Biophotonics
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Lecture No. 48
Design of Optical Tweezers

Welcome back. In the previous two classes you have seen how strong enough focused light on a nanoparticle can be utilized to lift up the nanoparticle as well as move it around, move its position and here the nanoparticle could be a single cell or a group of cells. Today, we are going to discuss how to design such a system, how to design an optical tweezer. So, welcome to the class and we will continue our discussion on optical tweezers, scissors and traps.

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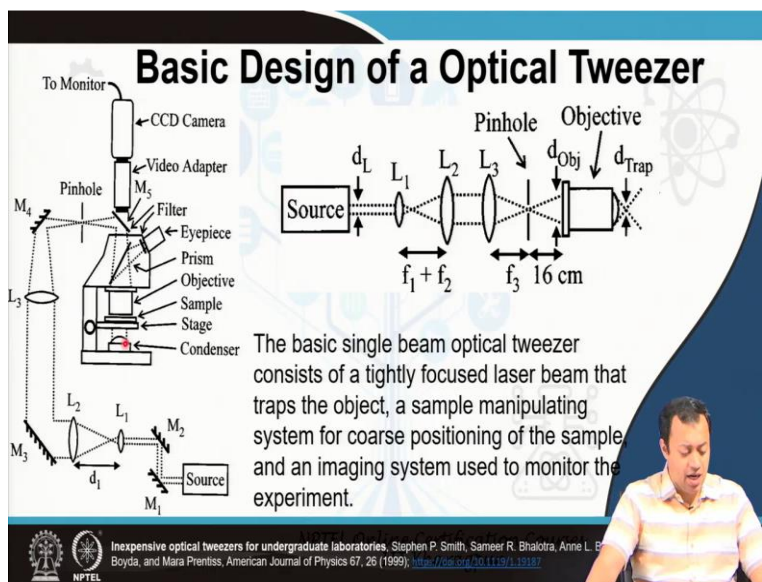

So, the idea of the design or the basic design of an optical tweezer is very straightforward. If you have a good enough microscope, something that is fitted with say good enough objective of 50X or 100X or something of that matter, it could be upright microscope, it could be inverted microscope, it does not matter for that regard. If you are able to align a laser through it, so that the laser, like the normal light that is getting focused onto a particle through the eyepiece and through the objective, if you are able to align.

So, this is an inverted microscope, for example, where the objective is below, you are putting your sample here. If you are able to put a laser source somehow aligned, so that the microscope's light, illuminated light as well as the laser's focal point are more or less fixated or more or less

aligned together, you can bring in an optical tweezer. You can have an optical tweezer in your hand. Now, make no mistake. There are several thousand sophisticated examples and alignment and modification to this design. But the crudest and the basic and the cheapest method is where you put a laser source through an objective creating some kind of a trap.

So, you put, this is the laser source, you put through several different lenses to add just the focal length per se, and finally, put it under an objective. You can put pinholes or condensers there so that those lines, you, by this time you know why pinhole is required, why a condenser is required etcetera all those concepts have already been covered. And thereby you are able to tightly focus the laser beam on to a specific spot. So, this is the spot of the focal length. So, all the light is focusing here and obviously, once light gets focused here, it will also diverge away. So, we see some kind of a V shaped structure. So, this is the interface where the where the light is.

So, this sometimes causes confusion in students I have seen. So, if you have a lens like that and light is going and it is converging into a point, it does not stop at that point. All the light has converged on to a point after that what. Usually most of the textbook shows concave lens, convex lens, all of the light going and converging into a point. What happens after it has converged? It has not simply ceased to exist, it has not vanished after it has reached a point. It then again diverges out.

So, this is exactly what is happening, all the light is converging at this point and then diverging out. So, you get X, two inverted Vs like structure with the intersection point is the focus. And this very, very tightly focused, because you have used so many different lens, change the focal length, try to put it through a pinhole, even though it is a laser, even though it is already condensed and already modified etcetera, it is still requires further modification, further polishing we sometimes say, so that the overall light beam, the optical tweezer, the laser beam gets tightly focused onto a specific spot.

Now, if this is the same arrangement at that of your, if this objective is the same objective of your normal microscope, it is even better. You save, you save some money. Otherwise, you can add this thing separately through separate objective or a separate eyepiece and focus it on to a position on the stage of your microscope. And then somehow you have to manipulate both the lasers position as well as the microscope stage and you have to have some kind of a coordination between them.

But the overall basic, basic crudest, simplest design is when you have the same illumination, the same two lights are going through the objective, one is the illuminated light of the microscope, the light, the normal light of the microscope that illuminates the stage, that illuminates the sample and through that also you are sending a laser light so that both of them are aligned.

Now, the overall light, the microscope light is at a broader area and it has, well, its focus is not that tight and it spreads, whereas your laser, if you are able to focus it, within that broad illuminated region is focused on a tight spot. And this tight spot is where you are putting your target, where you are putting your sample, where you are putting your analyte, where you are putting your nanomaterial, nanoparticle and either you move the stage per se or you move your laser, change the focal length by changing these kinds of lenses, so that the particle can move in X, Y and Z direction.

So, the particle is trapped here. And this is based on all of these focal lengths etcetera. So, if you are able to manipulate, if you are able to control them, the focal length can also be changed, it also be controlled, and thereby your particle that is put here is also going to move. So, these are basically the controlled knobs, these are basically the steering. So, of course, they help in focusing and etcetera. And there are other ways in which you can change the focus as well. So, that is still there. I do not want you to come back and say this design is completely different from what you have said. There are 1001 design. So, let us not fall over semantics.

But overall, the basic and the easiest thing could be done like this. I am giving you a basic example so that if you have the resources, if you have the interest, you can create maybe in your own laboratory, maybe you already have a good enough upright microscope, all you need to do is align a laser and laser these days does not cost much.

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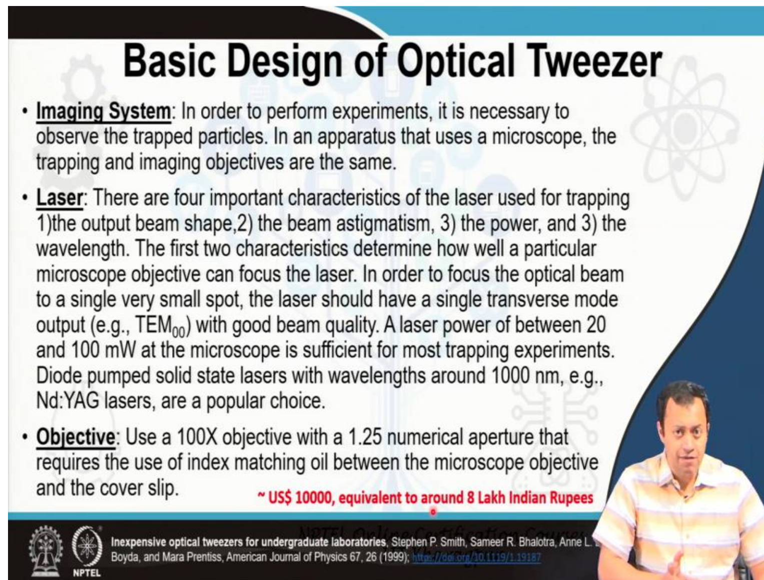

You need to have a specific power. Say, for example, 3 joules, 3 milliwatt giving you something like 4 to 5 joules per centimeter square. And if it is working at a wavelength of say, 1043 nanometer, you can easily trap cells, because these are the usual frequencies, near infrared frequencies where cells or biological matter for that regard, most biological matter for that regard do not absorbed. So, basically you need three things, an imaging system, a laser and an objective.

Imaging system is obviously the microscope, which we do, because otherwise how you are going to see a nanoparticle. A good enough microscope, so that you are able to see individual cells so that you know what you are trapping or if the trapping has happened or not. Of course, the laser, the 3 most important thing of laser are, 4 more important thing. This should be 4 instead of 3. The output beam shape, your beam has to converge rather than do something else. The beam astigmatism, you know what a stigmatism is.

Obviously, the power, so the power should be strong enough to lift it up, but power should not be that high that scattering, and all those other things comes up and thermal heating and so that the cell does not get destroyed and the wavelength. The first two characteristics determine how well a particular microscopy object can focus the laser, i.e. the beam shape and beam astigmatism in order to focus the optical. We have astigmatism in our eyes. You should know by this time. I am not going to tell you every single detail. By this time, you should know what astigmatism is. It is,

we do vision correction in our eye because of astigmatism as well. It is different than myopia, hypermetropia. So, anyways, figure it out if you are unclear about what astigmatism is.

So, anyways, we try to use a single transverse mode, transverse electromagnetic mode 00 so that in the direction of propagation, there is neither an existing electric field or a magnetic field so that any unwanted noise or anything such that does not affect. So, your, the direction of the propagation of the light is only there to trap the object. The presence of the electric and magnetic field or even its component in the same direction may disturb the molecular structure of your nanoparticle. Thereby, TM00 is quite required.

So, for those of you who do not know, this, these are the modes, TM, transfer electromagnetic modes of, transverse electric mode or transverse magnetic mode, this is where in the, during, in the direction of propagation, say for example, Z direction, the electric and magnetic field or its components are mostly in X and Y direction. It is just an example. There is no component of either electric or magnetic field in the direction of the propagation. So, that is what it is.

So, that it prevents disturbance. So, that the molecule that you are trapping, its electron cloud is not getting modified or it's not getting changed, so that the molecular structure will change and thereby the cellular structure might change. So, that is what we are trying to prevent. Laser powers of 2200 milliwatt, I said, 3 milliwatt make it 30. So, 3 milliwatt at the microscope is sufficient for most trapping experiments.

So, I was strapping something even smaller for that 3 milliwatt was required, but for all intent and purpose go for 10 to 100 milliwatt and wavelength around 1000 nanometer, 1043, 1043, 1011, 1011 nanometer etc. are a popular choice and a 100X objective with a 1.25 numerical aperture index matching oil between microscope objective and the cover slip. All of this can give you a good enough laser tweezing system.

The overall cost I got it from this, this is my reference, inflation corrected is $10,000 approximately equal to 7 to 8 lakh Indian rupees. Remember here, usually 4 to 5 lakhs is the cost of your microscope. So, almost 60 percent of this is the cost of the microscope. So, if you have a microscope already in your laboratory, say in your institution, in your college or in your university or in your laboratory somewhere in a hospital, somewhere, by spending 4 to 5 lakh rupees, you can have a laser tweezer in your hand.

Now, I am not asking you individually, you students individually to purchase it, but for a laboratory of any semi-decent college, any semi-decent engineering college or medical college or any university per se or any scientific institute per say, 4 to 5 lakhs at an instrument is doable to the best of my knowledge, is doable. I am not talking about the two extreme ends. I am talking about average, average laboratory, average college, average institute as say, average from the point of view of the financial conditions there, middle class, the middle of the road, not on quality, of course.

This much amount could be affordable, 4 to 5 lakh Indian rupees for a particular system. And thereby, you will be able to align a laser on to the microscope. And then with the help of lifting the laser up and down, changing the focal length, moving it here and there, you can look into a particular cell, put it at the same focus as your microscope objective, and then study it, rotate it in three dimensions.

Remember, if you are looking at a cell in a petri dish, you have no control of the orientation of the cell. If the cell is in the petri dish, you are trying to look it from here. What if you want to see in this direction or in this direction or in this direction, you might have to take it to a different microscope, something that illuminates from the bottom that is inverted microscope or you have to do something with the stage and that is difficult.

Here, you simply pick the cell up, rotate it, change it, move it and you will be able to observe it completely. And not just a particular cell, what about observing a particular bacterium. You can see bacteria under microscope. I am not talking about virus, but say bacteria, an individual bacterium, which is uninfected and you pick up another bacterium, which has been infected by some kind of virus, and then try to image, get a three-dimensional image of it by simply rotating it.

You will pick it up, pick up the bacteria using your laser tweezer, rotate it, just like we rotate any object in our hand which we have picked up with our tweezer, take an image of every possible angle, every possible orientation and using your some kind of an image processing skill, image processing program create a 3D construct and then see shape-wise, does the bacteria changes once infected versus non-infected. What about a cell? Do you think it will change? Think about it.

All of these things, all of those experiments very easy, very nice science experiment could be done at a lab, any engineering college lab, any semi-decent medical college lab at an affordable enough cost, at an affordable enough cost of 4 to 5 lakh Indian rupees, say 5000, 6000 US Dollars equivalent of 5000, 6000 US Dollars is just a thought. It is, and I could be wrong. I could be saying that for some people this price is too high and maybe there are other pressing needs for you to spend on to do that I completely agree.

All I am saying that such a beautiful device, such a beautiful equipment could be achievable at a semi-decent cost. That is the all of the thing and what are the other, just instead of simply viewing it simply rotating it and viewing it what are the other things that we can do?

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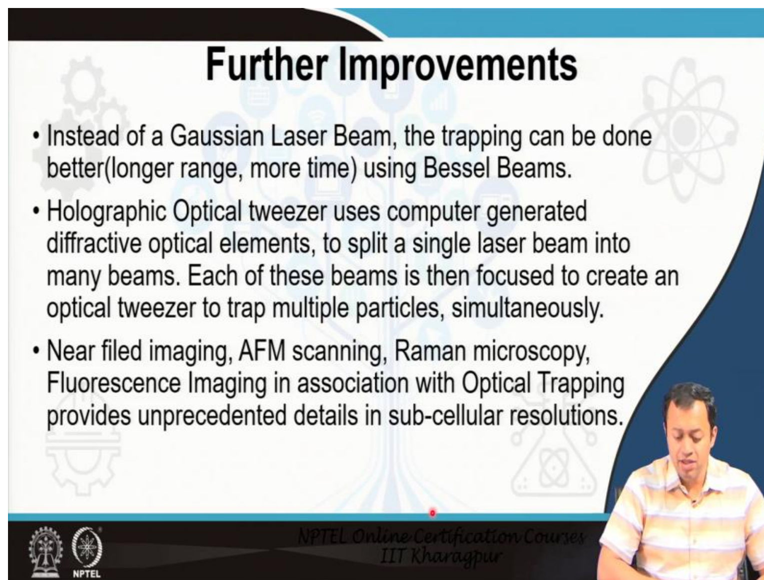

So, let us understand that how you can further improve it. Instead of a Gaussian beam you can give, go for Bessel beams. Bessel beams are one that do not diverge. So, Gaussian beams most lasers usually, are Gaussian beams, but you can go for Bessel beams. Bessel beams remain straight. They do not convert. So, focusing is bit easy. You can go for computer, everything is computer generated diffractive optics and you can split single laser beam into many beams thereby. You have many traps at a time. So, that multiple beams can be, multiple particles can be attached simultaneously.

And if you have huge amount of resources, you can add atomic force microscopy, Raman microscopy, fluorescence imaging, so that the individual molecule, individual cell that you have

picked up, you are doing a Raman, you are doing an FTIR of that, you are doing an AFM of that so that you get the internal information all of that associated with it, but this requires obviously the cost will further increase if you are adding things. But overall you get the idea.

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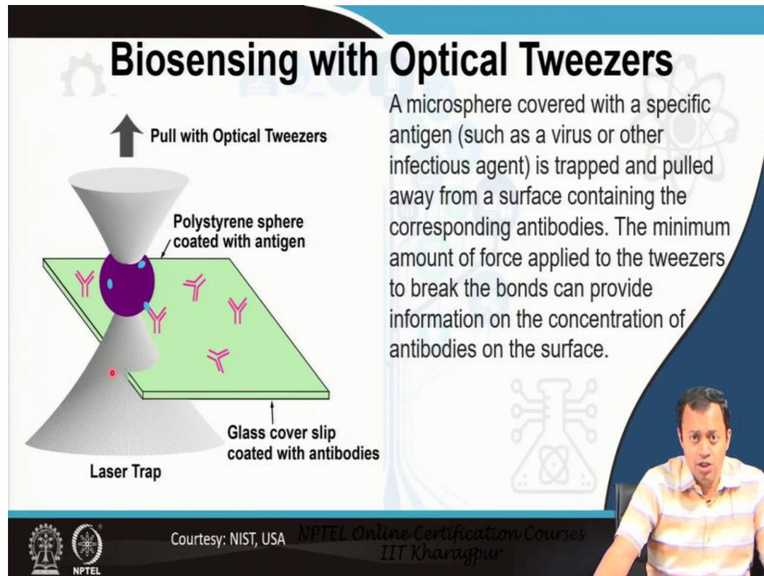

But I have a cool experiment for you to perform and that is biosensing with optical tweezer. Think about this. So, you have a polystyrene sphere. You know polystyrene spheres, we made it in the AFM-IR imaging system. You have a polystyrene sphere. The polystyrene sphere is coated with antigen. Antigen is pathogen, say for example, a virus or any other kind of infectious agent. And you have trapped, instead of the single virus, you have trapped a slightly bigger polystyrene. A polystyrene ball, a polystyrene sphere has been trapped by your laser. This is the laser and this sphere has beads of viruses or bacteria attached with it.

You have glass a cover slip that is coated with antibodies. Remember, antibody, antigen attaches, antibody and antigen attach, remember the paratope we discussed, antigens are specific to antibodies, though there are specific sets of antigens, specific sets of antibodies, there are lakhs of antigens. There are specific sets of antibodies. Antibodies will have paratopes. These are the specific hook protein that can attach like a lock and key mechanism which specific part of the antigen which is part of the pathogen as such and thereby they get attached. So, see this is attaching and these are very, very specific. You know that from biosensing lectures.

Now, so, they have been attached. You have brought this sphere coated with virus and you have brought this sphere close to the antigen and now the antigen has attached to the sphere, attached to the, the antibody is attached to the antigen which is coated onto the sphere. Now, if you want to pull with optical tweezer, you have to exert some additional force because now they have formed a bond. The antigen, antibody has formed a bond. Previously it was non-bonded. So, you can pull it up like this. The sphere can be pulled up and down.

Now, the sphere or some part of the sphere which is the antigen is attached, is locked. So, now, you have to add some extra energy, extra force to pull it away. Will this force, this additional force that you are asking for the optical tweezer to go through, will it give you information on the bond between antigen and antibody and can it provide a concentration of antibodies on the surface.

Forget about concentration, concentration is very nice, but can you make a fundamental guess, a fundamental information on the bond that is formed between antigen and antibody. And if you know that this particular bond, this particular piconewton force that I am adding extra is because of the antigen, antibody bond, can I modify this force? Can I modify this force? So, that maybe my antigen becomes stronger or maybe for certain cases my antigen become weaker.

You know the viral spike proteins hooks to cells, can I take such a cell which has just been hooked by a virus and try to pull the virus through my optical tweezer, thereby telling me the piconewton force that the virus' spike protein has employed to hook to the plasma membrane of the lung cell, why not. There is a part in the plasma membrane that is attached to the spike protein of your virus.

So, what kind of bond has formed there? It is not covalent bond. You know in biological components, usually it is not covalent bond, is either hydrophobic bond or hydrogen bond or even Van der Waals forces or a combination of several different forces. So, all these forces cumulatively add up and there is a total amount of force that by which the spike of the virus has attached to the cell. And you are now able to determine with the help of optical tweezer how you can pull it up, how much force you have to exert to pull the virus out of the cell without destroying the cell or the virus.

If you can conveniently pull it up, you will be able to understand the force. Now, what is the force? This is a bond. What is a bond? It is basically sharing or overlapping of electron clouds, that is basic of any bond, that is the basis of any bond. Some kind of overlap of electron clouds or this electric field or the magnetic field whatsoever is possible with the electrons that is overlapped and that is causing the bonds to happen. That is causing atoms and atoms to combine together, molecules and molecules to combine together and matter has formed. You know this already from previous classes.

Now, into the system you have a knowledge that say 10 piconewton is the force that the virus is exerting is the force that is between its spike proteins, its hook and the cell membrane. Can you not add or introduce some foreign particle into the system which weakens the bond? Say, there is a foreign particle which simply disturbs the electron cloud, the electron cloud that has formed the bond. So, a foreign particle has come that has either attracted electron cloud towards it or modified it or have done some other kind of a bonding. As a result, this bonding is weak. This bonding is weakened.

So, the spike protein is no longer holding on to the cell. So, the cell is still alive. The cell machinery, the cellular reproduction machinery has not yet been hijacked and the virus is getting somewhere else or it is getting weakened or this, we are basically preventing the spike protein to bond itself to the plasma membrane. And if it is, if that is done, if we are preventing it, then that is half the battle won. If the virus is not able to adhere, not able to attach itself to the plasma membrane, well, it is unable to then open the cell up and penetrate inside, take over the nucleus machinery and reproduce itself. Think about it.

These are some of the ideas that we are thinking and maybe one of you will be able to implement it much better than me using some kind of a laser tweezer that you can have in your, that you can build in your own laboratory. Maybe in your laboratory you already have a laser such as this. If you can have a microscope in your laboratory, several engineering colleges have a laser laboratory, why do not you join these two together, just a thought.

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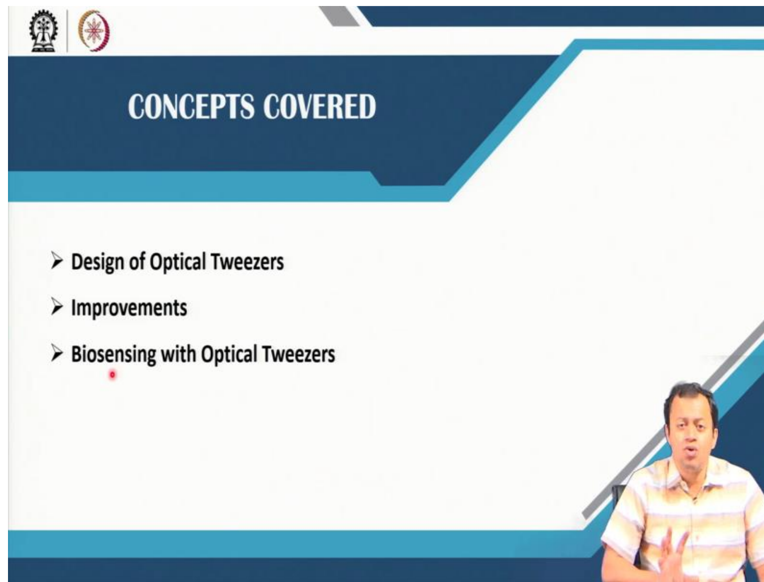

So, that is overall the idea what I was telling you. The design of optical tweezer, very easy and relatively moderately cheap. There are one or two improvements that you can do. And an idea I discussed about biosensing with optical tweezers.

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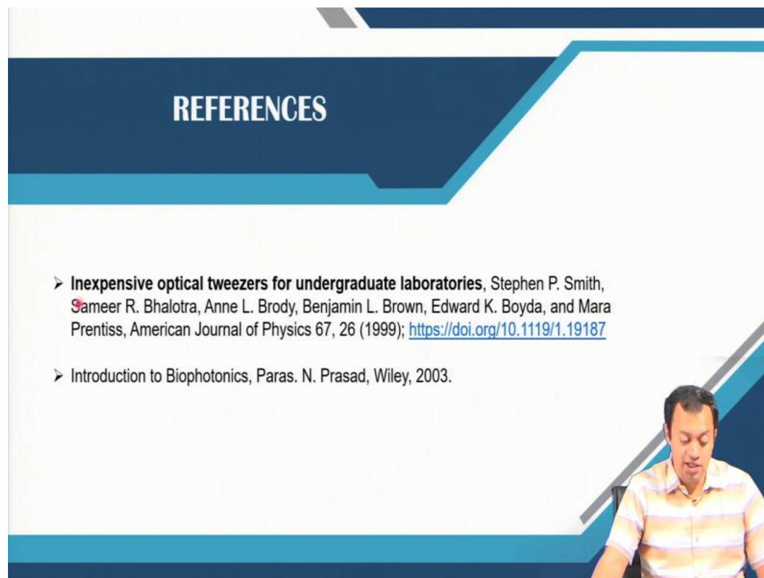

So, these are the topics. These are the references I have. Please go through this particular paper published in American Journal of Physics long time ago, 20 years ago. This was simply stating inexpensive optical tweezer for undergraduate laboratories. So, see the Americans were making

undergraduate laser tweezer for undergraduate laboratories back in 1999. We can at least give a try now. Maybe some of you are labs already equipped with it. You do not know.

But if you have this equipment a laser tweezer in your laboratory, please, please, by all means use it. If you do not have, but you have the resources separately, combine them together or ask the person in charge if anything of that kind could be made so that several of these ideas that I am talking about could be implemented. Several of your own ideas that you have thought off you could implement it yourself.

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So, thank you. Thank you very much. I will see you in the next class.