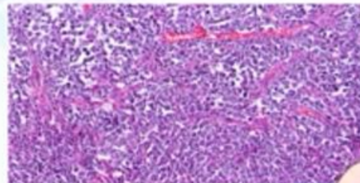
The panel typically includes markers that are commonly expressed in B cells, bone marrow cells such as CD19, CD20, T cells. We used to ask what T in T cell stands for, it is basically thymus. So the presence of the blood cell WBCs is in bone marrow, then it comes out and through the blood go to the endocrine gland of thymus, from that it acquires weapons, it become weaponized and then it fights diseases. It is a very very simple term, blood is something people make their carrier out of it. So, what I just said to you in one sentence is the most diluted and simplest form.

Biologist will say that there are so many much more to it, but overall that is T stands for thymus, we have first identified them coming out of thymus, then we use the term T cells. So, the sample cells are incubated with fluorescence level antibodies specific to the chosen marker, CD19, CD20 molecular proteins, protein macromolecules. Data analysis involves getting strategies to identify and characterize population. The flow cytometry analysis results are interpreted by comparing the marker expression pattern of the patient cell to normal cell populations. You need to have a reference which you have done previously, calibration, normal thing we have discussed in last class, compare it with and that is it, that is your answer.

B-Cell Markers and Associated Cancers in Flow Cytometry Analysis

- B-Cell Markers and Associated Cancers:

1. CD19 & CD22:
   1. Diffuse Large B-cell Lymphoma (DLBCL)
   2. Burkitt Lymphoma
   3. Chronic Lymphocytic Leukemia (CLL)
   4. Mantle Cell Lymphoma (MCL)
   5. Follicular Lymphoma
   6. Waldenström Macroglobulinemia
2. CD20:
   1. Diffuse Large B-cell Lymphoma (DLBCL)
   2. Burkitt Lymphoma
   3. Follicular Lymphoma
   4. Mantle Cell Lymphoma (MCL)
   5. Waldenström Macroglobulinemia
3. CD79a:
   1. Diffuse Large B-cell Lymphoma (DLBCL)
   2. Burkitt Lymphoma
   3. Mantle Cell Lymphoma (MCL)
4. CD5:
   1. Chronic Lymphocytic Leukemia (CLL)
   2. Mantle Cell Lymphoma (MCL)



Diffuse Large B-cell Lymphoma (DLBCL)

Similarly there are several B cell markers and associated cancers like different, there are various various types of blood cancers. So, CD19 and CD22 are connected with these types of cancer, CD20 is connected with these types of cancer. So, you identify this, the presence a huge amount of this in your blood, then you can be certain that one of the five is affecting and thereby you need to do some further test to be absolutely absolutely sure. So, these are the markers associated with various types of blood cells. So, similarly T cells markers are also associated with different types of cancers, mantle cell lymphoma, large cell lymphoma, various other blood-based diseases etcetera.

Lecture 24 : Application of Flow cytometry in Biology

Acquired Immuno-deficiency Syndrome(AIDS)

HIV
Binding
Fusion and entry
RNA
Reverse transcription
cDNA
dsDNA
proviral DNA
Integration
Transcription
Protein synthesis, processing and assembly
mRNA
RNA
Budding
Maturation
Weeks
Years

So, you can read it at your own leisure. And finally, we come to HIV, Acrylidimino Deficiency Syndrome. What it does as you probably know, but let me repeat it, these virus attacks your immune system, it attacks basically the WBCs. It destroys the immune system completely, destroyed white blood cells completely, destroyed the T cells etcetera and this

lymphocyte WBCs lymphocyte the virus comes in attached with the one cell part of the cell membrane of the WBCs, injects its own RNA, this RNA goes and hijack the nucleus, the reproduction mechanism of the white blood cells and this then instead of producing or dividing into more WBCs produce more and more AIDS virus. So, this is the graph, this is the primary infection as you see, the blue is the lymphocyte count that amount of WBCs, amount of white blood cells per millimeter cube and this is the viral RNA copies per millimeter of plasma. As you see that at the beginning the amount of virus, amount of AIDS virus is very low and the amount of WBCs is very high, but as it progresses from weeks to years, weeks to years you see there is of course, a spike the WBC spike up to attack the virus, but well here blue is WBCs, there is a spike, but overall there is a complete breakdown of white blood cells in your body and a huge huge huge huge increase in the amount of virus.

So, this is the point where your body is left with absolutely no immunity, think of it as a country which has no police, no military, nothing left, what will happen? It will be invaded and taken over by outsiders. So, if your body's immunity is completely gone all WBCs have simply ceased to exist, any normal virus or bacteria, common cold is enough to kill you. So, this is precisely what happens, the number of virions insert itself and starts replicating. So, the red curve goes up that is amount of virus HIV RNA copies and the amount of WBCs white blood cells reduce, lymphocyte counts reduces finally to 0, finally to 0 by 9 to 10 years since it has been affected. Hopefully, we now have got medicine, we now have also vaccines to the best of my knowledge for AIDS, but this is the overall procedure very very scary.

Lecture 24 : Application of Flow cytometry in Biology

Watch later Share

Antibody panel design for HIV

Marker	Remarks
CD3	It is a marker for T cells, including CD4+ and CD8+ T cells. It helps in identifying the total T-cell population and determining the CD4/CD8 ratio, which is often altered in HIV infection.
CD4	It is a critical marker for identifying and quantifying CD4+ T cells. HIV primarily targets and infects CD4+ T cells, leading to their depletion. Monitoring CD4+ T cell count is essential for HIV disease progression and determining the need for antiretroviral therapy.
CD8	It is a marker for cytotoxic T cells (CD8+ T cells). Changes in CD8+ T cell subsets and their activation status can indicate immune responses and disease progression in HIV infection.
CD45	It is a pan-leukocyte marker that helps in distinguishing lymphocytes from other cell types. It is useful for gating on lymphocyte populations during flow cytometry analysis.
CD38	Elevated levels of CD38 expression on CD4+ and CD8+ T cells are associated with HIV disease progression and immune activation.
HLA-DR	It is an activation marker expressed on antigen-presenting cells and activated T cells. Increased HLA-DR expr[illegible] observed during HIV infection and can indicate immune activation.
CD69	It is an early activation marker expressed on activated T cells and natural killer (NK) cells. It can provide[illegible] early immune response to HIV infection.

We can now try to understand or try to detect the overall amount of WBCs and we have now gone that far that we are even able to detect the amount of WBCs compromised by AIDS virus, human immunodeficiency virus. We do a panel, all these markers are there

and each marker have been identified as one symptom or one expression of HIV, human immunodeficiency virus and by doing a full blood panel, you have heard of blood panel probably. So, basically we try to see all of these specific proteins available or not and based on the presence or absence of these proteins, we can come to because at the end of the day everything is protein, virus is also protein, your blood WBCs also is protein or also contain protein. The amount of which protein is higher or lower can be used to determine what kind of disease you are suffering from.

Lecture 24 : Application of Flow cytometry in Biology

Other Viral Infection & Respective Markers

Disease	Markers
Influenza	CD3, CD4, CD8, CD19, CD56, CD25, CD69 & HLA-DR ON T & B Cell
Cytomegalovirus (CMV)	CD3, CD4, CD8, CD56, HLA-DR, Antibodies specific to CMV antigens, such as immediate-early antigen (IEA) or pp65
Epstein-Barr Virus (EBV)	CD19, CD20, CD3, CD8, Antibodies specific to viral antigens, such as Epstein-Barr nuclear antigen (EBNA) or viral capsid antigen (VCA)
Hepatitis C Virus (HCV)	CD3, CD4, CD8, CD69, CD25, CD56, HCV Core and NS3

Other types of infections are also possible to be looked into, these are the markers influenza, cytomegalovirus, Epstein-Barr virus you probably know and hepatitis C virus.

Hepatitis is also one of the killer viruses. So, you identify these small proteins in blood, you identify the presence of this virus. So, these proteins will be connected with a specific fluorescent marker. If it is attaching an antibody with a fluorescence marker attaching with CD3, CD4, CD8 etcetera and depending on that you understand the presence or absence of hepatitis or Epstein-Barr or influenza. Similarly, cell cycle analysis could also be done.

You have read cell cycle, I request you to look into it once more. If you find it too boring, do not go into that, I found it bit boring myself. So, cell cycle has different phases G naught, G1, S, G2 and M. Do not worry what they are for the time being, I will just quickly describe G1 phase is when a cell is growing to get divided. Cell grows and then divide mitosis and meiosis, you have read about mitosis and meiosis part of cell division. Forget about meiosis for the time being, mitosis is the one of what we are talking about.

Cell first grows that is the G1 phase, it is still one single cell. S phase is when DNA is getting copied, DNA is getting replicated inside the nucleus. G2 is when the cell have started to divide and M is actually the cell division phases. So, you want to look into what sort of cells present in a body fluid are at what particular phase for division. Usually you have more cells about to divide, less cell usually divided, provided it is not cancer.

Cancer cells proliferates or divides rapidly. So, you can try to understand from this the presence or absence of cancer, if this graph is very high as compared to the G1 phase, if G2 and M phase are very high, may be perhaps if you are from medical background, correct me if I am wrong. Because look I am in uncharted territory, I by no means am a medical personnel, but I have just read it, but correct me if any of this is wrong, if this graph become very high that it is rapidly dividing, then you need to investigate further. I cannot immediately claim cancer, but you need to investigate further.

Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI)

- The fluorescent ubiquitination-based cell cycle indicator (FUCCI) system consists of two fluorescent proteins fused to cell cycle regulatory proteins. A green fluorescent protein called mAG is fused to a fragment of hGem (1/110), while an orange fluorescent protein known as mKO2 is fused to a fragment of hCdt1 (30/120).
- These fusion proteins contain nuclear localization signals and ubiquitination sites for degradation, but they are not functional proteins themselves. They are designed to produce fluorescence during specific phases of the cell cycle and undergo degradation during other phases.
- The green fluorescent protein (mAG) is produced and fluoresces during the S, G2, or M phase of the cell cycle. It is subsequently degraded during the G0 or G1 phase.
- Conversely, the orange fluorescent protein (mKO2) is produced and fluoresces during the G0 or G1 phase and undergoes degradation during the S, G2, or M phase.

So, yeah various different type of fluorescence materials is available, the green fluorescence protein is produced and they attach to different stages of the cell cycle. G naught is when the division has occurred, when it is finished, when it is like normal it is lying down.

Conversely orange fluorescence protein is for G1 phase or undergoes degradation. So, using different types of proteins, different types of fluorescence you can understand if the cell is at what particular stage it is about to divide, it has finished division or it is DNA replications is going on and the rate of this can be utilized for detection of several different types of diseases with respect to time, how rapidly they are proliferating, how rapidly they are breaking down.

Lecture 24 : Application of Flow cytometry in Biology

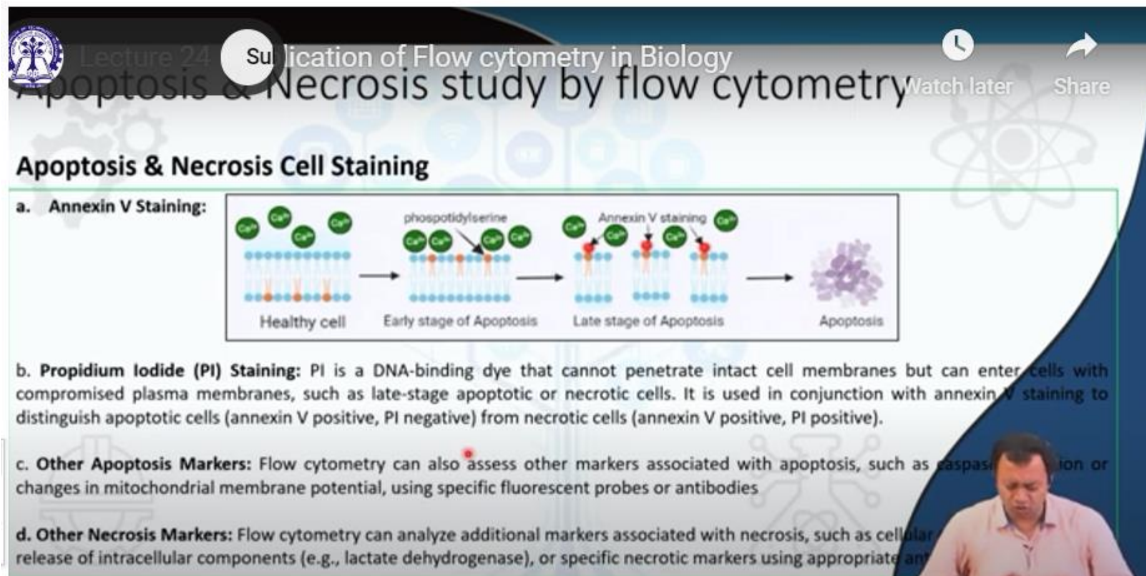
Watch later Share

Apoptosis & Necrosis

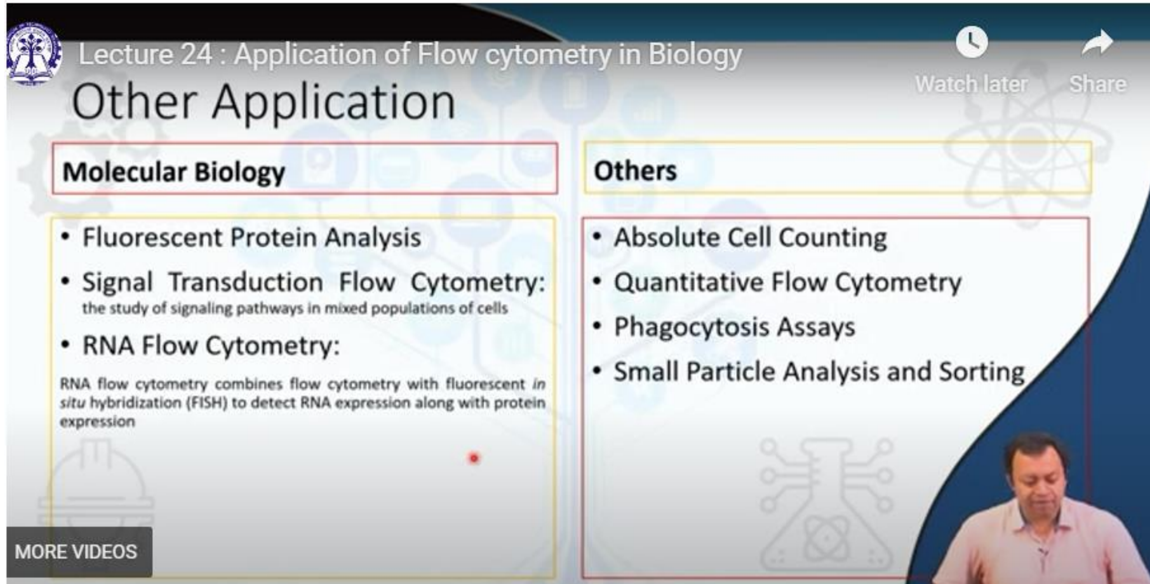
Nucleus
Normal cell
Organelles
Blebs
Blebs
Small blebs form; the structure of the nucleus changes.
Small blebs form.
The blebs fuse and become larger; no organelles are located in the blebs.
The nucleus begins to break apart, and the DNA breaks into small pieces. The organelles are also located in the blebs.
The cell membrane ruptures and releases the cell's content; the organelles are not functional.
The cell breaks into several apoptotic bodies; the organelles are still functional.
Necrosis
Apoptosis

Apoptosis and necrosis can also be discussed, apoptosis means that it is you know breaking from inside. I think necrosis is murder, apoptosis is suicide, where cell grows up some kind of mitochondria or something has broken down and cell simply implodes, whereas necrosis is when cells have gone through an injury, a trauma, a murder and something has the outer cell membrane has punctured and inner material goes out. So, it final count is how many number of cells are presents, what are the different size structure plus what are the individual characteristics.

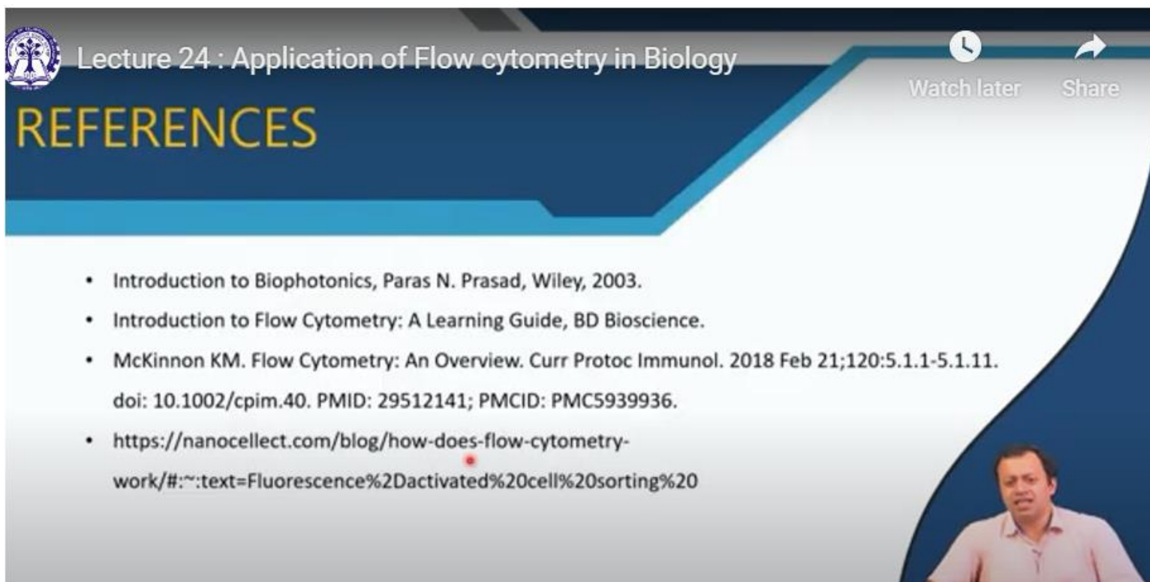
So, based on that the size, the granularity you can also understand if your cells have been injured, traumatized or if it has internally imploded something has destroyed it from inside, apoptosis inside, necrosis outside. Suicide you do you kill yourself, necrosis you are murdered. These analogies help you understand especially if you are not from the background, this is simplification and whenever you try to simplify truth some amount of inaccuracy comes in, you have to tell me whether I should continue with or not.



Apoptosis and necrosis cell staining they can also be used other type of fluorescent material thereby try to understand which is going on in what particular, how much of your cell is injured, how many of your cells are imploding from inside.



There is other type of applications are as well we have RNA flow cytometry, fluorescence protein analysis, small particle analysis and sorting if there is anything other foreign bodies present.



So, that is basically the example that I wanted to give you. These are my references kindly go through them

Lecture 24 : Application of Flow cytometry in Biology

Watch later

Share

CONCLUSION

- Flow cytometry is a versatile technique used to analyze various aspects of cellular biology, including cell cycle dynamics, apoptosis, and necrosis.
- Cell cycle analysis by flow cytometry involves staining cells with DNA-binding dyes and measuring their DNA content to determine the distribution of cells in different phases of the cell cycle.
- Apoptosis can be studied using flow cytometry by assessing markers such as annexin V and propidium iodide, enabling the identification of cells undergoing programmed cell death.
- Necrosis, a form of cell death characterized by cell membrane disruption, can be detected using flow cytometry by analyzing markers like propidium iodide.
- Flow cytometry provides quantitative and qualitative data, allowing researchers to investigate cellular processes, monitor disease progression, and assess the effects of various treatments or experimental conditions on cell fate.

MORE VIDEOS

and these are the final conclusions of my lecture for today. Thank you very much.