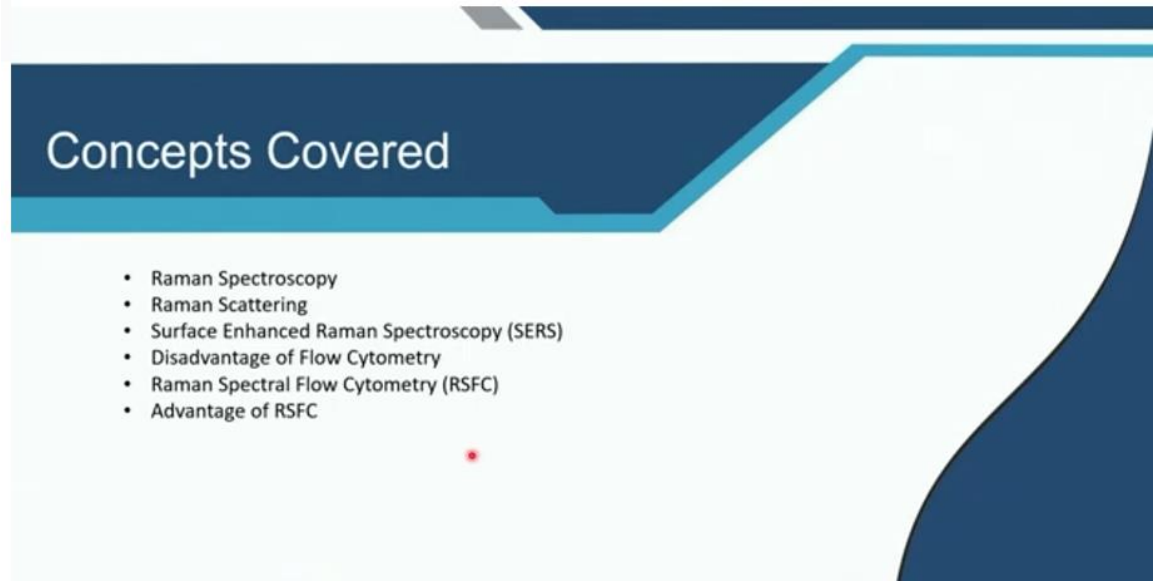
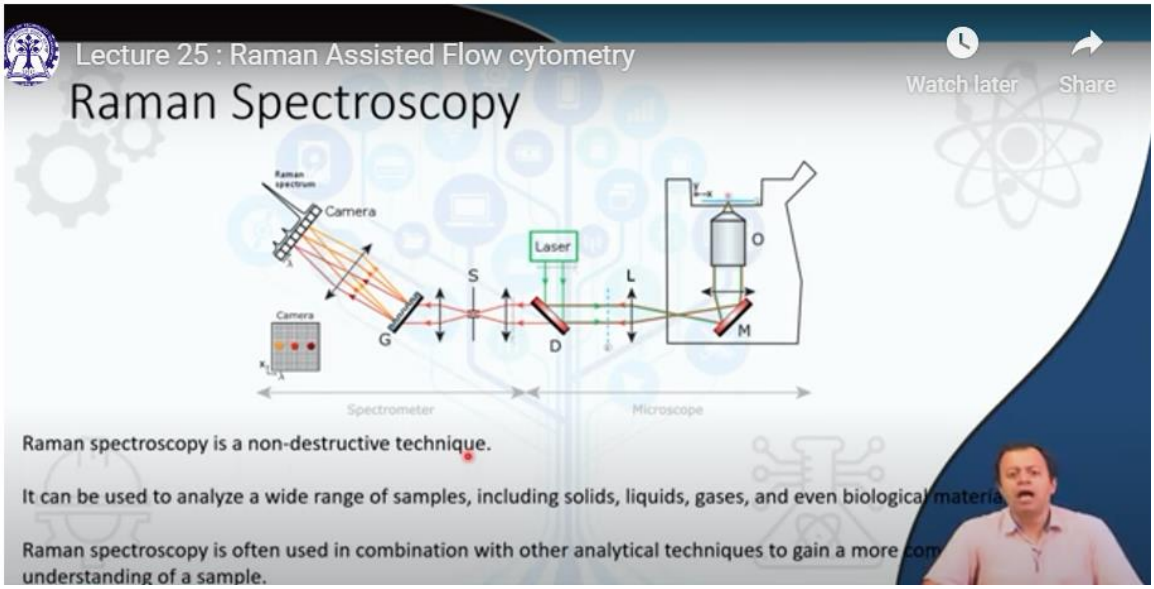
Nanobiophotonics: Touching Our Daily Life
Professor. Basudev Lahiri
Department of Electronics and Electrical Communication Engineering
Indian Institute of Technology, Kharagpur
Lecture No. 25
Raman Assisted Flow cytometry

Welcome back we are at the last leg of our module bio photonics for disease diagnosis and today we will be finishing the flow cytometry. Thus far we have mostly discussed about flow cytometry where fluorescence is utilized FACS fluorescence assisted cell sorting. Today a new thing we will discuss which is Raman assisted flow cytometry the process the property the mechanism, everything remains as it is. There is absolutely no problem, except instead of fluorescence, what you are doing is you are doing a Raman spectroscopy.



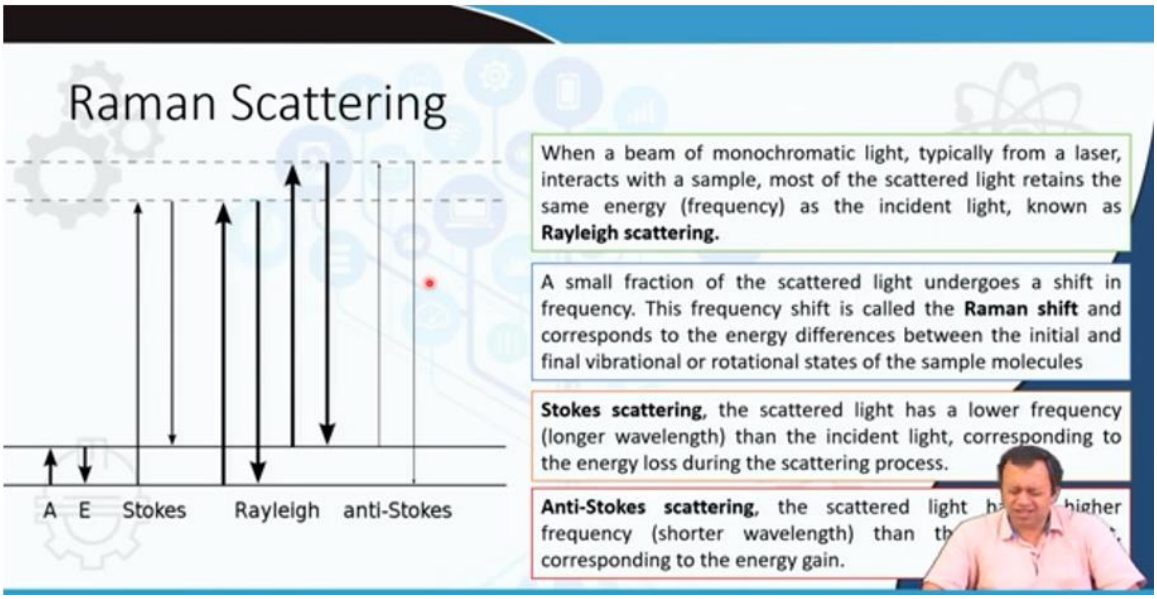
So, these are the concepts that I am going to discuss.



So, let us start you all know about Raman spectroscopy I have discussed Raman spectroscopy is a non destructive technique we are trying to look at the scattering of the photon from the molecule incoming photon gets scattered by a molecule. The output photon the energy of the output photon is then measured usually the energy of the output photon is either less than the input photon or more than the input photon depends on how much energy the photon has lost either given to the molecule or in certain cases it has extracted from the molecule the molecule was not in the ground state when the input photon has hit it because of the interaction of the photon with the molecule which was not at a ground state. It has now gone to the ground state and the difference of energy is then imparted the difference of energy is imparted into the output photon. So, the energy the frequency of the output photon is more than that of the input photon we have discussed this. it actually helps you to understand the various energy levels available in the molecular states what are the different energy levels if the photon comes it will go up that much amount of energy the photon will lose if it is already present it will come down so based on that you calculate what are the different energy quantum states different energy levels the molecule possess It is tremendously helpful when we are trying to detect the allotropies.

So, carbon double bond, single bond, triple bond or you want to detect the difference between say diamond versus graphite or graphene these days, we can we can very well determine the different energy levels and thereby differentiate it. so it could be analyzed for a wide wide range of samples including solid liquid gases and even biological material most importantly water is raman inactive i told you ah where water absorbs huge amount of infrared light so if you are trying to do infrared spectroscopy for a biological material which contains substantial amount of water it gets saturated water is not Raman active i e its molecule do not scattered in elastically to photon i e whatever photon is scattering H_2O water molecule more or less same amount is given in the output. So, Raman spectroscopy

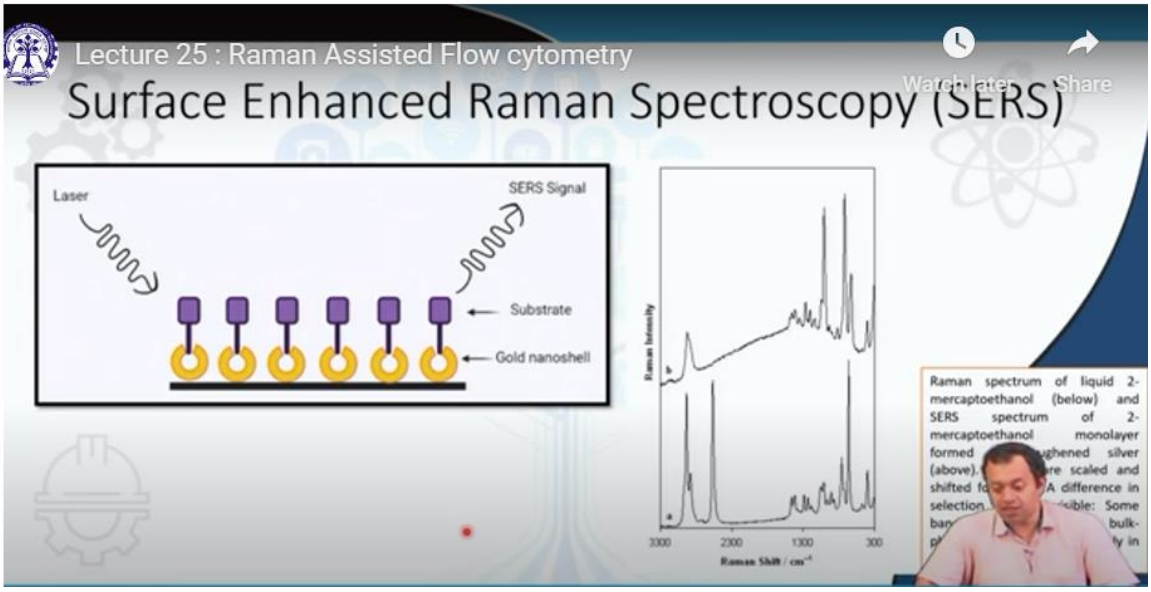
often used in combination with other analytical technique to gain more comprehensive understanding of a sample meaning Raman spectroscopy is usually very very weak. very few molecules in a particular Raman active molecule Raman active compound will undergo this scattering these scattering phenomena right. You know this entire process you excite the signal it then you know pass through the grating we have discussed this in previous class biosensing and bioimaging.
So, just go through it I am not going to describe it in full detail we also add microscope inside to focus on a particular area and the light that is scattered out of that particular area is then passed through the spectrometer to find out what are the specific wavelengths, what are the Raman shifts as compared to the input light. So, we have we have discussed this part.



So, just for the sake of it, this is what we call as stokes and anti-stokes. Rayleigh's thing is elastic as in whatever goes up comes down, whatever goes up comes down. but there is a problem with anti-stokes and stocks whatever goes up it does not come down to the same place or it goes up somewhere else and comes back to the ground state. This was not ground state when the photon has hit the molecule and it has come back to the ground state in this particular case the molecule was at ground state. but the scattering of the photon allowed it to come back to a non-ground state. When a beam of monochromatic light typically from a laser interacts with sample, most of the scattered light retains the same energy, rallies scattering. A small fraction of the scattered light undergoes a shift in frequency. The frequency shift is called Raman shift and correspond to the energy difference.

So, you basically calculate these values, you basically calculate these values by doing the same thing over and over stroke scattering the scattered light has a lower frequency than

the incident light correspond to energy loss anti stroke scattering the scattered light has a higher frequency than the incident light correspond to energy gain this amount of energy has been gained this amount of energy has been lost you calculate you measure both are equally possible for the same molecule based on this usually stoke is generally more more prominent than anti stokes. This is what the boldness of the lines have given what is more probable and what is more intense, but your computer is sophisticated enough to make these measurements make these calculations and thereby figure out all these energy states. So, molecule was here then here then here. So, what are the band gap, what are the energy states, what are the difference between homo and lumo all of those things can actually be calculated with Raman scattering, but nevertheless the process is weak, nevertheless the process is weak. So, what we do? We do surface enhanced Raman spectroscopy, So, what surface enhanced Riemann spectroscopy does is produce some kind of a metallic thin film or metallic nanostructures on top of a glass slide.



On top of that nanostructures these kinds of nanostructure perhaps some of you know what they are they are splittering resonators I work on splittering resonators. produce their own plasmonic effect i told you metals have this electron cloud moving through them and they try to screen any incoming light that falls into it. So, metals have this electron cloud moving inside it the electrons in metals are very very loosely bound to their parent nucleus the electron of a metal atom belongs to the entire bulk not just one particular atom it is very highly energized. So, it contains all those electrons from all those atoms form some kind of an electron cloud and move around the entire bulk of a material. They move around with a particular frequency called the plasma frequency.

The plasma frequency is usually for most metals in the ultraviolet range. So, any times any other electromagnetic wave comes, these plasmas frequency-based electron cloud tries to screen it, tries to prevent it from entering through. We have discussed this. so, metals

are shiny metals are shiny because as soon as any light lower lower than the energy of ultraviolet comes and hits it it gets scattered back it is not simply reflected it is scattered back because all the electron cloud there tries to scatter tries to screen it because their frequency is higher than the frequency of the incoming light visible light has lower frequency than ultraviolet. So, any visible light that falls into ultraviolet electron clouds is scattered.

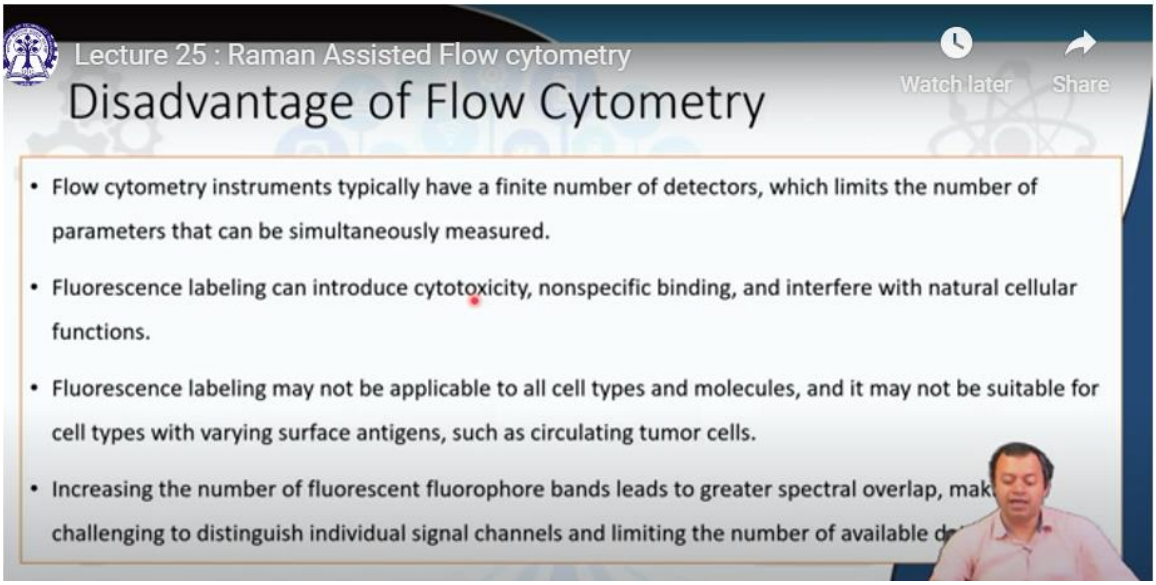
Another word of scattering is shiny, gold is shiny, silver is shiny. So, it is getting scattered. So, that is what exactly happens. ah the question comes what if your incoming light has a higher frequency than ultraviolet will it penetrate through metals the answer is yes it will penetrate through metals it will penetrate through metal x-ray penetrates through metal you might say that you have done an x-ray and some kind of metal has been detected it was blocked It was simply blocked because the x-ray used for medical applications have a very very low intensity. X-rays are very very highly energetic.

It fries biological tissue its givens in proper surface. It will penetrate through metal, it will penetrate through your body. So, we give very very low intensity X-rays and that somewhat passes through our body thinking that it will not cause enough damage, but it still does and metals block it, but otherwise X-rays or ultraviolet simply pass through. So, what are SARS? SARS have these metallic nanostructures. on top of which you put your samples on top of which you put your samples and then illuminate illuminate that sample on top of your metallic nanostructures using laser that laser has some light but that light is getting screened that light is getting scattered by the presence of these plasmonic nanostructures ah plasma because the electron do not listen to its parent atom electron is semi independent from the nucleus basically plasma hence metallic nanoparticles or metal based structures are also called metals are basically plasmas metallic behavior is plasmonic behavior so this plasma assisted see this plasmon assisted or surface enhanced thing actually enhances, actually zaps the molecules Raman response.

So, surface enhanced Raman spectroscopy, meaning previously you have your molecule here. Light was coming, some of it got scattered properly, some of it got scattered improperly and you are getting the output now you are using an amplifier whenever light is coming into the substrate which contains gold film or gold nanoparticle the gold nanoparticle is producing its own field the plasmonic field this field incoming and the inherent field then combine together to scattered the to interact with the molecules of the substrate that is present yeah This incoming light is creating some sort of a field, some sort of a plasmonic effect electron cloud waste because of the presence of metallic nanostructures onto the substrate. so both combine together both of these the energy from the laser as well as energy from the metallic substrate metallic nano structures combine together if you are clever enough you can match these two you can match these two very

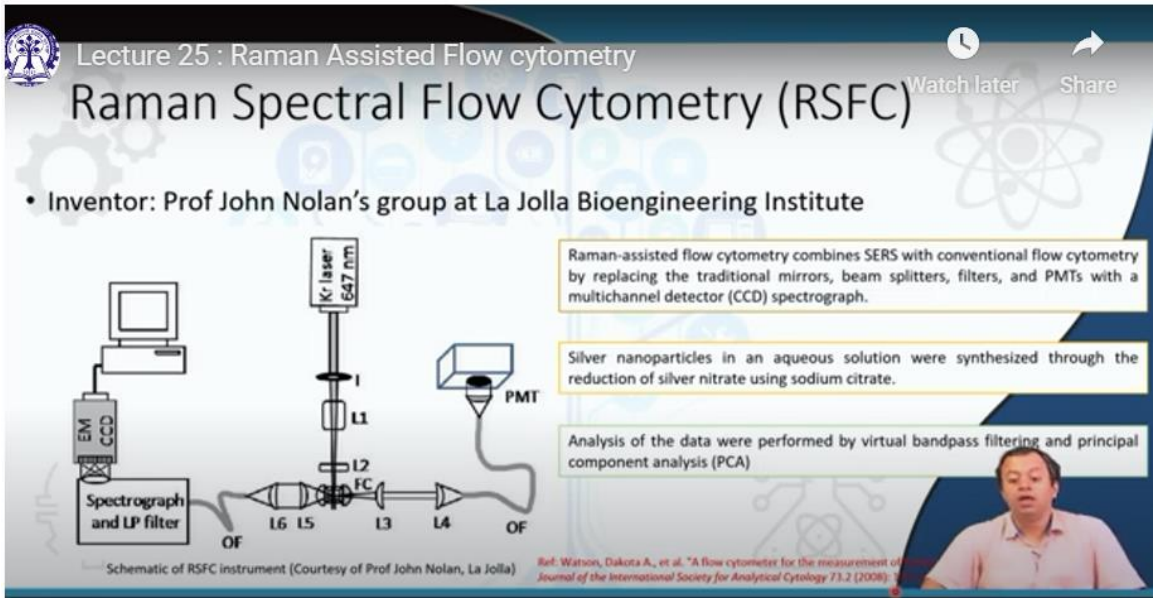
close to one another they will match and thereby they can amplify in fact ten to the power four ten to the power eight times the incoming light could be amplified so thereby they are zapping they are interacting they are exciting all the molecules all the type of different samples it should be sample not substrate this is substrate this is sample the sample is getting more. zapped, more number of molecules have had been scattered and you are getting a very high surface enhanced Raman spectroscopy is what we get in. Basically, we amplify the Raman signal.

Raman is inherently weak, but by putting plasmon nanostructures we can amplify it. So, based on all of that



Do you not think that we can utilize it on flow cytometry? Because see flow cytometry has several disadvantages, flow cytometry instrument typically has finite number of detectors which limits the parameters, fluorescence leveling can include cytotoxicity, you have labeled antibody with a fluoro chrome that has connected with protein of a cell, but the fluorochrome might itself is killing the cell it is cytotoxic. Cyto is cell toxic you know non-specific binding and interfere with natural cellular function because of the presence of this. fluorochrome into the cellular structure the cell is behaving differently labeling kind of creates some sort of artifact. Fluorescence labeling may not be applicable to all cell types and molecules and may not be suitable for cell types with varying surface intagents such as circulating tumor cells do not worry what circulating tumor cells are these are the new type of cancer related information that is coming up that the tumor cells actually circulate through blood any cancerous tumor cells actually circulate through blood, but they have different types of antigens multiple types of antigens they can be utilized for detection meaning you them, fluorescence leveling them is difficult.

Increasing the number of fluorescent fluorophore bands leads to greater spectral overlap making it challenging to distinguish between individual cell signals and limiting the number of available data channels.



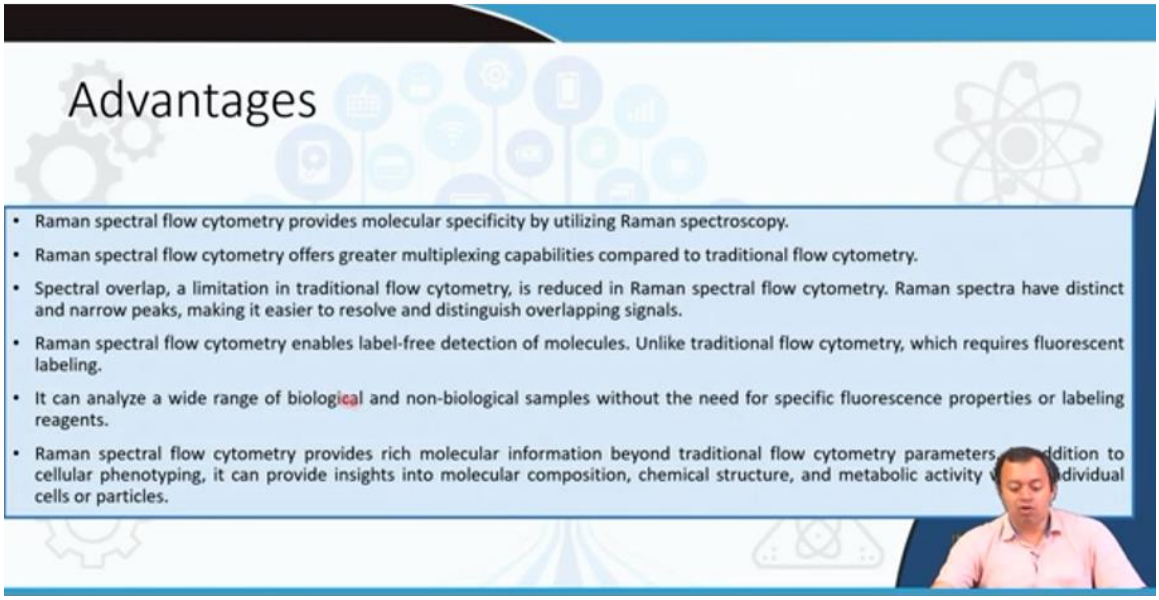
So, voila the result is by Professor John Nolans group at Loyola Bioengineering Institute worked on Raman spectral flow cytometry. I think Professor Kesuke Goda of Tokyo University took this to a completely new level, but the process is exactly the same. You have your flow cytometer, you are zapping it with a laser only thing is the output of the laser is going into a Raman spectrometer and you are trying to understand the process the raman signal of such samples that are getting passed through think about it how how how clever it is how clever it is you can make flow cytometer with ah metallic nano structures etcetera into the same place you can coat it in the wall or put it into the liquid etcetera all of that thing but previously the laser light was the fluorescence light excitation versus emission was different here there is no excitation no emission no fluorescence except auto fluorescence you are sending an input light and you are collecting the scattered light out of it stokes versus anti stokes And the scattered light is then passes through normal Raman spectrometer and trying to see the Raman characteristics. Each of these molecules, the one which I showed you in previous classes, WBCs, white blood cells, red blood cells, etcetera, all of them have their individual Raman signature.

DNA have Raman signature. dna have individual raman signature you can simply without labeling without changing or without adding any antigen antibody fluoro chrome you can simply pass laser light to detect the raman signal coming from each individual cells and this is a very very hot topic this is a very very hot topic these days please please go through professor john nolan's group as well as professor goda kesuke goda university of tokyo the type of work these gentlemen are doing in converting flow cytometry taking it to a

completely different level is fantastic i am very very impressed i am very very impressed by the type of work they do raman assisted flow cytometry combine sars with conventional flow cytometry by replacing traditional mirrors beam splitters and pmt photomultiplier tubes with multi-channel This is Raman spectrometer. Silver nanoparticle in an aqueous solution were synthesized through the reduction of silver nitrate using sodium citrate. Silver nanoparticles produce those plasmonic nano plasmonic effects. They combine with the laser effect zap the molecules that is passing through.

Analysis of data were performed by virtual band pass filtering. and principal component analysis, principal component reduces individual spectra, individual spectra such as these into individual points the dimensionality is reduced. So, you basically get some sort of a dot plot and those hits determine a particular type of Raman signal as compared to something normal or otherwise. that identifies your molecule that identifies the type of your molecule the granularity the size the chemistry most importantly this probably will not give you the sized information but granularity as well as most importantly the chemical information present would very much be determined by Raman assisted spectral flow cytometry. So, this is a brilliant, brilliant piece of work, I cannot praise it enough.

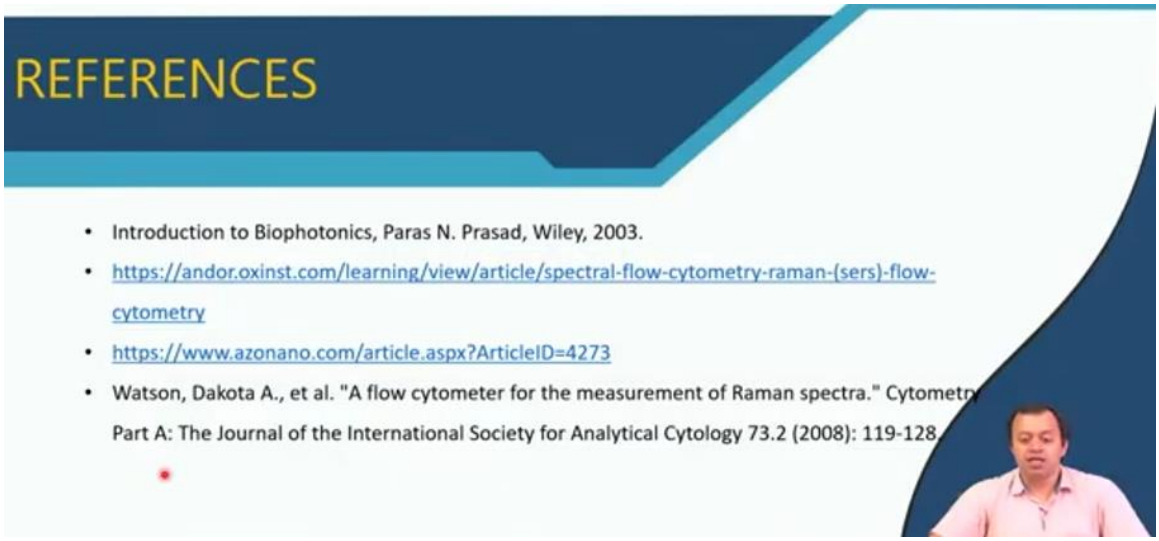
So, please go through it, please see some of the videos associated with it. These are very hot topic, those of you who are interested to do research on this, please, please look into this Raman spectral flow cytometry along with infrared flow cytometry is going to be the future of flow cytometry. right.



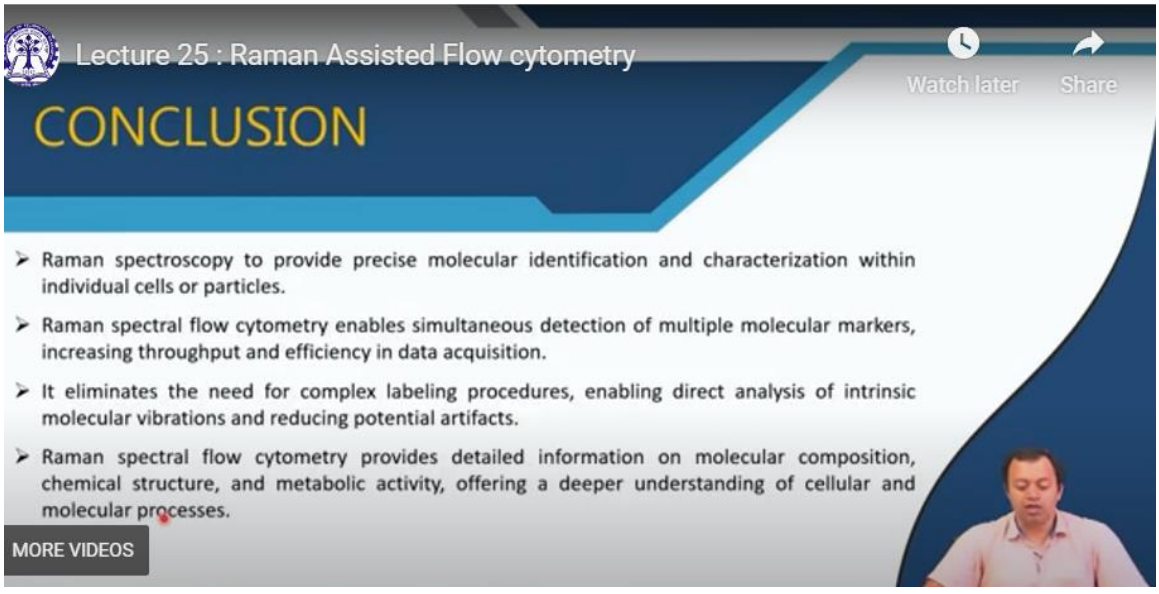
There is several advantage Raman spectral flow cytometry produce molecular specificity it over offers greater multiplexicity you basically do not have to label it can analyze wide amount of biological or non-biological sample without the need of specific fluorescence

properties. Unlike traditional flow cytometry labeling is not required it provides rich molecular chemical information beyond what traditional flow cytometry can offer.

It can additional to cellular phenotyping it can provide insight into molecular composition, chemical structure, metabolic activity with individual cells or particles.



So, these are my references with this I conclude module 5 that is flow cytometry and



these are my conclusions which you can read at your own leisure. And with this I conclude this chapter. Thank you very much.