Biophotonics
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Lecture No. 49
Laser Scissors

Welcome back. So, today, let us discuss Laser Scissors.

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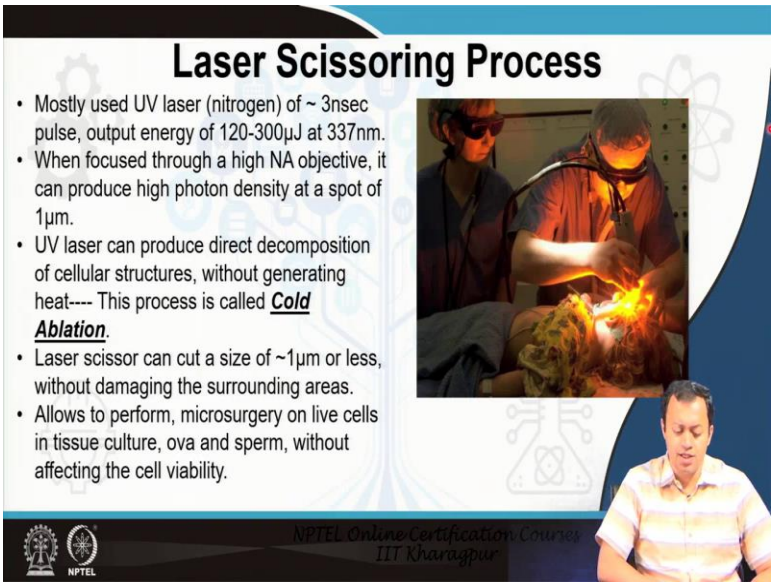

You have already some idea of this laser scissoring process. In lasers scissoring, we mostly use UV laser, UV laser of ultra-short pulse at a 337 nanometer or even below 151, 160 nanometers have also been used. So, the concept has already been taught by me. You know that most UV lasers or if you have a UV light, it could be used for direct destruction of the cellular structure. Usually in infrared or visible light, the laser focusing agitates the molecules. The molecules then start vibrating and they dissipate the heat. The heat travels and causes the damage.

Whereas, in UV light, as I have discussed previously in femto laser surgery, this is femto laser surgery for all intent and purpose, the UV laser is directly exciting the electrons. The electrons are moving away. There is a strong ionization of the molecule a plasma is formed. The plasma moves and overall there is an overall destruction, because the molecule is no longer there. Why, because the molecule is formed because of the overlapping of electron cloud. If the electron has got enough energy that it has drifted away then there is no reason for the molecules to exist in the original position.

So, you can simply make that by sending UV light. UV is mostly absorbed by most materials. And since it is a direct decomposition without generating any heat, we call it cold ablation, because there is no heat generated per se. Remember heat is generated when molecules are there and molecules vibrate, molecules rub against another molecule and it generates heat. Here the molecules are static or they are, they do not have even the chance to vibrate, because you have sent something of a very short pulse less than its thermal relaxation time much, much less than the thermal relaxation time of the molecule or the cellular structure and at a very high frequency, very high energy, very low wavelength.

And there is no time for production of heat, simply put. There is no time for production of heat. And without generating heat, you simply decompose a direct decomposition of the cellular structure. So, laser scissors can be cut, can be made to cut a size of 1 micrometer or less. I know people are these days making punching holes by focusing these laser lights, pulses very high energy in cell membranes.

I will show you several pictures cell membranes of sizes less than 1 micrometer, I am aware that 500 nanometer or below holes have very faithfully been used. I asked you to search into the minimum size. And this performs, this allows the perform, to perform microsurgery on live cells or tissue culture ova and sperm without affecting the cell viability.

So, the idea here is if you have a cellular structure, say 5-micron, 2 micron, 1 micron and if you have made a cut on to the cell membrane, which is 1 micrometer or less 500 nanometer hole, you have punched a 500 nanometer hole onto the side of the cell membrane, you can artificially inject some other material perform microsurgery, take away something, add something. And since it is a 500-nanometer hole or 1 micrometer hole, the cell membrane will heal up rapidly.

The cell membrane will heal up rapidly, why, because biological material heals up. If I have a cut in my skin, after certain period of time, it will heal up, it will, there will be blood clot, there will be thrombosis all of those things and then the skin will heal up there might be some scars etcetera. But the biological material, living biological material have a tendency to heal up.

Now, imagine the same cut, same hole, same injury, same punch happening at a scale of 500 nanometers at a cellular level, a cell wall, there is a small perforation at the cellular membrane, at this plasma membrane. It will also heal up. So, there is a very, very little long-term damage.

There is a very, very little long-term damage and without damaging the cells, you can perform microsurgery. Think about it.

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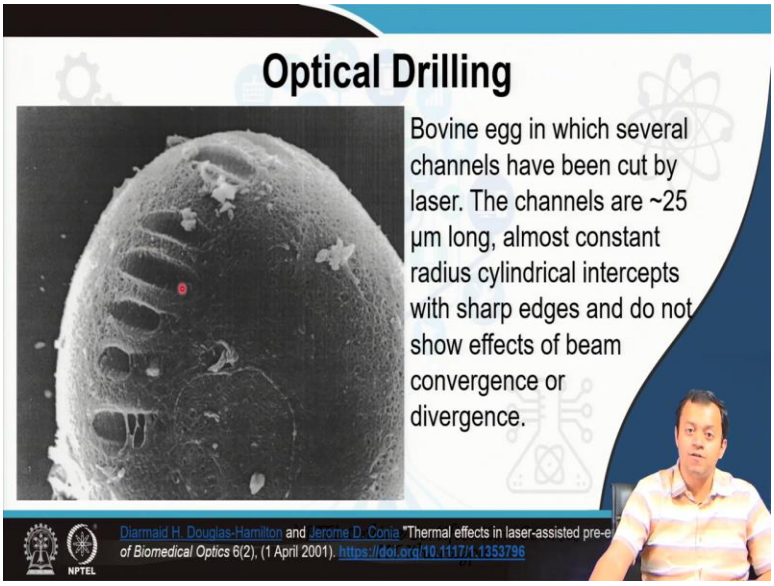

So, this is something that they have been able to do. This is a bovine egg. I will be discussing reproductive medicine. So, this is a bovine egg in which several channels have been cut by laser. So, these are several channels that had been cut on to this egg cell, on to this egg, this bovine egg and they are of 25 micrometer long. This is a slightly longer. This was done in 2001, 20, 21 years ago. And they are almost constant radius cylindrical intercept with sharp edges and do not show effect of being convergence or divergence.

You can make these kinds of punch hole on to the overall cell membrane of some kind of a big cellular structure, big tissue structure etcetera and then penetrate some extra external material through these holes and these will heal up very quickly like your skin or any other injury heals up given time. These are small enough for certain material to penetrate. We, therefore, call it optical drilling, but not strong enough to cause a long-term damage. You are simply damaging the outer wall. You are not sending the light inside.

And if you are able to ensure that the wavelength of the light is simply being absorbed by the tissue, by the external wall and not by internal material even better or reduce the focus or reduce the energy so that the drilling, so that the depth of the drilling is up to a specific, specific

nanometer scale, nanometer height. So, that the entire light does not penetrates, because it does not have enough energy, even though it is absorbed, UV is mostly absorbed by most material.

So, maybe you will not have that much luck with wavelength selection, but you can have power selection, intensity selection and how long you are focusing it. So, if this tick 1 second will go up till this, 0.5 second will go up till this, 0.1 second will go up till this. Thereby, you can make selective cutting, selective drilling onto it. So, this is the actual images of bovine egg which has been done.

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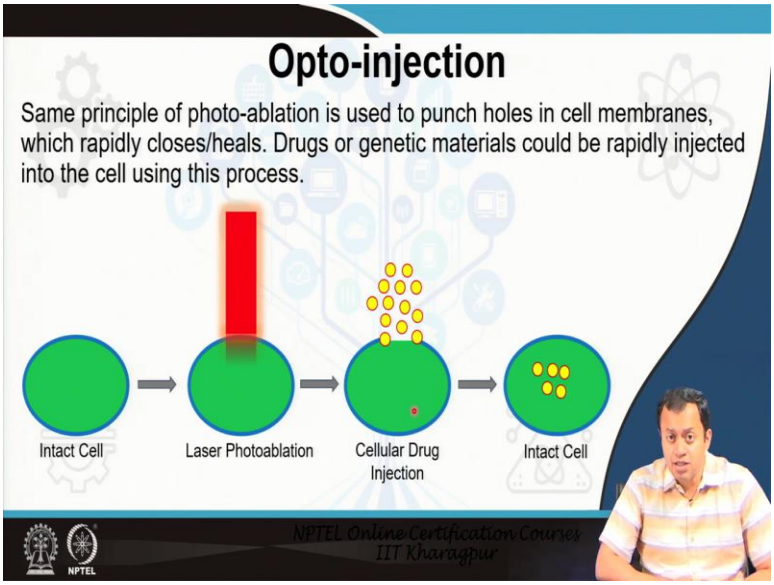

And thereby, this new thing came called opto-injection, where you have an intact cell. You send some amount of laser light to open up just the cell membrane without damaging the internal part or with minimal damage of the internal part and then drugs or other genetic material could then be injected, this is Opto-injection is injected inside and that guts inside the cell and this cell membrane after few seconds repairs itself just like your skin heals itself. It is a living biological system. It has a property to heal. A living biological, living cell, living tissue has a property to heal itself. So, it heals itself with your cell intact as it is.

Now, remember, we can also put genetic materials, different kinds of nucleic acids. And in that case, instead of Opto-injection, the term that is preferred is optical transfection. Biotechnology students or any person from a biology or life science background know what transfection is. For the engineers here or the physics students here very crudely transfection is where we are adding

some kind of a different genetic material, different nucleic acids into the cell non-virally. So, it is not a viral infection, the virus, viral DNA is not getting inside the cell. That is not transfection.

Transfection is something that is purely controlled and we are bringing in either a genetic material inside it by opening up the cell membrane some external type of nucleic acid, some external RNA, some external DNA has been put inside the cell to modify the overall genetic composition, the overall gene of the cell. Of course, you can understand from this that there is a variety have different subfields of transfection as such.

People again do their PhDs in transfection studies, but this is the general idea. This is a very simple and very general idea for those of you who are not from life science building. I believe transfection is a separate subject for a few biochemistry students. So, I cannot explain it in 2 or 3 minutes completely. So, this is this is the overall idea. Instead of putting drugs, we can put genetic material inside it to overall change the genetic composition of the entire cell.

That could be done once say, for example, you are making transgenic organisms, transgenic are, well, we mostly make transgenic mouse or transgenic materials, but you must have heard it in agriculture as well. There was a huge controversy GM food product, GM brinjal or GM food crops, so they are genetically modified. So, they are basically, well, up to a level transfected or transfection could be utilized there. So, they are genetically modified.

So, they have a different sort of genetic property than the native species, than the original species, which may be very subjective. It might be susceptible to particular pest, particular diseases, but now you have been able to do some kind of genetic modification on to that species, onto that cell so that it becomes more robust, it becomes, it has its own capability to, from that gene develop antibodies to destroy the bacteria or destroy the pathogen, destroy the virus per se.

But then again there are obvious controversies associated with transfection as in you are, what your gene, remember this is what I have been discussing. If you have changed your gene completely, are you still you. So, the brinjal that you are eating is it still brinjal, genetically modified crop. So, it is a, so there are arguments on both sides. I will not take any side. I will just inform you what it is. And there are obviously, different arguments, more arguments on both sides. So, I will ask you to read about it and make an informed decision.

But the point that I am making here is Opto-injection can be utilized to open up this hole, open up the cell membrane and thereby inject something, some foreign particle into the body, inside the cytoplasm of the cell and then observe what is the modification going on. And thereby, you can develop drugs, you can test side effects, you can see if the cell is able to defend itself against a specific pathogen, a specific bacterium, a specific virus and thereby see the viability, is it just one type of cell or a large number of cells. So, all of those things can be done using Opto-injection.

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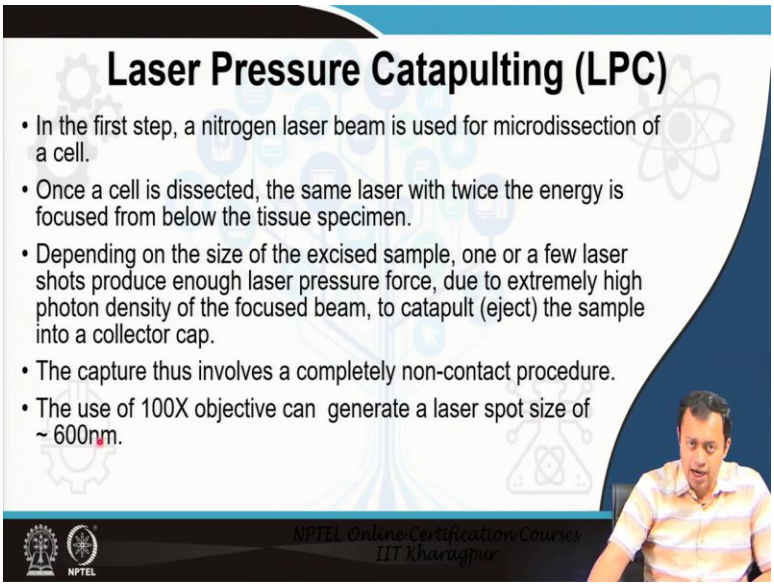

Then there is also the laser pressure catapulting. I do not think, frankly speaking that is that important, but this is catapulting, catapult that we used to play as child. So, here, it is basically picking up specific cell from a group of tissues. Here, first you use a laser beam for microdissection of a cell. Once the cell is dissected, the same laser with twice the energy is focused from below and these few laser shots produce enough laser pressure force for the, eject the sample on to a collector trap.

So, if you have a tissue like structure, you use laser to cut the one portion or one single cell and then you push other, well, the same laser with a different frequency from below, so that the cut part simply opens up. That is sometimes done to pick up a specific cell from a group of tissue. So, say, for example, you want to observe a cell that is going through necrosis among a tissue, you just want to pick that up. So, that could also be done.

And these are several fancy procedures that have come up in recent days. And as they say, using 100X objectives and betterment of different types of lasers, excimer laser, nitrogen-based lasers, you can generate a laser spot size of around 600 nanometer or even below, 500 nanometers could be done. There are several papers that talk about making cut or punch holes directly without any thermal procedure, without any thermal damage of around 600 nanometer size.

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So, that is again what I discuss. This Opto-injection is quite important both from the exam point of view as well as for, people keep on asking me the latest topic or the hottest topic optoinjection is quite important. It has been tested or it has been used for a long period of time in biotechnology, but the other engineering sections are firstly waking up to the joys of optoinjection.

From a laser point of view, what are the different types of laser that can be put so that we have a standardized version of different sets of lasers to do different sets of microsurgery or nano-surgery at a cellular level, at a tissue level which should be now, that is at least the aim of a standard operating procedure for surgical operations or for surgeons. So, instead of using scalpels or scissors or something like that, you will use lasers. You will use lasers to do micro-drilling or micro-cutting or micro-ablation as such. So, that it becomes more precise and you will have bit more control without harming the surrounding areas.

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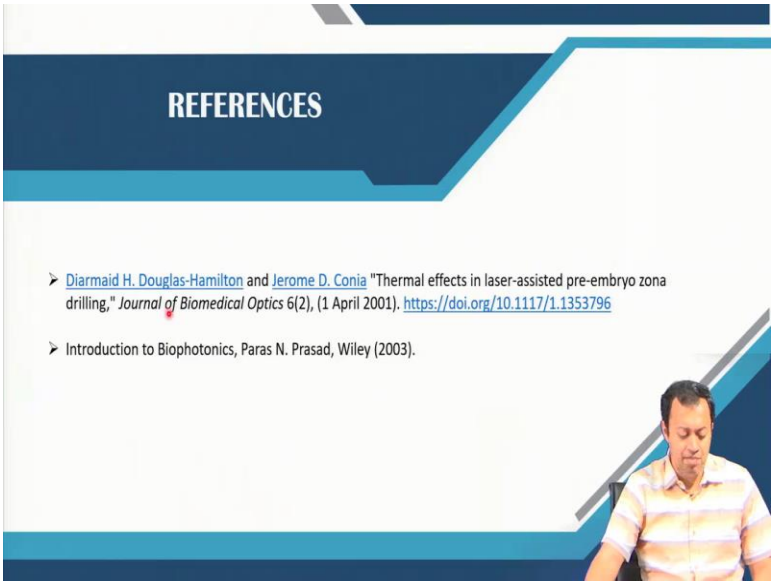

So, please go through the references that has much more important images as well as concepts which maybe I am not discussing here. If you are interested, just go through these references. And of course, this is a very handy book. And in the next class, I will give you some quick examples of what needs to be done.

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Thank you. Thank you very much.