

Biophotonics
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Lecture 47
The Optical/Laser Tweezer

Welcome back. So, we were discussing about laser tweezers and laser scissors. I give you the premise. The overall premise, what it is, how it can be utilized and what are the possible applications through it.

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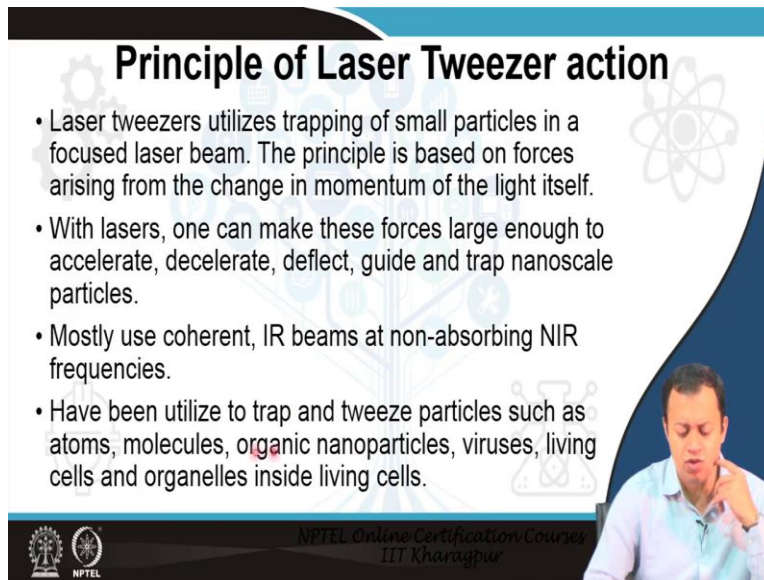


Today, I am going to go a bit of detail on the optical tweezing part, optical tweezer. Now, I will be interchanging the word optical and laser tweezers, both are equally popular, both are equally used. You understand that mostly the light that we are utilizing in some kind of a tweezing action is laser. It is coherent. It has a specific polarizability. Its energy, wavelength all of this could be controlled with the intensity etc. You will see intensity is quite important here. With a normal lamp it gets a bit more difficult. So, the, however, nevertheless laser is at the end of the day light, light amplified, something that got amplified.

So, both optical tweezer and laser tweezer are more or less the same thing. People use them interchangeably. I have kept both because some people prefer seeing optical tweezers. You must have seen or read papers, scientific papers talking about optical tweezing action, but when

companies try to, try to sell you some equipment that performs the same optical tweezing function they call it laser tweezers. We sell laser tweezers which will help you manipulate cells at an individual cellular level. So, both words are used interchangeably.

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Principle of Laser Tweezer action

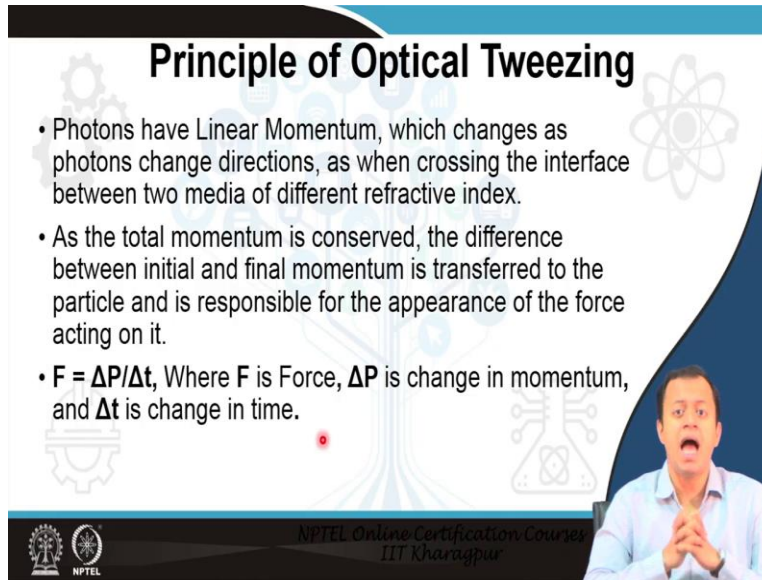
- Laser tweezers utilizes trapping of small particles in a focused laser beam. The principle is based on forces arising from the change in momentum of the light itself.
- With lasers, one can make these forces large enough to accelerate, decelerate, deflect, guide and trap nanoscale particles.
- Mostly use coherent, IR beams at non-absorbing NIR frequencies.
- Have been utilize to trap and tweeze particles such as atoms, molecules, organic nanoparticles, viruses, living cells and organelles inside living cells.

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So, let us try to understand the basic principle or the basic theory behind it. So, what are this, the principle behind laser tweezer optical tweezer action. So, the optical tweezer, see I have written laser, but I will say optical tweezer utilizes trapping of small particles in focused laser beams. You focus a beam of laser light onto a particle and the particle gets trapped into the laser beam and by moving the laser beam you can move the particle. The principle is based on force arising from the change in momentum of the light itself.

With lasers one can make these forces large enough to accelerate, decelerate, deflect, guide and trapped nanoscale particles. Mostly use coherent IR beams that are non-absorbing. If it starts absorbing the light, then the molecule or the particle will show some modification in its property. It gets excited. Molecules will start vibrating more. Here the idea is to prevent the molecules or prevent the particle from modifying its property, any property that is and we have used to trap and tweeze atoms molecules, organic nanoparticles, viruses and everything similar.

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Principle of Optical Tweezing

- Photons have Linear Momentum, which changes as photons change directions, as when crossing the interface between two media of different refractive index.
- As the total momentum is conserved, the difference between initial and final momentum is transferred to the particle and is responsible for the appearance of the force acting on it.
- $F = \Delta P / \Delta t$, Where F is Force, ΔP is change in momentum, and Δt is change in time.

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So, let us understand the basic idea. So, imagine this, a bunch of photons, which have a specific energy and specific momentum is changing direction, how, by moving from one medium to another medium. Whenever light moves from one medium to another medium refraction happens and refraction happens with a specific law. We call it Snell's law. And you many of the time defined refractive index as the change of the velocity from one medium, denser medium to rarer medium, rarer medium to denser medium, however, you want to define it.

So, whenever light moves from rarer medium to denser medium, there is a change in its direction. It moves away from the normal or towards the normal depending on whether it is moving from rarer to denser or denser to rarer, Snell's law, we all know this. $n_1 \sin \theta_1 = n_2 \sin \theta_2$. The idea here is the velocity changes and optically rare medium, for example, vacuum has a maximum velocity of light, photon particles that is.

And whenever the photon enters in media something which is optically denser medium, air or water or glass or any other matter, for example, there is a change in velocity. Now, understand this. If there is a change in velocity, there is going to be a change in momentum. Momentum is mass into velocity at the end of the day. So, if mass into velocity changes, if momentum changes, we also know that overall the momentum needs to be conserved. This is like the oldest theory in the book, conservation of momentum. It is like coming from thermodynamics and whatnot the total energy of the system needs to remain fixed.

So, the total momentum of two scattering bodies, two interacting bodies, Newtonian mechanics, two billiard balls, we have seen this middle school physics. One billiard ball is hitting another billiard ball. One atom is hitting another atom. At the end of the day, input momentum and output momentum have to be same. Somehow the momentum has to be conserved. Total energy before and after has to be same. Total momentum before and after has to be same.

So, how is that change of momentum, momentum in and momentum out that ΔP will be enacted upon the particle it is hitting. The ΔP , mass into velocity, the change in momentum will be enacted upon as a force, because force is ΔP by Δt , that is basically the force that hits the billiard ball and the billiard ball move, something moves upon acted by a force. The direction of the force is where the particle goes. Why is the billiard ball or the carom disc or the carom coin falls on to the pocket at a specific direction? The pocket, the hole is in a specific direction. You send, you use some kind of a force to move it into that specific direction, into that specific target, into that specific pocket. What do you do?

You send some kind of a force. The force is rate of change of momentum with respect to time. And this is happening because the force is exerted by the change in the direction of the photons. Direction at the end of the day is something to do with momentum, do not you think, momentum is mass into velocity. So, again, let us recapitulate. Photons while moving from say rarer medium to denser medium undergoes a change in velocity. When they undergo a change in velocity, they also undergo a change in momentum. Momentum, Newtonian mechanics, mass into velocity, but nevertheless, there will be change of, even from a quantum mechanics point of view, there will be change in momentum as well.

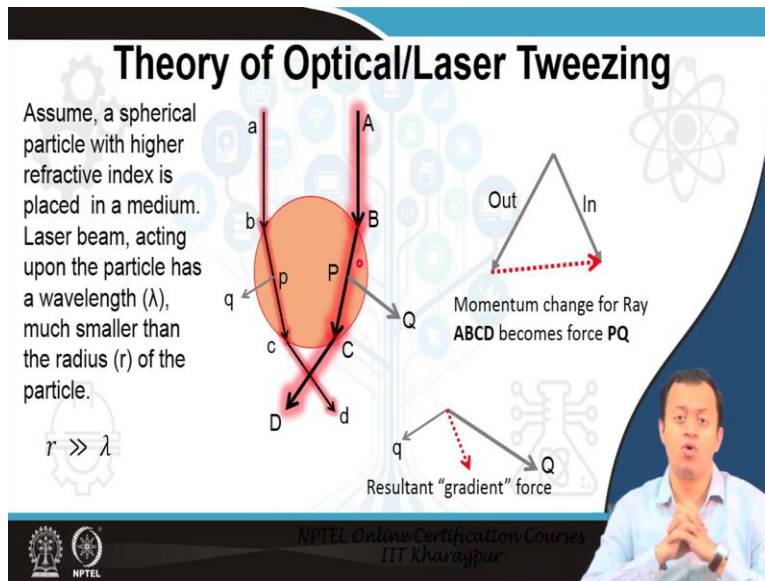
In quantum mechanics, the mass is not constant, mass is relativistic. The mass in quantum mechanics is not constant depends on the velocity per se. So, there is a thing. However, you should not be concerning yourself with that understand this much. Whenever there is a change in the direction of the photon, there is a momentum change. The momentum change has to be conserved somehow. In momentum has to be out momentum and that change of momentum is enacted, the conservation of momentum is enacted as force onto the particle.

Usually the force onto the particle upon which it is enacting is negligible, some piconewton as such. Piconewton has absolutely no concern whatsoever on a macroscopic particle. The force is

there, but the force is unable to dislodge. The force is there, but it is unable to move its property or change its direction or change its position, because the force is incredibly negligible, but it exists, because conservation of momentum has to happen. The change of momentum is very, very, very small. So, the force in acted upon the particle is also very small.

So, for all intent and purpose for most particles, it bears no consequences. However, when we are talking about nanoparticles, it does have consequences. And we manipulate that, we manipulate this force to manipulate the position of a nanoparticle. That is the overall story. I will try to give you a bit more.

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So, suppose you have a nanoparticle, for simplicity sake, we call it a sphere. Sphere of radius r . And this radius is much larger than the wavelength of the laser light that has enacted upon. So, the radius of this particle is much larger than the wavelength of laser light it has enacted upon. So, there are a specific case. Let us take a specific case here. You have one single photon enacting upon this particle, enacting upon this nanoparticle in the direction small a, b, c and d. This is the direction in which a single photon is moving.

This was air or a surrounding media. You can consider it as water. Usually we put cellular structure inside water for it to live. Usually it is difficult to put it in air, but forget about the timing, the surrounding material, forget about the timing for the surrounding material. So, you have a particle whose size is larger than the wavelength of laser. A photon comes, it hits the

particle according to Snell's law refraction. It will undergo refraction and thereby its direction will change, thereby the velocity will also change, it hits, and then it returns to its original position and comes back to d. So, the direction from a, b, c and d will be like this. This is how it happens normally.

The idea here is the photon needs to simply transmit, simply pass through one single photon without getting reflected, without getting scattered, without getting absorbed, without doing any of that. Simply a photon is coming, it is getting refracted and it is returning coming out and no it will not be in this direction. There will be no course correction like this. Why, read Snell's law. This will go in this direction itself.

So, while there is a change of momentum from media one to media two, i.e., when the photon has moved inside the particle, the velocity has changes, the velocity has made the momentum change, the momentum has resulted in some kind of force been enacted upon the particle and the force here is p, q, small p and small q. So, the direction of the force that has enacted upon this particle is given by this arrowhead p to q in this direction, p to q in this direction. While this process is going on a, small a, b, c and small d, small a, small b, small c and small d, two photons this time simultaneously have come up in the other direction capital A, capital B, capital C and capital D. This is small is one photon, capital is two photons. Lowercase is one photon, upper cases two photon.

So, two photons are moving in this direction, refractive index is same. So, similar kind of effect will happen. B will go into C direction, capital B to capital C, and capital C will move into capital D. So, this is bit bloated, because it is two photons as compared to d. Intensity is twice. So, the momentum is also twice, one photon, two photons. So, the momentum is twice. So, the force per unit time is also twice. So, capital P capital Q, this is the force that will enact, that will act upon this nanoparticle. So, two sets of forces are acting upon the nanoparticle small p to small q and large p to large q.

So, if this is small p or capital P, one is taking it in this direction, small p to small q, one is taking it in large P to large Q direction, normal vector mechanics, high school physics. Remember, a particle is enacted by two forces. One force is trying to throw it in this direction. One force is trying to throw it in this direction. These two forces are not balanced, i.e. one force is weaker, a,

b, c and d, small p, small q is weaker, this force is weaker, but it exists, it is finite, it is not 0. And one force is slightly stronger and it is moving in another direction.

What do you think will the resultant be? A particle is enacted upon two different types of forces. One is weaker. One is stronger. There will be a resultant gradient force. This gradient will depend upon will be more towards the stronger section with a particular direction. There will be an in and out. Remember, these are the math you used to do in, I think, first year physics or class 12 I believe high school where a billiard ball has been enacted by two separate forces from two separate direction. This is the same thing only in nanoscale, two different forces. This is less intense. This is more intense. This one photon. This is two photons. This momentum and this momentum are twice. So, the force is twice that of the force.

So, overall the resultant is a vector superposition or a vector calculation of force p, q small and P, Q large, resulting in the movement, overall movement of the nanoparticle in a specific direction according to the vector calculation that I have by two different forces. So, if I can control this intensity a and b, small a and small b, and large A and large B, if I can control in which direction I will be focusing the laser to the particle, can I control the overall gradient force. If I can control the resultant gradient force, can I control the direction in which this particle will move? You control the force. You control the direction.

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Theory of Optical Tweezers

- Particle is forced towards region of more intense light.
- This is called gradient force.
- The overall balance of forces determines the overall movement of the particle.

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Next case, you have the same force, but acting oppositely A to B, C to D, the same exact two photon, same intensity, same coherence, same polarization, but in another direction. So, you have Q, P, Q and P, S. I forgot to put P here. So, these are the forces that enact upon this nanoparticle Q and S. So, you are pulling this by a balanced force in both directions. And the force is small enough so that it does not damage, does not stretch it, does not tear it apart, but both the forces are same, balancing one another that direction. They are equal and opposite.

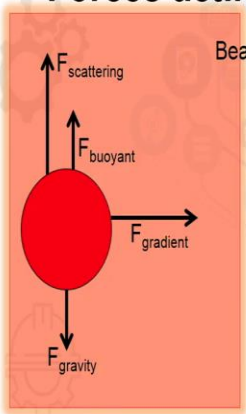
What do you think will happen to the particle in that particular case? Can we then consider the particle either, according to the vector calculation, static, not moving? Can we, therefore, call something that is not moving as trapped? And if it is trapped, we are putting some kind of a lens on top of it, focus in it separately to image it properly. A live issue, a live cell, a live bacterium, a live virus has been enacted by light force and you are simply trapping it because the light force here, big pardon, and the light force here are completely same, equal and opposite.

The overall balance of forces determines the overall movement of the particle. So, the total combination of forces that is enacting, it does not have to be just two understand this. It does not have to be just two photons falling in different direction. Remember the size or the wavelength of these photons are much, much smaller than this. So, you can have up to you a uniform distribution of photon falling on two all over the surface or a non-uniform, a heterogeneous.

The overall balance of forces will determine the overall movement of the particle. If you can control this, you can control the overall forces that are enacting upon the nanoparticle. And thereby, you are controlling the movement of the particle. Remember the video that I showed you. How beautifully they are moving that. Some kind of a dance. Look into Wikimedia Commons they have beautiful videos of manipulating cellular structures. In fact, they manipulated red blood cells into some kind of a matrix 4 by 4, 3 by 3 and all the cells were moving in tandem like a choreographed dance. You can do it. Why not? It is the same principle.

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Forces acting upon the Nanoparticle



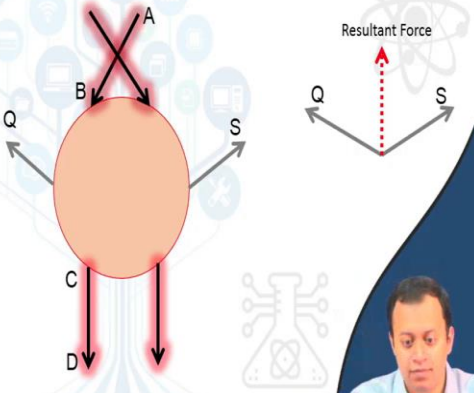
The diagram shows a red circular nanoparticle with four force vectors: $F_{\text{scattering}}$ pointing up, F_{buoyant} pointing up, F_{gravity} pointing down, and F_{gradient} pointing right. A vertical dashed line labeled 'Beam Axis' is to the right of the particle, with a downward arrow indicating the direction of the beam.

The restoring gradient force is larger than the scattering force, and the gravitational force is balanced by the buoyancy force. Therefore, the particle can be **TRAPPED** by the laser beam.

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The diagram shows a particle being pulled towards a region of higher light intensity. Two beams, labeled A and B, converge on the particle. Two other beams, labeled C and D, are shown below. A resultant force vector is shown pointing upwards, with points Q and S also indicated.

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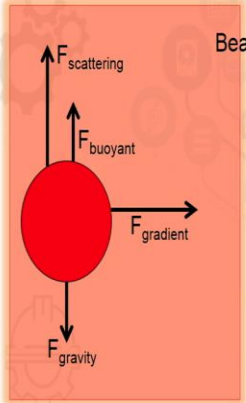
What are the other forces that acts upon it? So, remember, the overall total forces that is acting upon the particle which you are trying to manipulate are thus far. You cannot escape gravity. So, if this is a beam on which a nanoparticle has been placed. This is the laser beam with the beam app axis like this, you will always have gravity. Gravity is going to pull. If you have put it in some kind of a liquid medium, say water, if your cell structure, if your nanoparticle is in some kind of a liquid medium, there will be a buoyancy. Buoyancy is always there, that is how ships float, things float. There will always be some amount of photon that will get scattered.

You can prevent absorption to an extent that no light is getting absorbed inside, because it does not match the energy levels, vibrational energy levels or the electronic energy levels, but chances are scattering can happen. But the overall momentum change will also occur because of the presence of these many number of photons. So, this entire thing are photon beams. Previously, I have shown only one or two, but actually you have several of them.

And you can, if you can change the intensity profile and make the overall gradient force, the overall gradient resultant force, the overall resultant gradient force greater than gravitational attraction, buoyancy force that is simply floating it in a direction and the scattering force, well, your problem is solved. You are moving the particle in the direction of this force. You are controlling this. And thereby, you are controlling this. You are controlling this force. This is the light force. Think about it. This is the light force that will overall control the direction.

Now, any time there will be a gravity working on it. If it is in a liquid medium, there will be buoyancy working on it. Some of the light will get scattered. The overall idea is the total balance of forces has to be such that the gradient forces are greater or is able to overcome all the other forces that is enacting upon it. If all of that comes to some sort of an equilibrium position, your particle is trapped in a laser beam. Overall, that is the answer.

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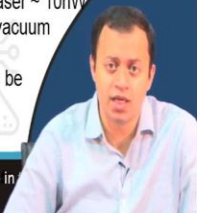
Gradient Force


The restoring gradient force is given by

$$F = Q \left(\frac{n_1 P}{C_0} \right)$$

Where, F is the maximum force exerted by the trap
 Q is the scaling constant that depends on the particle size and the difference in refractive index between the particle and the surrounding media
 n₁ is the refractive index of water at 1.33
 P is the incident power of Laser ~ 10nW
 C₀ is the velocity of light in vacuum

All these, gives the Trapping force to be approximately **10 PicoNewton**





A. Ashkin, "Forces of a single-beam gradient laser trap on a dielectric sphere in Biophys. J. 61, 569-582, 1992

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And this force, the overall F gradient force is given by this particular formula. Check out this paper, force of a single beam gradient laser trap on a dielectric sphere ray optics, not quantum optics regime. This is a fantastic paper. Long time ago they predicted it. This is the force that is the overall that comes up that determined. So, the force is a maximum force exerted by the trap. Q is the scaling constant. This is a constant that depends on particle size and difference in refractive index between the particle and surrounding media. There is a whole list of this. This is basically the constant, the proportionality constant.

P is the incident power. N_1 is the refractive index of water. They have put it in some kind of a water medium. Usually cellular structures are put in water medium for them to survive, for them to leave, as well as we do not want a very abrupt change in refractive index between these to this. The momentum change could be disturbing, could cause scattering, could cause some kind of a problem. So, if the water having a refractive index of 1.33, the cells have a refractive index, a combined overall effective refractive index of 1.35. So, the direction change is less abrupt. So, the cells can survive better or the force can be better controlled. And C naught is the velocity of light in vacuum.

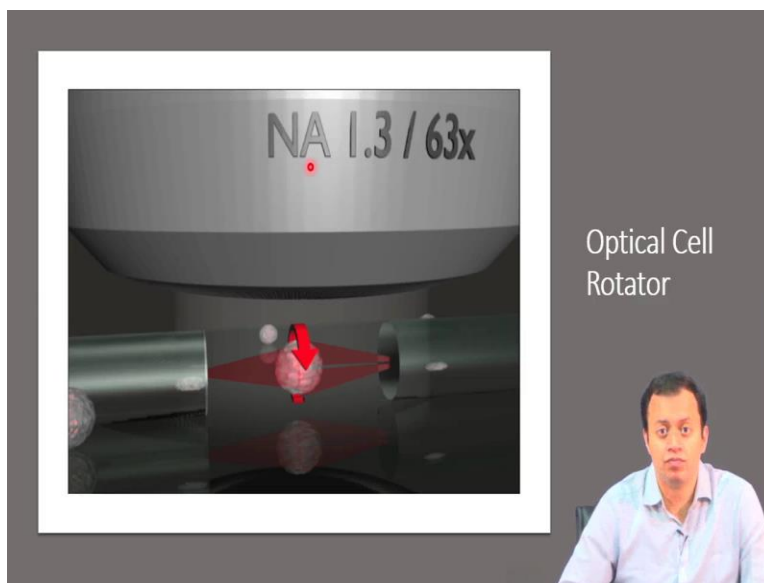
If you put all those values including Q , you will see a force of 10 piconewton is acting upon your nanoparticle and this 10 piconewton is enough to negate the effect of scattering, buoyancy and most importantly gravity. Well, technically gravity is somehow negated by buoyant forces anyways. That is how things float and do not sync. Cells also float and do not sync, Archimedes

principle. So, this gives the trapping force of 10 piconewton. So, that is the force the 10 piconewton. Think about it. 10 to the power minus 12 newtons. Do you know the force that acts upon 1 gram of pebble that you throw from top? Force is equal to mass into acceleration. Think about it. Here acceleration is G, 9.8 meter per second square.

And if the mass is 1 gram, what do you think is the force here? What kind of a mass you need for an acceleration of say G for 10 piconewton to do some kind of an effect. Force is mass into acceleration. I am giving you the force. I am giving you the acceleration as 9.8 meter per second square. What should be the mass of the material that you can manipulate? So, this is the overall general principle that light produces some kind of a force when it enters a medium. That force is in credibly small piconewton as such, and thereby for most macroscopic elements, most macroscopic matters it is purely neglected.

However, we utilize this force in manipulating nanoparticles or materials, which has sizes in nanoscale region as well as mass or in this particular case weight in pictogram, nanogram, atto-gram region.

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So, can we use a cell rotator? So, you have trapped a particle, but now instead of the laser light going like this, laser light is going like this rotating. So, can you rotate a particle that you have trapped? Thereby, can you take a full three-dimensional image of the entire molecule, entire cell using your microscope lens near scale microscopy, very close to it, bring it very close to it and

there is a laser beam that has trapped it and the laser instead of like this is moving in this particular direction. Can you do it?

We have an experimental setup that exactly does that. If you can individually rotate, trap and thereby rotate a single cell, can you rotate a single virus? Can you rotate a single bacterium? Try to look through the microscope which is obviously connected with a camera which is again connected with obviously image processing tools, try to see the vulnerability, the weakness of this cell or this virus and thereby know where to attack if you want to attack or where to put reinforcement if you want this cell to survive. Remember this is live. You switch off these lasers, it goes back, returns back into the petri dish where you have taken it from.

Only problem is this has to be outside the body, in vitro in a petri dish. You cannot put all of this thing inside human body. Theoretically possible, but still optical tweezing to the best of my knowledge has thus far been mostly done in human body, in a petri dish in a laboratory. But still it gives us huge amount of input. Think about it. This cell, this cell, they are not touched. You are picking up one single which has fallen into this trap, which has fallen through the path of this beam and you are rotating it thereby checking it in three dimensions.

Can you move this microscope, this lens from top to bottom or to sidewise and this laser which has trapped it goes up and then it moves like this? So, you can move the cell in any axis you want. These are several of the flexibilities that you can get with optical tweezing. And remember you are not touching it with any particle whatsoever except photon. Generally, photon will not contaminate it. Photon can excite it. But if you have taken care of a particular lambda which simply allows light to penetrate it without exciting, without absorbing, your problem is solved. This is the basic of laser tweezing. Newtonian mechanics is more than enough to understand it. You can use quantum mechanics, but I do not think it will give you any other information or much extra information. Surely it gives something, but that is the idea.

So, I asked you to read a bit about optical tweezing. In the next class, I will discuss about the instrument that is required for optical quizzing. Believe it or not, the instrument is simply a microscope enabled with a laser. And there are several papers in which very inexpensively within couple of Indian lakh rupees, you can build one of this in your laboratory and thereby image or trap single cells, manipulate their position, rotate them, trap them, maybe put some

amount of scissoring into them, punch hole and check their property out. That is what the essence of biophotonics is all about. You will see you in next class. Please go through it. And we will be discussing some more in next class.

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- Principle of Optical Tweezer Action
- Theory of Optical Tweezer
- Gradient Force
- Cell Rotator

So, these are some of the concepts that I discussed.

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- Kirkham, G., Britchford, E., Upton, T. *et al.* Precision Assembly of Complex Cellular Microenvironments using Holographic Optical Tweezers. *Sci Rep* **5**, 8577 (2015). <https://doi.org/10.1038/srep08577>
- **Inexpensive optical tweezers for undergraduate laboratories**, Stephen P. Smith, Sameer R. Bhalotra, Anne L. Brody, Benjamin L. Brown, Edward K. Boyda, and Mara Prentiss, *American Journal of Physics* **67**, 26 (1999); <https://doi.org/10.1119/1.19187>
- A. Ashkin, "Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime," *Biophys. J.* **61**, 569–582, 1992, [https://doi.org/10.1016/S0006-3495\(92\)81860-X](https://doi.org/10.1016/S0006-3495(92)81860-X)
- Introduction to Biophotonics, Paras. N. Prasad, Wiley 2003.

And go through several of this paper, especially this one, inexpensive optical tweezer for undergraduate laboratories written in 1999, 20 years ago. This price has further reduced or you

can reduce it. You can make your own optical tweezer. Trust me, it is very easy. Just read about it. I have given the DOIs of it. You can check them up yourself.

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And I will see you in next class. Thank you.