Nanobiophotonics: Touching Our Daily Life
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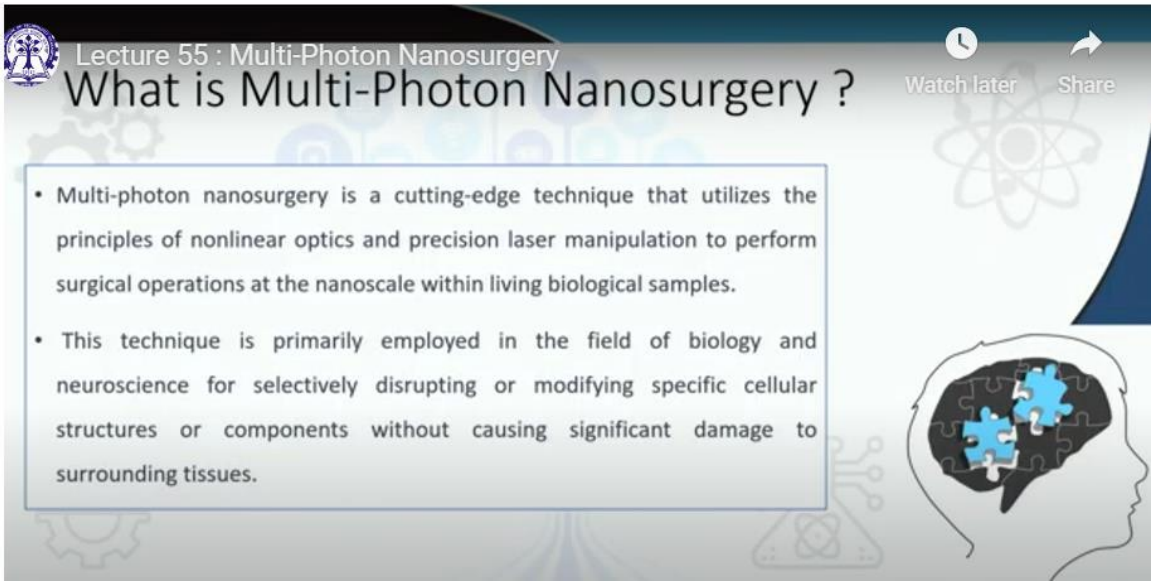
Lecture No. 55
Multi-Photon Nano surgery

Welcome back. Continuing our discussion from Multiphoton Microscopy. I think it will be apt to close this chapter, close this module by talking about multi photon nano surgery. If you can send you know multiple photons and then ah you can look into the emitted a specific emitted photon out of it and try to image it, what will happen if a high intensity laser beam of similar multi photons, 2 photon, 3 photon together are sent at a specific area which you have previously imaged and you think that that needs to be eliminated surgically taken out, surgical strike if you say ah at the specific area why cannot we do that. So, that is today's topic we discuss about multi photon nano surgery. So, think how far or how advance ah our our our knowledge and our technology is going where noninvasively by sending laser light we are attacking specific specific areas of the brain few neurons at a time either removing it all together or we are modifying it right.



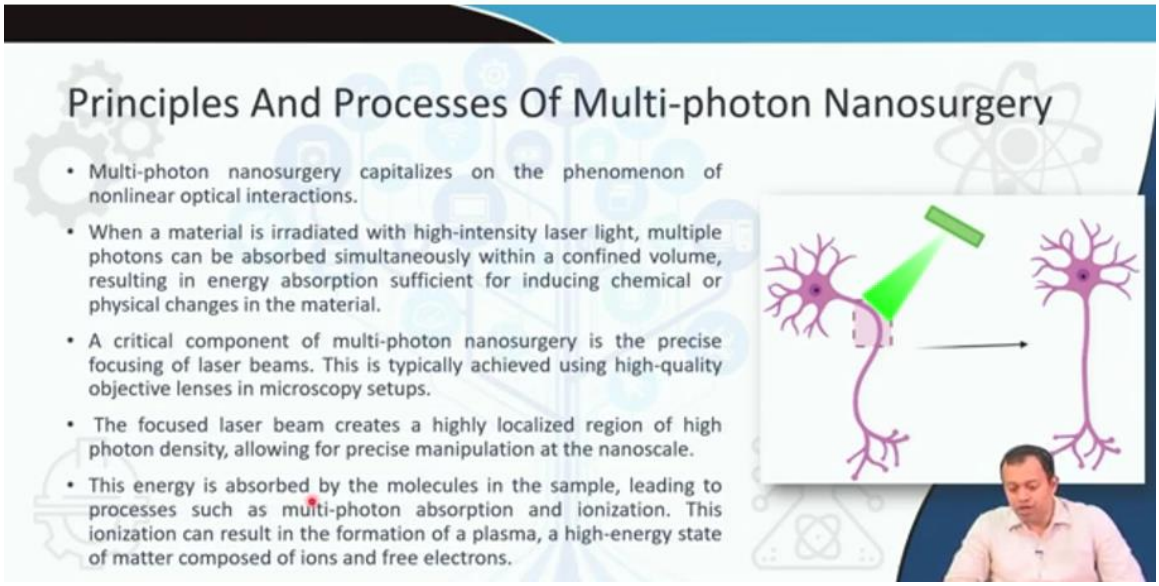
So, what is multi photon nano surgery? Multi photon nano surgery is a cutting-edge technique that utilizes the principle of non-linear optics and precision laser manipulation to perform surgical operations at a nano scale level. You are sending laser light highly focused laser light of resolution few microns at specific areas of the brains and perform surgical operations in in living biological samples. This is important you are not simply taking the portion out putting it in a slide and then trying to do it you practice using that, but the real deal is when you have inserted into living biological samples. This technique is primarily employed in the field of biology and neuroscience for selective disruption or

modifying specific cellular structure or components without causing significant damage to surrounding tissue selectivity.

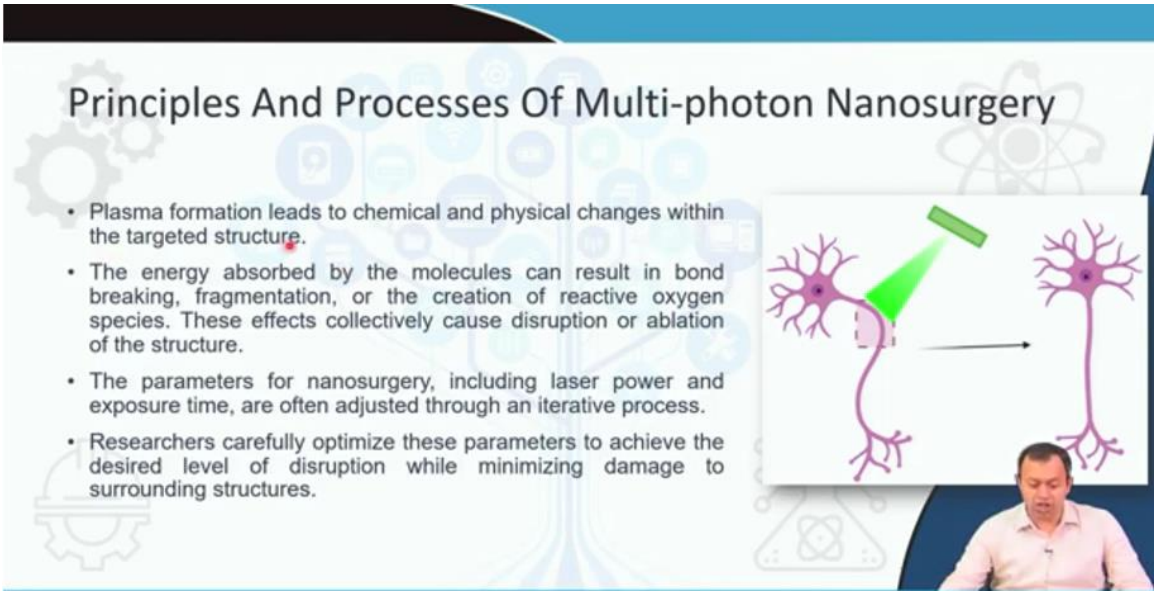
Selectivity is the key word here either you disrupt you burn it off or you modify now you might ask how it is going to get modified. So, the process is pretty simple all these ah neural transmission at the end of the day they are biochemical reactions yes even your transcription translation the central dogma part these are biochemical reaction reaction nonetheless and every reaction has an optimal temperature where it performs the best hydrogen with oxygen will create water know everybody, but how much or how efficiently or how fast this process will occur depends on several environmental phenomena one of them being temperature. So, every chemical reaction has some sort of an optimum temperature range at which at which this particular chemical reaction works with most efficiency using laser light you can heat certain areas up. So, for example, if you see a loss of neurons right if you see a loss of neurons there is just like your printed circuit board where a connection has been broken a wire is cut off what do you do you replace with another wire what do you do when similar thing you find out inside the brain that a neural connection is broken neural connection is broken. Now, you need to recreate the neuron you need to connect it the neuron now for a time being several areas of the neuron you know about glia we have discussed about neuro glia glia they contains the stem cells these stem cells can convert themselves into you know neuronal cells and repair the damage sustained by the neurons, but you can aid this you can aid this by selectively increasing or optimizing the temperature around that particular neuron that particular tissue which can you know results in development of specific you know tryptase molecule or those enzymes that that helps in transcription or translations you can help a particular temperature which will attract more amount of those tryptase or that enzymes that helps in translation or transcription from DNA to RNA, RNA to protein and in term accelerate accelerate the process of protein generation which in terms will repair the tissue maybe it has so happened that the stem cells within that person and old persons brain has exhausted itself or it is not working.

So, you are simply giving it a boost by changing the temperature or even pH of the surrounding area how the pH is changed you create that bubble remember that bubble from previous section you create that bubble the overall temperature overall liquidity overall ah presence because you create an optoelectric field with this with this with this laser ah the ions the ions can you know be be be modified to come to specific specific areas there by the polarizability the pH ah etcetera can also be modified obviously much more complicated than said, but the potential is here and thereby you can help in not just breaking down something like a bad cell bad ah tumor bad neuron, but you can also modify and repair specific cellular structures or components without causing significant damage

to surrounding tissue. So, that is overall the essence of nano surgery that is the overall essence of nano surgery. So, principles and processes of multi photon nano

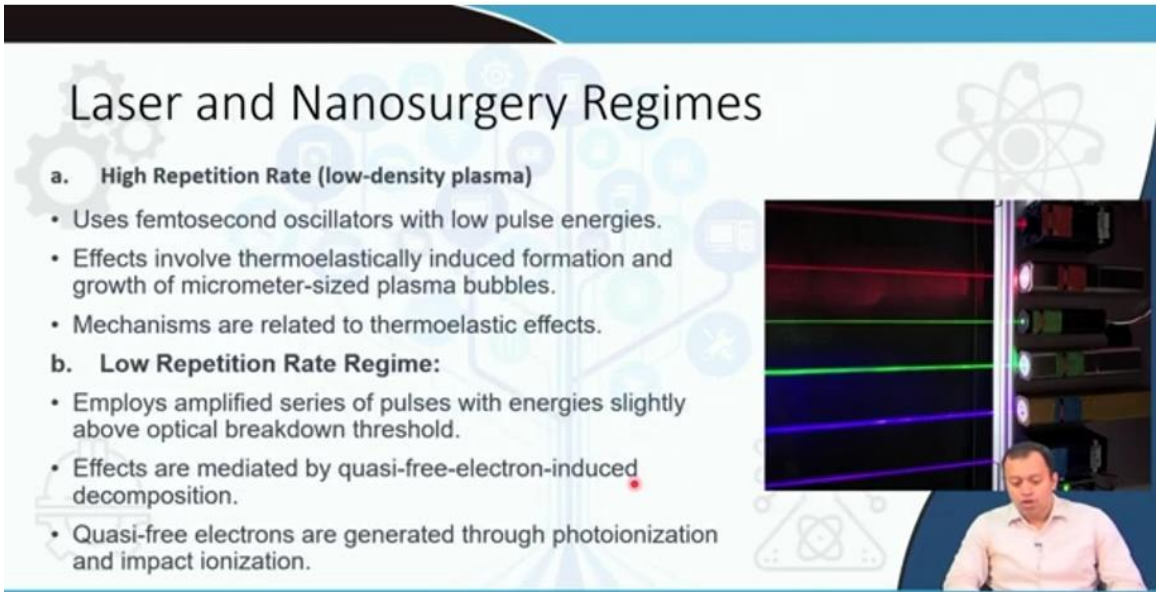


surgery multi photon nano surgery capitalizes on the phenomenon of non-linear optical interaction when material is irradiated with high intensity laser light multiple photons can be absorbed simultaneously within a specific volume resulting in energy absorption ah sufficient for inducing a chemical or physical change in the material. So, you you try to do this a chemical change or a physical change physical change burn it out damage it because of the absorption absorption results in heat dissipation heat dissipation can you know basically burn it or you can induce chemical changes into the material just like what I discussed previously. Critical component of multi photon nano surgery is the precise focusing of laser beams this is typically achieved using high quality lenses the focused laser beam creates a highly localized region of high photon density allowing for precise manipulation if you have focused only in this part using your sophisticated lens on these days you know you can break even the diffraction limit metal lenses and what not ah the photons are only you know activated in this particular region and this dendrites and axons this dendrites and this axons are no longer affected.

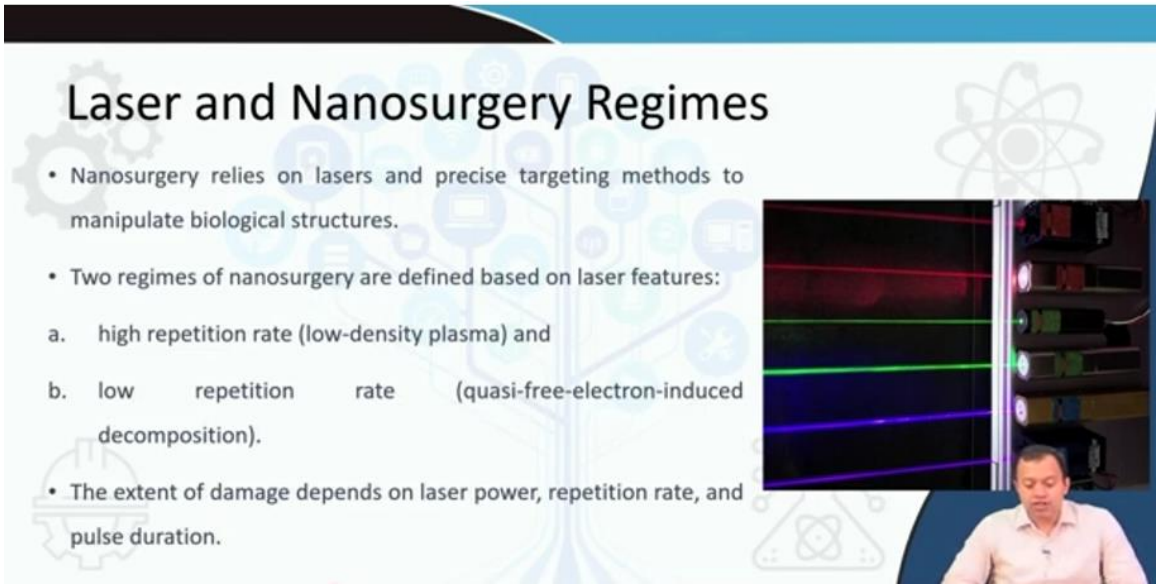


This energy is absorbed by the molecules in the sample leading to process such as multi photon absorption and ionization this ionization can result in formation of plasma and high energy state of matter composed of ion and free electrons. Plasma formation leads to chemical and physical changes within the targeted structure plasma you know the fourth stage of matter where electrons have ah generated sufficient energy they are flying away from the nucleus the nucleus is separate from the electrons these electrons can in turn ah react with the surrounding molecules mostly oxygen our body has huge amount of oxygen there or they can react with the water presence knocking out one of the hydrogen atoms out ah thereby creating a hydroxyl group OH minus or ah radical oxygen group ah reactive oxygen species O double dot radical oxygen O minus and they are highly ah you know highly energetic reactive oxygen species highly highly reactive they can simply be used to eat up they can simply be made oxygen oxygenation is by definition heating up or you know burning something they can be utilized for heating up a specific areas that you want to target that you want to remove, but at a neuronal level very few surgeon can actually do an operation and take out a single cell using laser perhaps they are more equipped to do that. The energy absorbed by molecules can result in bond breaking fragmentation or creation of reactive oxygen species ROS these effects collectively causes disruption or ablation of the structure the parameters for nano surgery including laser power and exposure time are often adjusted through iterative process. Now, both previous lecture and this lecture you have to understand they are still under development yeah, we are not simply putting lasers inside human brain ah yet it it is still to be standardized, but overall the potential is there researchers carefully optimize these parameters to achieve the desired level of disruption while minimizing damage to the surrounding area. Selectivity is the key you cannot simply you know burn away entire brain tissues and then ah say that ok the tumor is removed you have to be very very selective about what needs to be done and these selectivity these optimization computer science or even electronics know how

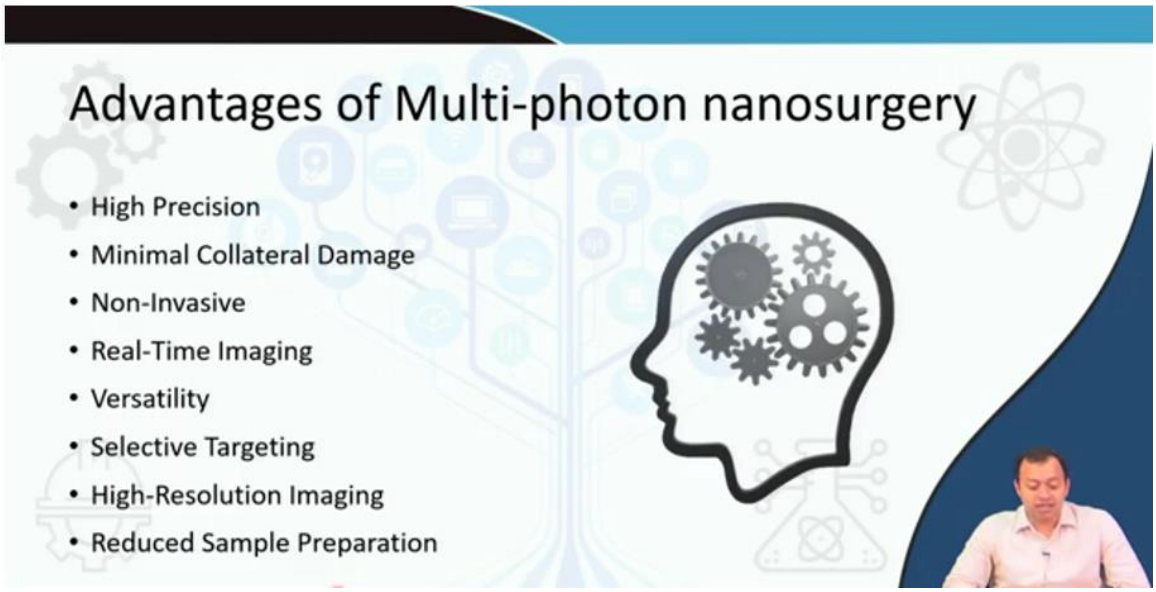
difficult optimization is, but we need to achieve optimization in this which is still lacking in in in mouse brain we are trying to do and hopefully we will get this soon.



So, lasers and nano surgery regimes using femtosecond oscillator very small pulses ah existing for few femtoseconds. So, that you do not damage the nucleus damage the atom the damage is minimum no matter how much localized your area is you are going to cause damage and sometimes you need the damage, but still you need the heat to be dissipated locally closely and as soon as possible effect involve thermo elastically induced formation and growth of plasma bubbles mechanisms are related to thermo elastic effect it absorbs it swells up and then dissipates the heat and comes back to its original position how fast this process works determine you know how successful your surgery and how localized your surgery would be low repetition rate regime. So, both high repetition and low repetition employs ah amplified series of pulses with energy slightly above optical breakdown point effects are mediated by quasi free electron induced decomposition and quasi free electron quasi free electron means they are almost free, but still have some amount of connection with the nucleus they are not completely out of the bulk just like your surface plasmons they are almost free, but they are not leaving the bulk free electrons are generated to photo ionization and impact ionization they react with surrounding areas ionize it creates a ross hydroxyl groups and cause some kind of a chemical disruption.

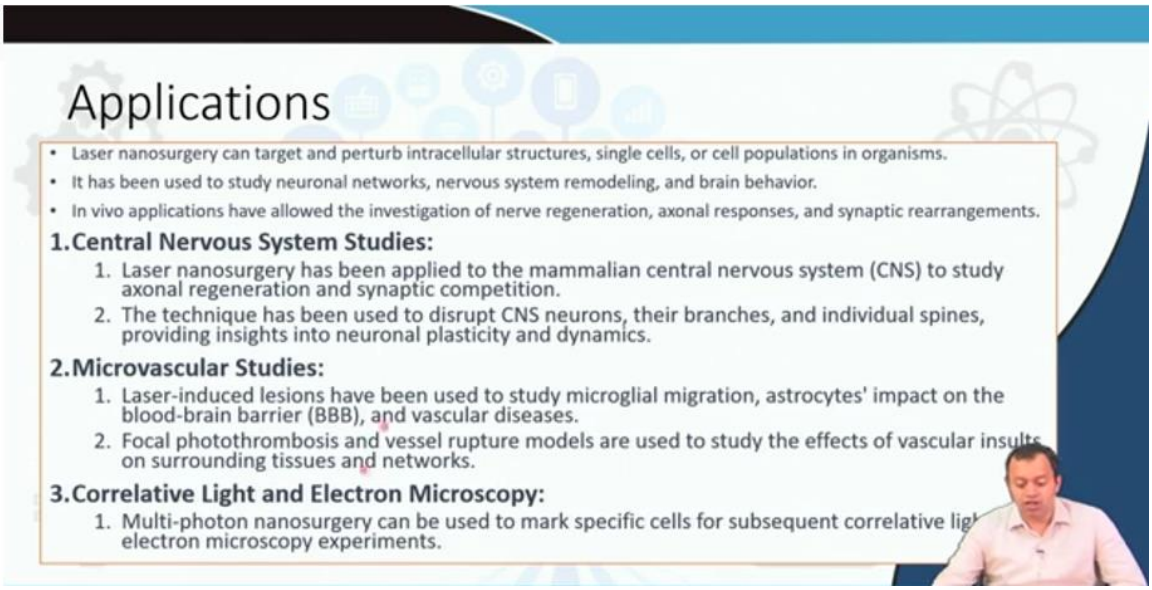


Nano surgery relies on lasers and precise targeting methods to manipulate biological structure both high repetition rate and low repetition rates are used the extent of damage depend on laser power repetition rate and pulse duration.

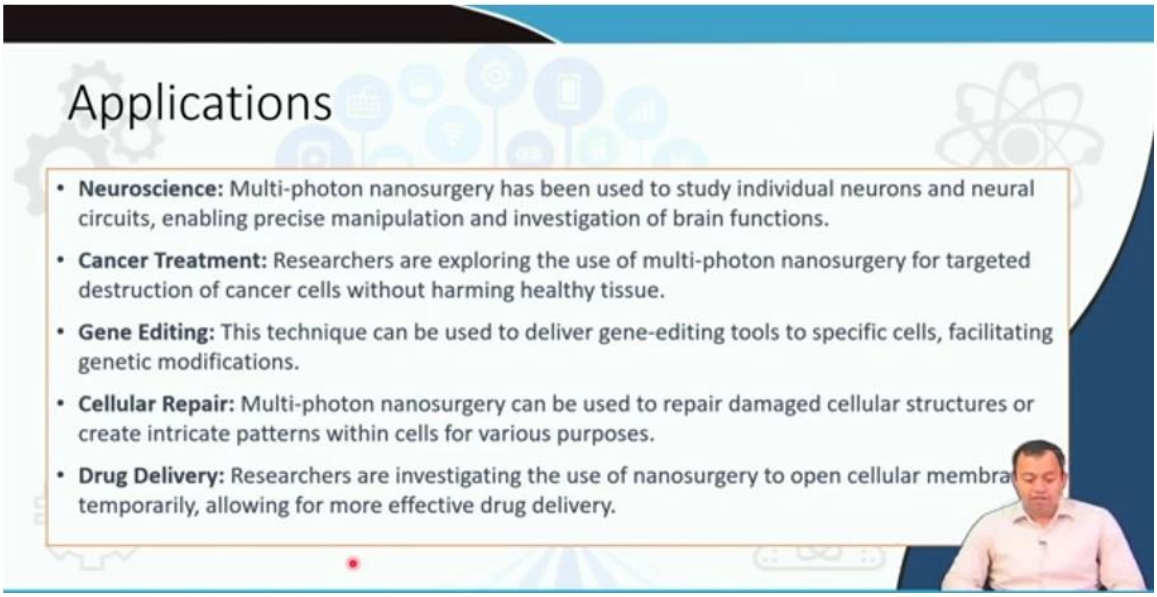


There are several advantages the one being high precision then you have minimal collateral damage or surrounding damage non invasive or I think it is minimally invasive rather than non invasive real time versatility you can target selective areas you can even ah you know be clever and allow some exogenous fluorophores or exogenous material like nano particles to be attached at specific areas of the brain and then send the laser light specifically there to create this bubble which will then in turn disrupt the laser.

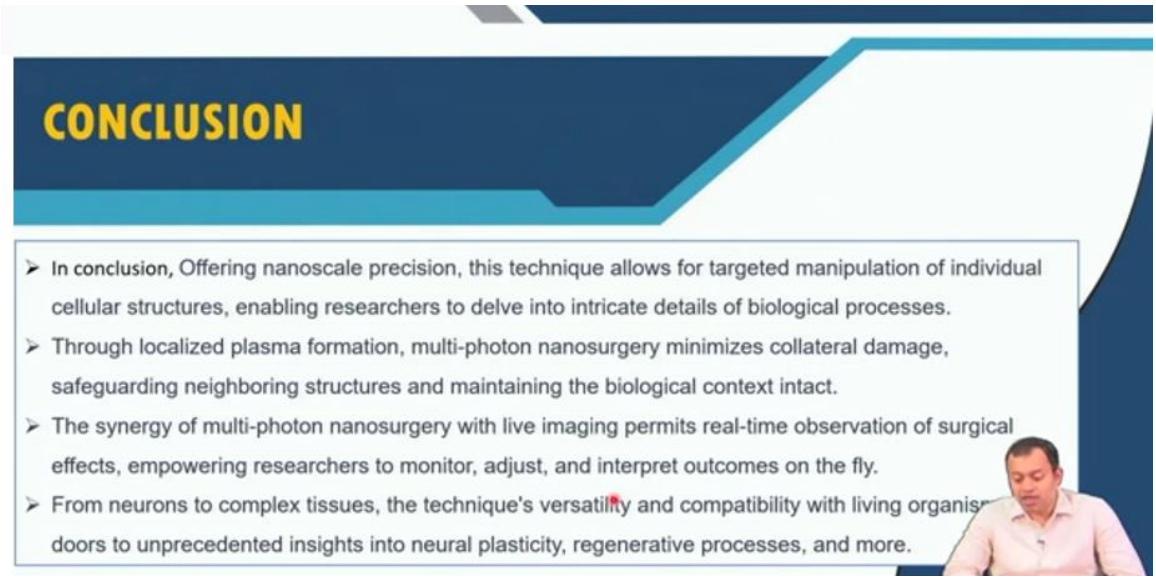
So, basically photo dynamic therapy photo thermal therapy of the brain. Several applications central nervous system studies laser nanosurgery has been applied for



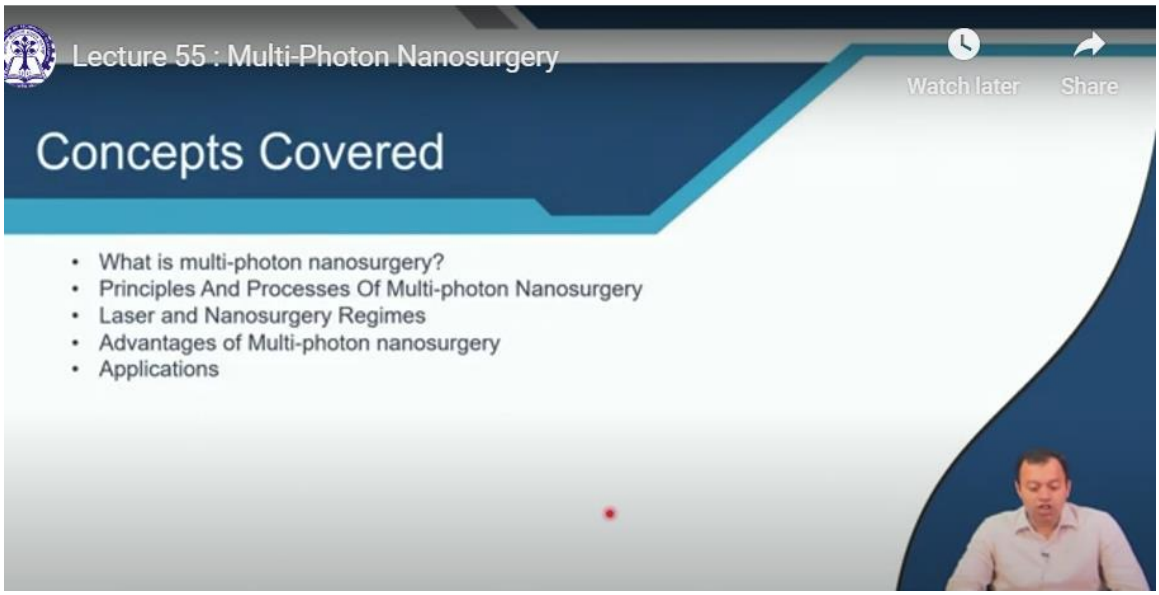
mammalian CNS to study axon regeneration and synaptic competition the technique has been used to disrupt CNS neurons you can both repair as well as disrupt. Molecular studies laser induced lesions have been used to study microglial migration astrocytes focal ah photothrombosis and vessel ruptures models these are you remember those ah port wine stain areas where in abnormal vesicles ah can be can be disrupted, but at a very very nano scale level. Electron nanosurgery along with multi photon microscopy can be used to you know mark specific cells for subsequent correlative light and electron microscopy experiments. You have application not just in neuroscience, but cancer treatment gene editing cellular repair as well as drug delivery.



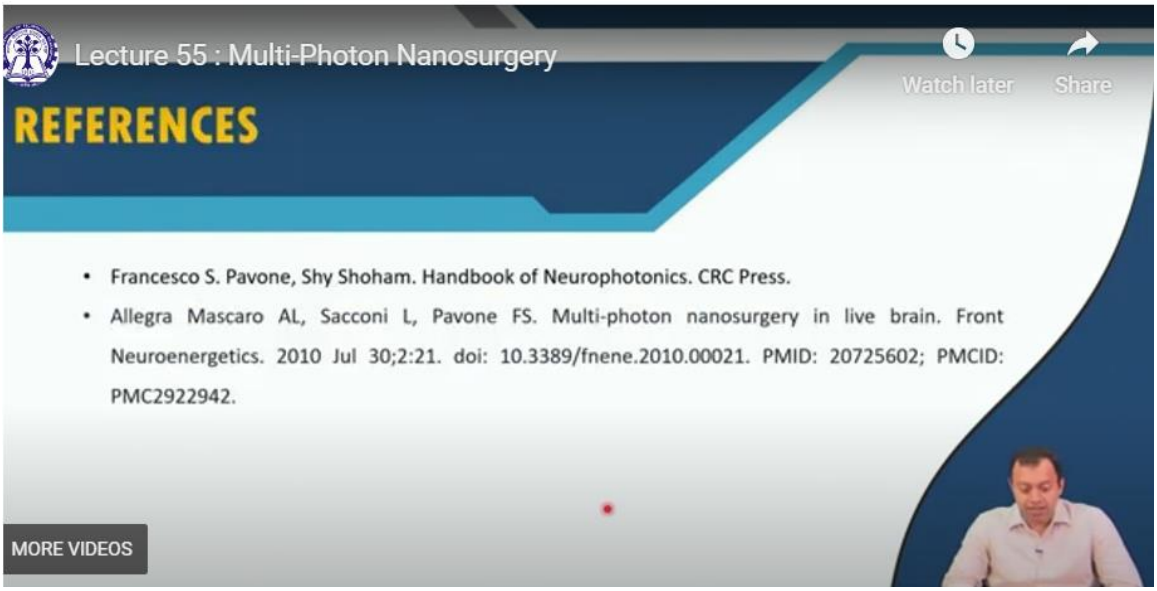
Researchers are investigating the use of nanosurgery to open cellular membranes temporarily allowing for more effective drug delivery remember we can open up certain areas we discussed in previous lectures certain gates inside the cell membrane and then allow not just ions, but come some some drugs to go inside and then remove the laser. So, that the membrane closes ah thereby the cell having more chance of retaining the retaining the drug cellular repair. So, repair remember is the far challenging one yes it is pretty easy to disrupt you know burn that area and damage that area and you know ablate that area all of those things, but cellular repair what do you do when the axons are no longer connected you can that repair part is the most complicated and the most challenging one and using lasers and nano particles and quantum dots if you are able to do it you are very close in saving some human beings life and that is overall the essence of nanobio photonics the aim of nanobio photonics why are we doing any of this this is simply to save human lives right. In editing optogenetics part is something that is coming up and cancer treatment nanosurgery to remove cancerous cells.



So, in conclusion offering nanoscale precision the technique allows for targeted manipulation of individual cellular structure through localized plasma formation multi photon nanosurgery is minimized collateral damage the synergy of multi photon nanosurgery with living imaging permits real time observation and from neurons to complex tissue the technique is pretty versatile right.



So, these are the concepts that I covered today and these are my references.



Thank you very much.