Biophotonics
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Lecture No. 50
Selected Examples of Applications

Welcome back. So, we are at the end of our module number 10, which was optical tweezers, scissors and traps. And today, I have decided to take few selected examples of application. And of that selected examples, I have chosen to discuss about reproductive medicine and I will be discussing about the human reproductive part. So, if you think that to risk a topic or if your religious or cultural sensitivities prevents you from doing that, by all means, you can skip this entire lecture. I will be discussing scientific aspects of it.

So, again, if you think that you are not mature enough or this offends you, by all means, go ahead and skip the class. We can take care of it in the exam as well. So, you have been warned. So, what are few real-life examples of this optical tweezers and optical scissors or the traps working, simultaneously.

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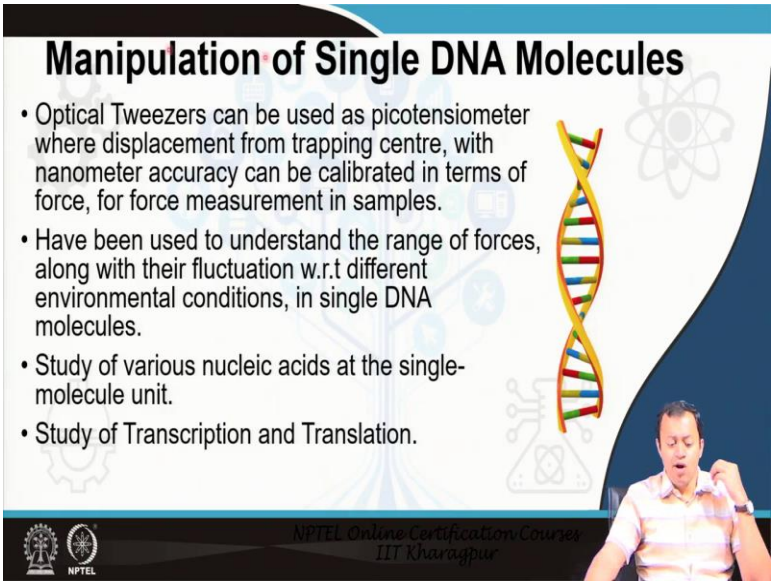

So, let us start with the manipulation of single DNA molecules. Optical tweezers have been used as a picotensiometer. Remember, I asked, I told you in the previous lectures as well that these various bonds between two different base pairs or the skeletal part, the skeletal frame, the

phosphate skeletal frame of the DNA, how these bonds are being formed, what are the different types of bonds, specific forces that can be measured using an optical tweezer.

We can have a full force measurement of the samples with nanometer accuracy as a picotensiometer, a tensiometer with a piconewton sensibility. And we will be able to or we have been able to understand the different ranges of forces. All these bonds are quite different from one another or all these bonds in a biological material, for example, can be quite different. It will be non-covalent per se, generally not always, but there will be a plethora of different bonds, hydrophobic bond, hydrogen bond, then Van der Waals bonds all these are coming together to form a complex complicated biomolecule or a biological matter.

We want to understand each of these forces and their effect. And most importantly, how does different environmental condition fluctuate these forces? So, there is a force of bond between say A and T, C and G and that is the inter bond that is allowing the two base pairs to join with one another and we understand the forces. And are the forces constant with respect to change in temperature, change in pressure, change in pH level or any other external factor, because this is important from a genetic point of view.

Remember, how these base pairs are generated or how these base pairs have a sequence, a particular sequence, remember your previous classes, they set up or they make a specific protein. If by any chance a mutation has happened, i.e. the arrangement has been broken or the arrangement has been modified of these base pairs of the arrangement has been changed, modified, destroyed, some sort of things have happened, it either does not produce the necessary protein or produces a bad protein.

The bad protein can cause several different diseases, including cancer. That is the overall idea, gene. But if we have understood what are the environmental condition, say, too much amount of alcohol is causing this kind of a mutation, too much amount of smoking is causing this kind of mutation, you know about carcinogens, things that can cause mutations. So, what are the overall environmental conditions? What are the overall changes?

So, suppose because of the too much amount of alcohol or too much amount of nicotine into the overall system, there is a change in the pH level or there is a local change in the temperature and that change is causing some kind of a mutation. If we are able to understand this, the fluctuation

of these forces, we would be able to understand the reason for the genetic mutation. And if we are able to understand it, maybe we can reverse it there by trying to make a therapeutic intervention for curing.

We can obviously study from basic point of view the nucleic acids at a single molecular unit, just a single strand of DNA or a single strand of RNA, you can basically break it apart structure by structure, nucleotide, nucleoside, etcetera, and try to see how the bonds are formed and which bond is resulting in which component or which structure getting in. And obviously, we can study transcription and translation. How DNA is converted to RNA or how RNA is giving rise to proteins, what are the different factors that are going on. So, overall, optical tweezers have a plethora, a variety of different uses.

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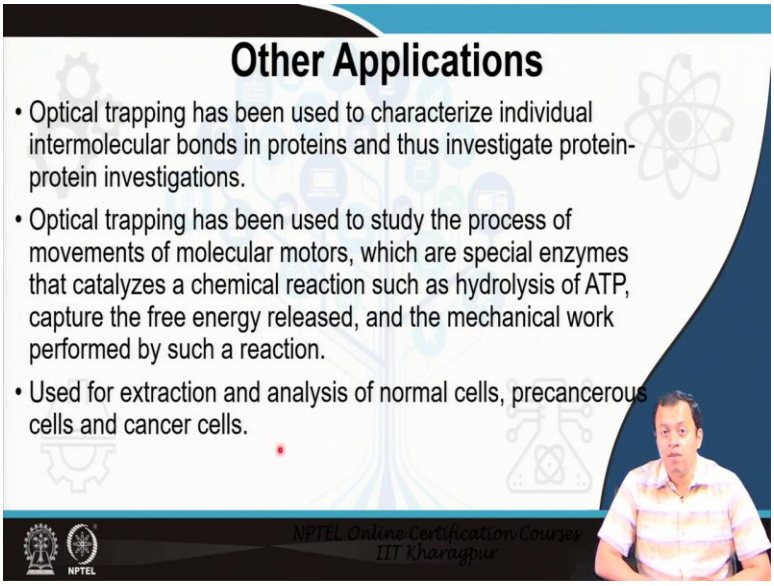

There are other applications. For example, intermolecular bonds in proteins, so two proteins, how they are connected together. Remember, overall complex protein like hemoglobin a, it has iron at its center. How is this iron connected with the other molecules of the protein? How are the tertiary structure is formed? Optical trapping has been used to study the process of movements of molecular motors.

Molecular motors are special enzymes, basically proteins again for that respect, which helps in hydrolysis of ATP, adenosine triphosphate, with currency, energy currency and the mechanical work performed by functions and you can use it for extraction of normal cells, precancerous cells

and cancer cells. So, there are a variety of different applications for optical tweezers, optical scissors and optical traps. But let me focus for a second on human reproduction. I have discussed about skin. I have discussed about eye. I have discussed a little bit about heart. Brain is coming, obviously, in the last chapter. So, let me discuss human reproduction and this is fascinating.

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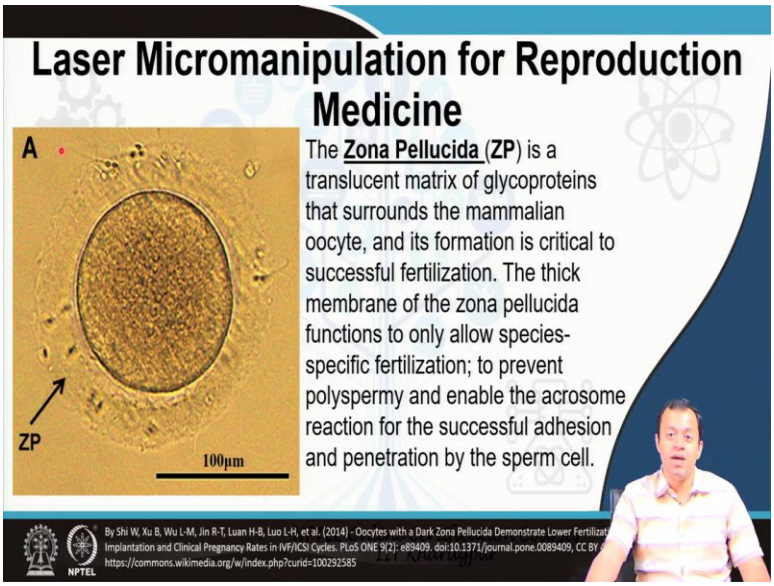

So, we use leaser micromanipulation for reproduction medicine. This is an example of an egg cell or an egg, ovum. Ova is the plural and this is an ovum, a female ovum and egg. And this internal is the oocyte this is the germ cell. This is what fuses with the sperm and the fusion creates the overall fertilization process. The egg gets fertilized. And finally, the blastomere and the zygote and the embryo, and finally, the fetus forms. We all know this.

The most important thing is this germ cell, this ovum, this egg has a semi-translucent barrier, which is called zona pellucida. Beautiful name pellucida if you are Italian, I was first told it by my Italian roommate. Zona pellucida is a translucent matrix of glycoproteins that surrounds the germ cell that is the oocyte. So, this is the cell. This is the genetic material. And it is surrounded by this armor, this suit of zona pellucida. This is ZP. This is zona pellucida. This is a translucent matrix of glycoproteins.

So, this is like a suit. This is like an armor. This is like the protection that the germ cell has, the oocyte has, here is germ as in germ has that prevents cell fertilization, that prevents the thick membrane of zona pellucida functions to only allow species-specific fertilization and to prevent

polyspermy and enable the acrosome reaction. So, for, let us break this one at a time. This is fascinating.

So, this is the semi-translucent layer, and see these are the sperms that are attacking it. In order to fertilize the egg, you have to drill, you have to penetrate through this protective layer of zona pellucid to form, to fuse with the egg that is inside. And from a scale point of view, see how small the spermatozoa are as compared to the overall oocyte, overall germ cell. But the sperm cell or the sperm has to penetrate through the zona pellucida.

Zona pellucida is a very, very selective membrane. Zona pellucida is an incredibly selective membrane. And first and foremost, it prevents species-specific fertilization only human sperm can fertilize the human egg or any specific mammalian sperm can fertilize the, that specific mammalian egg. We do not have interspecies fertilization, simply not, because of the presence of this barrier layer. This barrier layer is selective. It allows only a specific species-based fertilization to occur. It prevents polyspermy i.e. not millions of sperm can at the same time simultaneously fuse that will kill the zygote.

We see twins forming or quadruplet or several different types of that thing happened, but they are incredibly rare. We know that they are rare, because that comes in news. Nobody gets into the news if female or women have produced 1 or 2 children. It is only news if 6 or 7 babies have come simultaneously quadruplet, hexaplets etcetera. So, that is rare. So, this is because of the zona pellucida. The zona pellucida is selective. It is allowing only one type of sperm to inside, to go inside, only one specific species. It prevents polyspermy. And it enables the acrosome reaction. The acrosome reaction is basically the fertilization process where the genetic material of the father gets injected into the genetic material of the mother and the blastomere is forming.

The blastomere is the fusion cell. The blastomere forms into, I think, medical students can give me a better answer. I think blastomere forms into zygote. The zygote forms into an embryo. The embryo forms into a fetus. And when the fetus comes out, it is a full born baby. That is the overall processor. The blastomere is the fused cell between sperm and ova. And then it opens up and becomes zygote and then embryo and then fetus. But I could be wrong. So, please check it out. But the zona pellucida is fascinating.

So, couple of question. If we can have a species-specific translucent matrix of glycoproteins that already exist around a particular set of cells, just an idea, can we have these kinds of armor, this kind of a barrier artificially made for a limited period of time, it does not, it should not go on for forever, semi-permanent, around say any other cell, lung cell that is allowing only specific foreign bodies to enter. Rest of the foreign bodies are simply killed off.

You know where I am going with this. I have coated my lung cell with some kind of a translucent matrix of glycoproteins that is allowing my body's different material to penetrate, but is preventing any pathogen, any virus to go inside. We already have a selective translucent matrix and that is very, very selective. It knows, it prevents other species genetic material to go inside.

So, why cannot we apply similar idea to protect a specific area of an organ which we know will be affected by, which we know might get affected by a specific virus. We just coat it, not for a long period of time as long as there is a danger we just coat it and go into that viral environment, go into that bad environment. And then when we have returned back this translucent protein has been absorbed by the body.

Now, what I am coming to this, from this point of view is that in certain females, this zona pellucida is incredibly thick and it prevents any sperm that is human male sperm to go inside resulting in infertility and that causes severe amount of psychological problem. The sperm is unable to penetrate through this translucent matrix, through this zona pellucid, thereby go inside and fuse with the egg cell, fuse with the germ cell, fuse with the oocyte and the egg is not getting fertilized at all, because this is a bit too thick, it has to be semi-permeable. It should allow something, but not allow too much.

But under certain circumstances genetical modification, mutation as we saw previously, it will become so thick that it is preventing anything to penetrate. It is not allowing anything to go and thereby, preventing fertilization altogether. Result is obviously infertility. And infertility, I do not have to tell you, it causes huge amount of stress, cultural problem, religious problem, your own psychological problem will start, millions of couples suffer from infertility and infertility clinics are these days every corner and everybody has a plan or everybody has an opinion about infertility and how that can be prevented, etcetera.

There are several other reasons for infertility. Let us be honest. But one of them is thicker zona pellucida, a thicker version of zona pellucida which is impenetrable.

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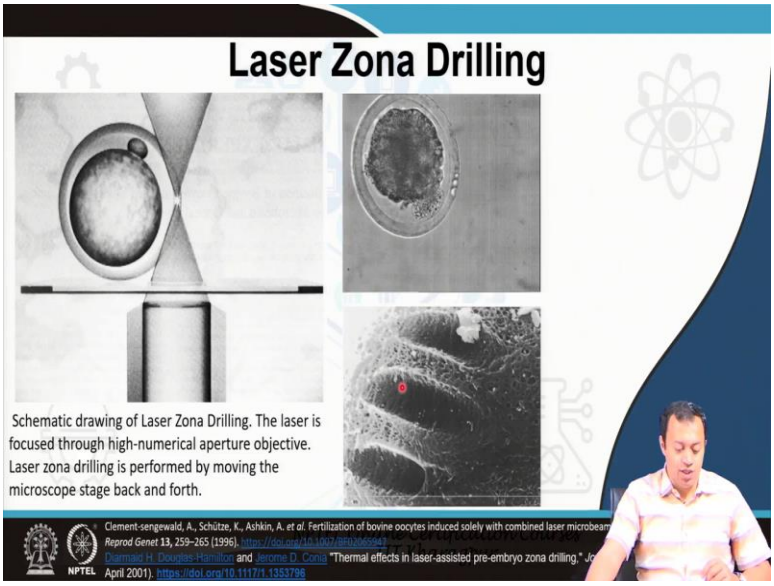

What we do, we use laser zona drilling. You saw that here we are bringing in the femtosecond laser near the zona pellucida and thereby make some holes into the outer area, thereby make some holes onto the zona pellucida. And a laser zona drilling is performed, remember we talked about optical drilling and by moving the microscope stage back and forth and you can open up holes inside the zona pellucida. This is a schematic and this is also a bovine egg. So, do not confuse it. But you overall get the idea. And then you have opened up holes in it.

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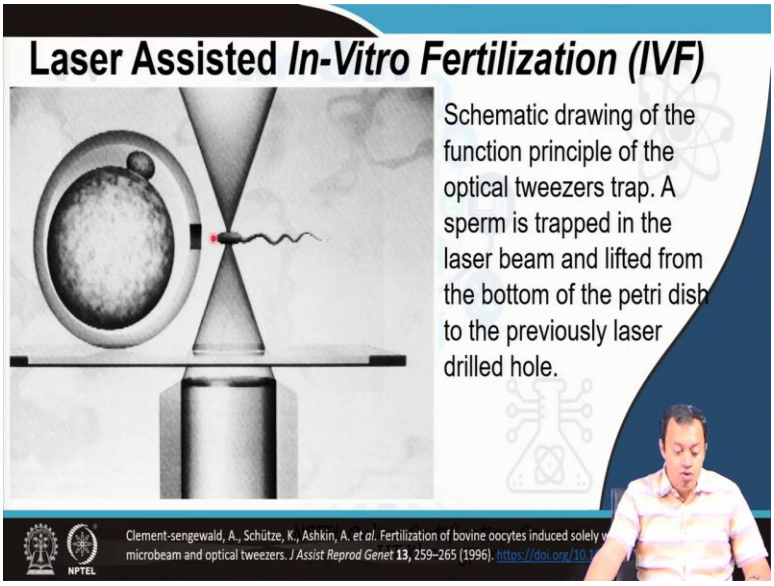

All you need to do is you have to trap a sperm and that can be made to penetrate through and thereby cause fertilization. You are doing the scissoring action here. You are doing the trapping action here. Here the laser tweezer is doing it. Also, there another reason for infertility is low sperm mobility. So, the sperm do not have the enough mobility to reach to the zone.

Here this is IVF, in-vitro fertilization, meaning you are doing the fertilization in a petri dish. You have taken the egg out from the mother. You have taken the sperm out from the father. You are doing it in a petri dish, say either of them or in both cases, either this is too thick or this does not move, you are moving it using a laser trap. You are moving it using a laser tweezer. So, you do not require its tail basically. I

have seen people cutting it off, cutting the tail off simply taking the genetic material i.e. which is present into the head of the sperm, simply taking the genetic material here, bringing it close to the egg or the egg which has already been drilled whose zona pellucida has already been optically ruptured, optically perforated have been put in some kind of a environment which contains large amount of sperms and one of them is close enough to bring it or if both of that is the case, you drill it first and then you pick up a sperm and put it inside through the hole, thereby it goes inside the germ cell, fuses with it, the fertilization occur, blastomere forms.

Countless amount of couples suffer from this kind of infertility and infertility treatment is a huge business and not all infertility is or because of this, but several of them could be treated using

biophotonics technologies, using laser technologies, remember. And once the cell has been fused, once the fertilization has happened after 2 or 3 days, when say a zygote has formed, you can put the entire zygote, the fertilized egg either back directly into the uterus of the mother or for surrogacy into the uterus of a different female, both of that can be done in in-vitro fertilization. So, the fertilization is happening in-vitro i.e. in a petri dish.

I have another question for you. Several women have problem with their fallopian tube, there will be a blockage, so the doctors directly implant this zygote on to the uterus wall. If the uterus is damaged, then we have to find a separate uterus. So, surrogacy comes in. A different female who has a healthy uterus, the zygote which contains egg and sperm from a couple, from a set of fathers that zygote is put into someone else's uterus, and that person carries it and then gives birth and that is how it forms. But there is a social or psychological problem associated with it as well. Surrogacy is a controversial topic.

So, these days, we are able to make an artificial womb. It is basically a condition. Sets of condition needs to be performed a particular set of temperature, pressure, pH needs to be maintained some sort of growth factor, the food, the excretion, all of that thing needs to be maintained as it is maintained in mammalian uterus. If we are able to create that uterus outside a human body i.e. artificially in which we are doing the fertilization in a petri dish, and the zygote that is formed is put into the sack, which we have created artificially, none of them are inside human body.

So, my question to you is the zygote that is getting formed outside a uterus, outside a human uterus, the fertilization took place in a petri dish and the growth of the zygote is happening in an artificial womb, in an artificial uterus, which you will simply open up and take the fully formed embryo, fully formed fetus up. Will you call that person a human being, someone who has been conceived as well as being nurtured completely in artificial environment? Let me know. Think about the possibilities of future. These are not science fiction. These are something that we can do with the help of nanotechnology, with the help of biophotonics. Let me know your comments.

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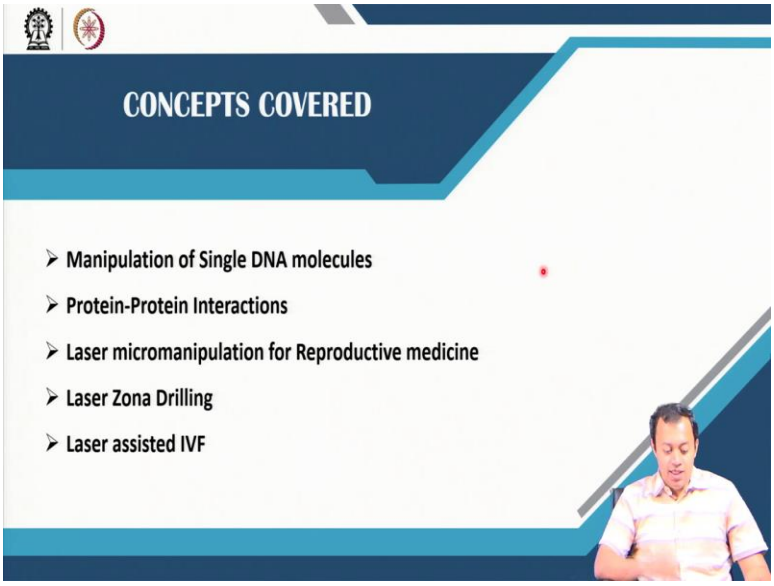

These are the topics that I have discussed.

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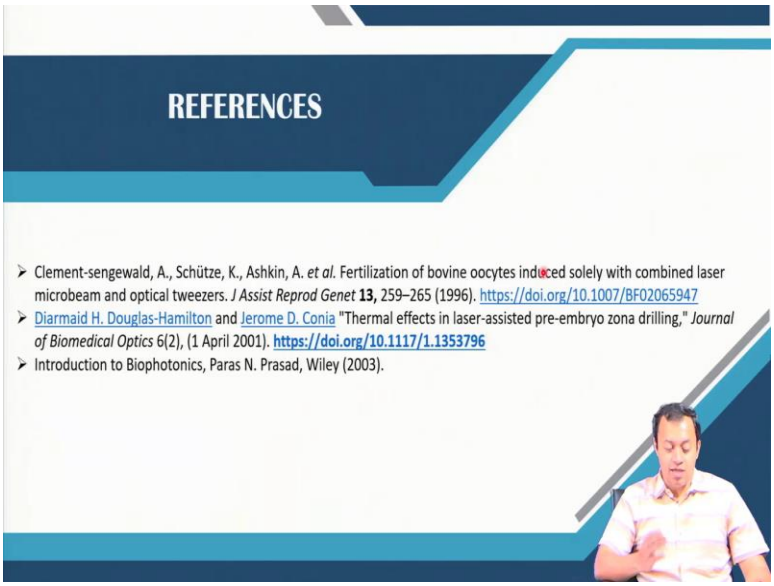

And these are some of the very fantastic references that I got to learn. And please go through them. I will see you in module number 11. We are almost at the end of this course, module 11 and module 12. And then that will be the end of this course altogether. I hope that you are enjoying it. My knowledge certainly is increasing. But since there is no direct interaction, I cannot judge your mood. So, I do not know where more I have to progress. Those of you who contact me in the forum, I try to help them as much as I can. But if you still require something,

please, please let me know. Unless you tell me, it would not be possible for me to simply get that idea and change it. You have to give me the feedback.

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So, thank you. Thank you very much.