Biophotonics
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Lecture 46
Tools of Micromanipulation

Do you know what this is? This is a tweezer. You pluck things with this. You can also call it scalpel perhaps. You must have seen people use it in the beauty or cosmetic industry. They pluck hair out of it. People who are jewelers use it to pluck small precious stones, diamonds, things like this. We in the business of nanotechnology pick our ICs, our silicon chips, some kind of silicon-based wafer out of this. You can without using your hand something that is very small or very fragile you can pick it up with this.

So, I have a question for you. What is the smallest item you can pick using these kinds of tweezers. You can obviously pick a strand of hair, just a piece of hair. You can just pluck it up. A very small piece of diamond, you can pluck it up. ICs, how bigger ICs, few millimeters by few millimeters, you can pick it up. But how small dimension-wise, size wise will be the material, will be the item, will be the sample that you can pick up with something like this? Can you pick up a single cell, a single bacterium? Can you tweeze up virus? What about molecules? Few molecules, can you pick them up using this, an atom?

Couple of questions here, A, can you do this, obviously the first question. And the second question is, do you need to do this? Do you need to pick up single atoms or single molecules or single cells or single virus? To answer the second question first, yes, you need to do that because, A, from a fundamental point of view, you need to understand the forces that governs these. So, you need to pick them up, examine it and then bring it back. You need to manipulate it. You need to modify it. You need to understand it. So, for that, you require them to be picked up, analyzed, put it under in front of your eyes or in a microscope for that regard.

And secondly, can you pick up cells or viruses or molecules or atoms using tweezers? Well, the answer to that is also, yes. Only, you will not require a or you will not need or you cannot use a mechanical tweezers such as this, but you will use light, you will use an optical tweezer, you will use a laser tweezer.

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Welcome to today's module. Today, I am going to discuss about optical tweezing and why and how we can utilize light as a tool for micromanipulation. It could be also nano-manipulation or pico-manipulation, where we use light or photons to manipulate that direction, the speed, the position of biological materials. Let us get started.

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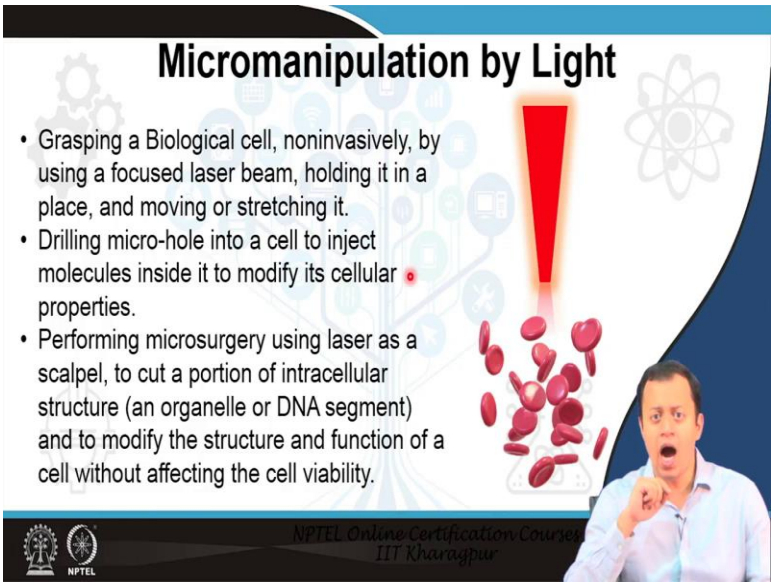

So, how or why we require micromanipulation by light? Well, first and foremost, by grasping a biological cell noninvasively. The tweezer that I showed you, that is invasive, that is there is a mechanical contact something is plucking you up, but using laser you can pick up a biological

cell, holding it to a place and you can move it or stretch it or using laser light you can drill a micro or nano sized hole into a cell and inject certain external material, maybe a nucleic acid, may be a particular pathogen, may be a specific chemical inside to modify the cellular structure and perform micro or nanosurgery using laser as a scalpel to cut a portion of intracellular structure and to overall modify it.

So, all of you know about microsurgery or surgery where the, some part of a patient's body is cut open using a scalpel and something is removed, say for example, a tumor, destroyed removed. What about surgery at a cellular scale level, meaning instead of cutting open an organ, a tissue, a flesh, we just perform surgery on a single cell. A cell that is going bad or a cell that has been infected, bad DNA or bad RNA it is producing because it got infected, we are simply taking a part say the nucleus or the mitochondria or some sort of a different organelle instead of trying to destroy the entire tissue or the entire organ doing some kind of an injury.

Can you do that with the help of light? Well, do not take my word for it. I have a beautiful video in which you will see single cells of size less than a micrometer, their position how it is manipulated using light.

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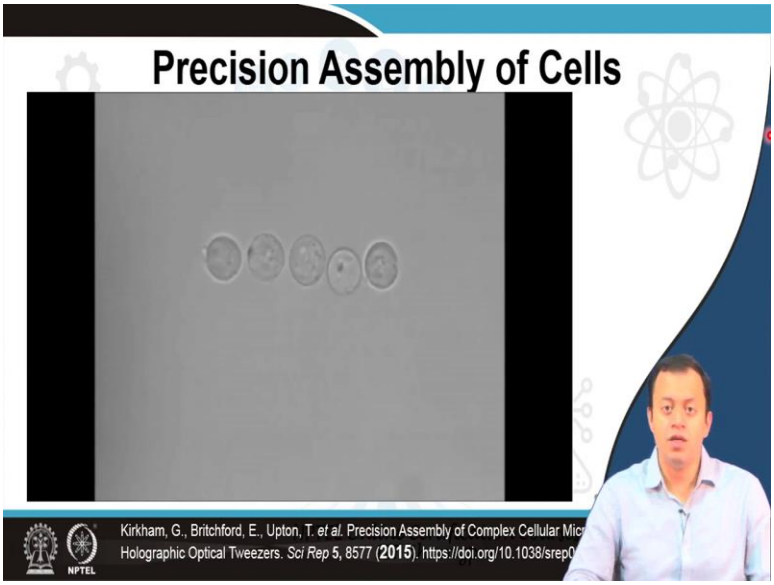

Here, you will see how the position of single cells, four or five of these cells are manipulated, are moved around their x, y, z position and some kind of cellular assembly is being done with the help of what we call as optical tweezer. Yes, it could be done. We could do it for single cells. We

could do it for viruses. We could do it for bacteria. Above all we can do it for molecules, sets of molecules. We can manipulate their size, their position etc. in an environment using just light or more precisely the force produced by light.

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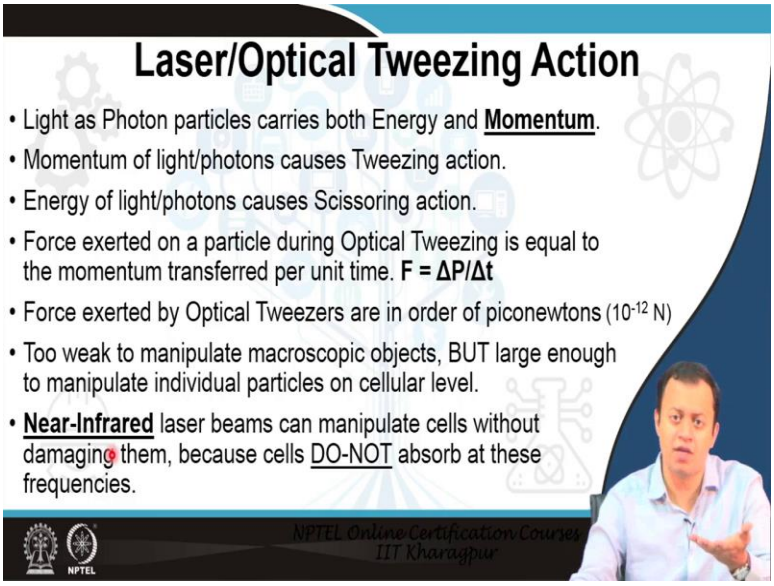

So, how does that happen? How is optical tweezing actually being done? So, you have to understand that light contains photons. We all know this. Anybody who has gone through this course we know that light contents photons. Now, photons contain both energy as well as momentum. It is the momentum present in the photons, remember from classical Newtonian mechanics, momentum is mass into velocity, momentum is simply mass into velocity according to Newtonian mechanics. We are not going into quantum mechanics. From Newtonian physics point of view, momentum is mass into velocity.

And trust me in this concept, in this module, Newtonian mechanics will be more than enough to explain you the overall basic concepts. You can utilize quantum mechanics, why not, but Newtonian mechanics will be able to explain for a period of time. So, just go with me. The momentum is mass and velocity and photons carry that momentum. Now, I do not want to go into that debate of the mass of photon, but you know there is a velocity of photon. Mass previously a photon was considered was 0, but now we have come to understand that it is non-zero, though very, very less.

So, irrespective of that even things with 0 mass can have according to quantum mechanics momentum. So, photons contain momentum. And the momentum is the most important thing that allows you for some kind of tweezing action. Momentum at the end of the day is a vector quantity determines the direction, it determines the direction of a particle in which some kind of a force has been acted upon. Again, Newtonian mechanics, second law of Newton's forces, force is mass into acceleration and Newton, and momentum is mass into velocity.

So, maybe there is a correlation between force and momentum. So, momentum of light causes tweezing action. We can also do, what we call as laser-based scissoring, i.e. you cut something up. So, if you can have a tweezer, you can also have scissor with light. Tweezer pick things up, scissor cut things up. We can have laser-based scissoring in which some amount of a portion of a cell or a tissue or an organ is cut off.

You know a little bit of that already. We have discussed that in the previous two cases, where light is being used to thermally burn, photothermal effect is done and thermally we are destroying a part of the tissue or a part of the cell. Here, strictly speaking, light based scissoring action is where we do some kind of cold ablation, where instead of converting some kind of a heat, we send very ultrashort pulses, femtosecond laser, you saw it in LASIK surgery for direct breakdown of the molecular bonds, i.e. the covalent bond that has formed or some kind of hydrogen bond or hydrophobic bond that has formed because of the presence of the electrons.

The electrons are getting highly charged and the electrons are moving away. As a result, some molecules are also simply drifting away, and as a result, some kind of a hole is created and through that hole you are sending something else. So, the force extended on a particle during optical tweezer is equal to momentum transferred per unit time.

So, force is rate of change of momentum with respect to time. Momentum is mass into velocity. Mass into velocity by time is mass into acceleration. Acceleration you know, high school physics or even middle school physics, acceleration is velocity, rate of change of velocity per unit time. So, overall you get mass into acceleration which is force is equal to ma, force is equal to mass into acceleration.

So, the idea here is that the force exerted by optical tweezers are very, very small in the orders of piconewtons, which is almost 0, 10 to the power minus 12 Newton, how small it is, piconewton.

It is too weak to manipulate macroscopic objects, but it is large enough to manipulate individual particles on cellular level.

A very large object or macroscopic object, large molecules, bunch of tissues or a big organ or any other non-microscopic object, this force, the light force, the change of momentum from light when it moves from one medium to another medium, the medium being the particle, the organ, the cell, the tissue, the force exerted by the photon beams, the photon particles on any object, a massive object, a non-microscopic object, a macroscopic object is 10 to the power minus 12 Newton it is negligible. You simply neglect it, 0.0001 percent, but when it comes to nanoparticles, how much do you think is the weight of a mitochondria, of a cell, 10 to the power minus 9 kg, 10 to the power minus 6 kg.

Will that smallest, ultra small nanoscale weight can be lifted by this piconewton force. What do you reckon? What do you think? Now, here couple of things needs to be understood that for optical twisting action we need to have a laser beam that is not absorbed by the cell or the particle which we are trying to lift up. For scissoring action, we obviously need the light to match the frequency of the energy gap of the electron so that electrons can take that energy move away or the molecules stick that energy start vibrating and if their vibration is too much, they can drift away.

In optical tweezing, we need the light simply to pass through. The light should not be absorbed. The light should not be causing any effect. It should not cause some kind of generation of heat, dissipation of heat. It should not generate molecules or electrons to get excited and just modify their response. We simply want light to pass through it. So, that the momentum that the difference in refractive index is causing, do not worry, I will tell you that, difference in refractive index is causing can be acted upon as simply a force, simply as a force on to the particle. And thereby, you can, since you are operating a force on a particular particle, you are able to move it. That is all.

So, mid-infrared you know more or less these are the molecular fingerprint region. They are absorbed by molecules and molecules start vibrating. Visible and ultraviolet is the frequency that is absorbed by electrons and electrons jump from one level to another level, but near-infrared around 1 micrometer, 1.1, 1.024, 2 micrometer, this is the near-infrared region, 900 nanometer

etc., where laser beams can manipulate cells without damaging them, without modifying their structure, without changing their property, because light at this frequency is too small to excite the electrons and too big to excite the molecules. There is a mismatch. So, this is a window.

Near-infrared simply falls through the crack which simply passes through the molecular structure without damaging them, without changing them, without changing their molecular vibration, without changing their electronic vibration. Now, by this time, through spectroscopic information, you know spectroscopic class that there, we go for molecular vibration, vibration state or electronic absorption state and infrared, near-infrared is neither, near-infrared, I am not talking about mid-infrared, near-infrared is neither and it can simply penetrate through the cell without damaging it and while penetrating it exerts some amount of force and we utilize that force, I will tell you in a moment how, we utilize that force to move, to manipulate the direction force, remember direction, force has something to do with direction, we manipulate the direction of the cell or in general a nanoparticle using light.

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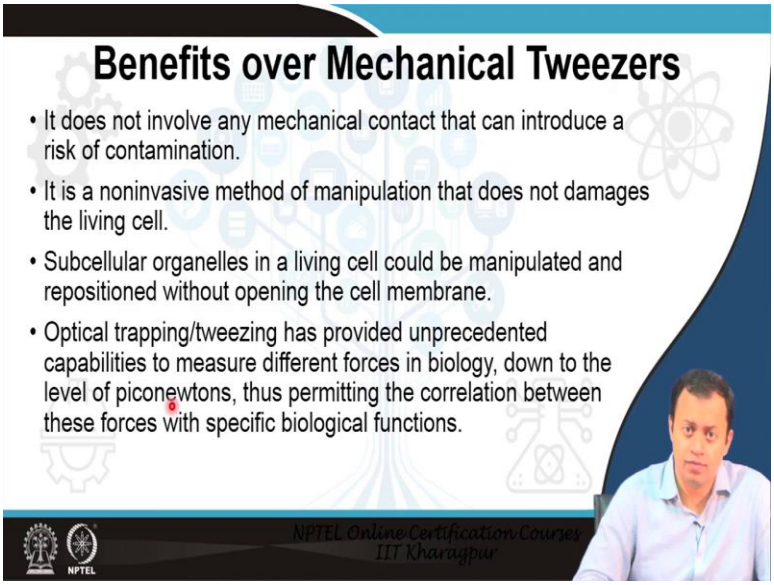

Why do this? What are the benefits over it? Well, first and foremost, if you use how small you can pick up that was my first question. How small of a material can you pick up using these kinds of mechanical tweezers. Granted you make it very small and you have handcrafting scales like surgeons' practice for a long period of time, but remember, they all come to some kind of limitation. You cannot simply pick up one single cell using micromechanical tool.

I know some medical student will say we have done that etc. Granted, there are certain sales which are big, but think about it, how complicated it used to be or how complicated it is to pick up one single cell among a plethora of cells very precisely, very accurately using some kind of a syringe, some kind of a micro tube, some kind of carbon nanotube. I am not saying that it is impossible. I am saying that it is difficult. It is complicated. Whereas, using laser-based tweezing, it is bit easy.

Secondly, it is a non-invasive method, meaning, well, light is all about noninvasive understanding of the structure. Spectroscopy is finding out what are the molecules inside or what is hidden inside the matter without actually destroying or invading the matter or opening it up, you are simply sending light, some amount of light is getting absorbed, some amount of light is getting transmitted. So, that absorption part is something that you understand that you try to analyze and that is the basis of spectroscopy here too.

You are doing some kind of a noninvasive way in which the molecule is or the cell is taken up. You are using light force, the force generated by the photons, the momentum of the photons to lift something up, which usually is neglected. But here you are using something that has an ultra-small size, ultra-small weight, a small organelle or a small virus 200 nanometer scale virus. You are simply lifting it up, putting it at a specific place, trying to see its characteristic, trying to analyze it, trying to modify it, punch some holes into it, send something inside it trying to see one virus at a time, one cell at a time, one molecule at a time.

Think how deep our understanding can go through that. Think how accurately we can thereby analyze to a molecular level what is happening inside instead of simply looking at symptoms that the entire area has metastasized, the entire area has got infected, we are now looking into individual cell. And if we can understand what is happening at individual cell, maybe we can modify it, maybe we can rectify it and we are rectifying it with the same light or similar light, a different frequency light which is getting absorbed, but that absorption is opening up some kind of a hole into the cell membrane. Through that cell membrane, we are inserting a particular molecular medicine.

All of you have heard of the term nanomedicine. How do you think that could be done? Sub-cellular organelles in living cell could be manipulated. Most importantly, it is a living thing. The

cellular structure is living. You are not harming it per se. When you are trying to use some kind of a mechanical tube to insert or try to eject particular cell or particular organelle, chances are you will kill it. And it has provided unprecedented capabilities to measure different forces in biology.

At the end of the day, you are enacting some kind of a force on to the cellular structure and thereby lifting it up. So, something has been attached properly. Groups of cells have been attached together to form tissues. Intracellular forces have made them attach to a specific tissue and then you are opening up or you are trying to pull up one single cell from the entire tissue matrix.

Do you not think this will give you information on the nature of the force that binds the cells? The intracellular junction, the intracellular forces in the forum, the biophotonics forum, someone asked me, I think Prashant it was, asked me use the forum. If you are not using it, you are missing something. I keep on asking you, use the forum. So, Prashant asked me, can we measure the forces by which a cell adhere to another cell to form tissue? Very good question. Here it is.

You take a bunch of tissues. All the cells are joined with another neighboring cells. You just pick up one cell out of it and the amount of force you require to eject the living cell from the neighborhood of its cells of other cells that it has combined together to form tissue will give you some amount of information regarding the overall forces. And remember that there is a correlation between these forces and their specific biological function. They are very, very specific. There is a specific force that attaches A, T, C and G. If you do not know what these are by this time, then I would ask you to revise previous lectures.

There is a specific force, a specific, specific force that binds the DNA or RNA base pairs. These base pairs are joined with a specific force. There is a specific force that is making the protein coil and uncoil having some kind of a folding reaction happening inside a particular protein, protein nanoparticle. There is a certain amount of specific forces that makes organelle stay inside cytoplasm. There is, of course, specific forces that makes cell adhere to its neighboring cell and form a tissue, all of them performing a specific, specific function.

So, these are the forces that have specific biological function. If we understand these forces, we can correlate it with the particular function it does. And by any chance if a particular function is

not being done, maybe we can rectify the forces governing it. You do know that viruses attach to the cellular structure using some kind of a receptor, a protein-based receptor, the viral spike attaches, a specific virus I am talking about, the specific spike protein of the virus is attaching with a cellular structure with, within the plasma membrane and thereby the virus is entering. So, that is a force. Some kind of a bond is formed, hydrophobic bond or some kind of a hydrogen bond being formed.

So, if we understand the specific forces that requires the specific force that allows for this bonding to occur. And if we are able to produce some kind of a counter force can we eject the virus out of the cell. Think about it.

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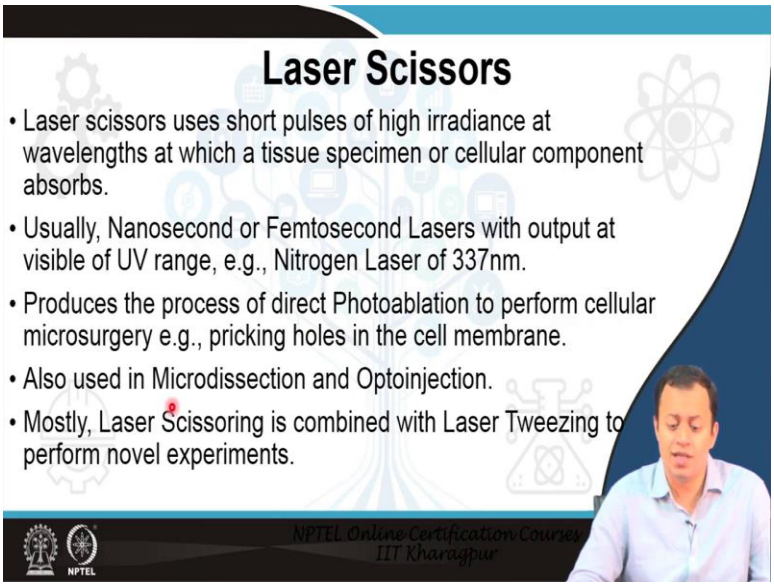

Optical tweezer performs that function. What are optical scissors or laser scissors? Well, as I said, they are short pulses of high irradiance, which needs to be absorbed by the tissue. Usually, femtosecond laser, excimer laser, though excimer term is misnomer, you can perform nano-surgery or microsurgery, pricking holes in a cell membrane and I will be telling you about optoinjection.

I will be telling you in the last class about optoinjection. These days we are combining laser scissoring with laser twisting. Twisting is moving a cell to one place to another. Scissoring is punching a hole inside that trapped cell or inside the cell which you are moving and putting

something inside it. A combination of all, a combination of both of that gives rise to unprecedented and novel biological application.

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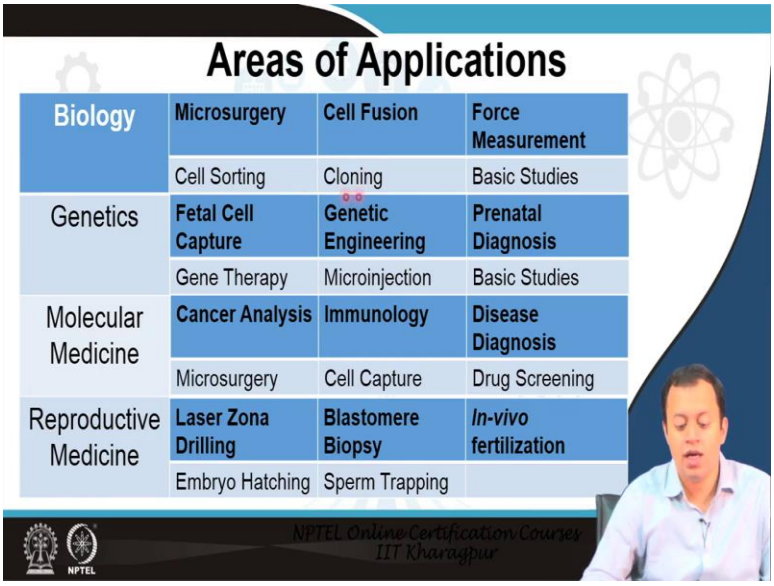

What do you say? Well, I have a full list and I will be discussing in the last class about the reproductive medicine. Boy that is going to open up a whole new can of worms and how controversial it is going to me. But I have taught you dermatology. I have taught you a little bit of ophthalmology, cancer biology, little bit of angioplasty, so why not reproductive medicine as well.

You can think of how to punch hole and putting some material inside a particular cell for some kind of a fusion. We can utilize optical tweezing and optical scissoring in molecular medicine such as, as I said, microsurgery, cell captured drug screening in a live cell, in a live molecule, live tissue you insert specific amount of medicine into the cellular structure and see if it is getting better or worse if the pathogenesis can be controlled.

Obviously, basic studies or there, disease diagnostics, you can do it immunology, genetics. Can we, using a laser tweezer open up the nucleus of a cell, look through some kind of a chromosome and say there is a bad chromosome something that cause sickle cell anemia, the genetic disease, we have figured it out how by human genome project by this time you know. We searched for the particular DNA strand, pull it up using laser tweezer, cut it using laser scissor. Can we do it in a fetus inside the womb? Can we do a fetal cell capture, fetal cell surgery, gene therapy,

genetic engineering, can it be done? Can we do some kind of cloning? Can we do cell fusion? Think about it.

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So, lots more to come. This was merely the introduction what it is the premise I gave, what it is why it is important.

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And in the next class, we are going to look into each of these in detail.

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So, thank you. Thank you very much.