Optical Spectroscopy and Microscopy
Prof. Balaji Jayaprakash
Department of Center of Neuroscience
Indian Institute of Science – Bangalore

Lecture – 49
Fundamentals of Optical Measurement and Instrumentation

Hello and welcome to this course on Optical Spectroscopy and Microscopy. So far, what we have done is that we have looked at the basic principles starting from the light matter interaction, how do we describe the light, how do we describe the matter, and the generation of the light itself using the laser light. Having generated the laser light, then how do we route this laser light through different optical elements or using mirrors.

How we can actually choose the path the light beam takes in a laboratory frame so that we can actually send the beam and construct different optical equipment using these mirrors and the light. In doing so, we were also looking at the different principles of optical microscopy, particularly the laser scanning microscopy. If you have to look at the laser scanning microscopy and the reason why we are looking at is the localization of the excitation and the emission.

I mean if you can do that in spatially in some manner, then something is of tremendous value and in doing so, I used without explicitly stating, I used some of the simple geometrical optic principle.

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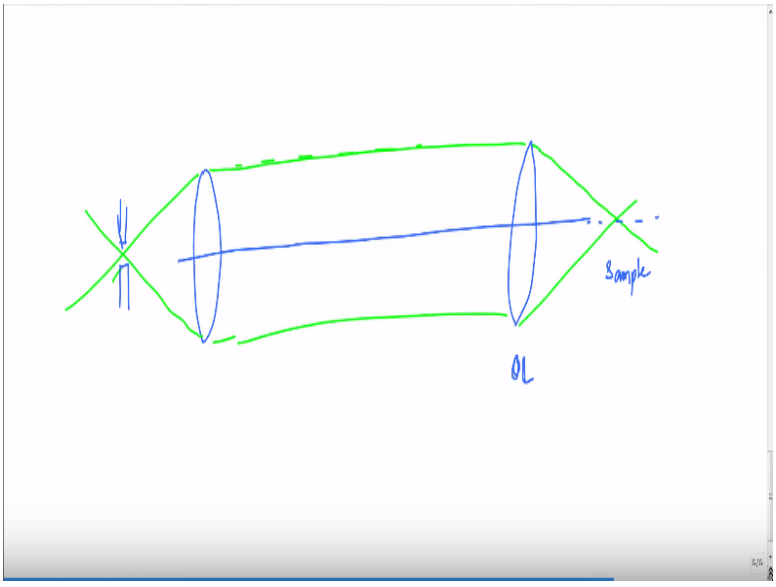

If you remember, we actually started of actually by saying imagine there are two lenses that are kept such that their optical centers fall in a straight line and then what we said is that to understand the focal principle, localize the emission photons in x, y and z all we have to think of is that, think of the race that are originating from the focus of this lens and what happens to it when it passes through another lens.

And then the idea is that we actually put in here aperture that selectively collects more of the light from the focal plane than before or after. Now, the point here is that if you look at this geometry, there are two lenses. The quint essential microscope that you can think of and one of the lenses, the lens that is closer to the sample plane, that is a sample, we call that as an objective lens, it is of paramount importance in any microscopic system for quite a few reasons.

As you will see in this lecture, all of this we are gearing up to be able to localize very close to the theoretical limits or the limits we can reach with an ordinary optics. So, if you have to do that, then a lot depends on the nature of this lens, and we would understand what are all the different artifacts or the effects that we need to keep in mind and how we can overcome these things in a modern objective lens is the subject matter of this lecture.

In order to get into that, let revisit our simple high school optics. In schools, when we want to actually understand the way in which light is propagating to the lens, we would use geometrical ray diagrams, so they follow very simple principles.

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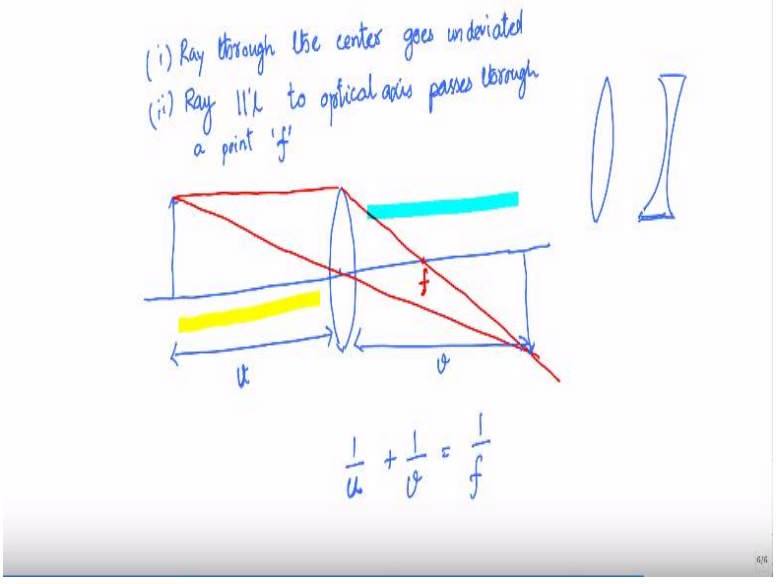

I am going to just list out few of them, not all of them, few of them that are essential for us to follow in this course, I mean follow in this lecture that may be useful. Number one, there are two kinds of lenses, we know it is a convex, the lens in this case specifically biconvex lens or concave lens. So, in order to understand the propagation through this, what we are going to draw at the least two rays, so let us take a simple biconvex lens.

Ray number one passes through the optical center of the lens originating from an object. A ray that is originating from a point in an object, so that is my optical axis. So a ray that is originating from a point in an object and that passes through the optical center goes un-deviated. So, that is rule number one. Rays through the center goes un-deviated. So, now the second most useful rule here is a ray that originates from the same point, but then that is parallel to the optical axis. May be you should draw this in a different color.

This is optical ray number one. Second optical ray that goes parallel to the optical axis for a biconvex lens goes through the focal point f on the optical center on the other side of the lens deviates such that it goes through on the other side of the lens. Now, this point is the focal point f. Whenever, these two light rays meet or wherever it meets, that corresponds to the image of the object that you have originally started.

So, I can think of this object consisting of many such points and then you can actually draw each of this and then construct this whole object, and we would have an object shown up like here. Now, the second rule there that we have used is this ray parallel to the optical axis passes through a point f on the other side for the biconvex lens. Now for the concave lens, the second rule stays exactly the same except what happens the line will be such that.

It will be deviating away from the optical axis and then you have to extend such that it meets on the side where the object itself is there. There are two sides. This side of the lens, which is colored yellow where the object space, you can think of that, the sample space and this is the other side that is colored light blue, you can think of that as a image space. I am coloring the opposite side with two different colors, so for the biconcave.

Everything would be more or less the same except now; this will be on the other side. We will not be using much of that as of now of the biconcave, so we will not specifically go into that, but it is important to know that, for the biconvex these rules are very simple. So, you

start from the object, goes through the center, goes un-deviated. Start from the object, go parallel to the lens, then it goes through the focus and wherever these two rays meet, then you form the image.

So, now in such a system, the distance between the object and the lens, and the image and lens are given by, let us call this as u and v and so you can write down this in such a system, it is given by 1 over u. So this formula again you have seen it before. So, the point for me is to say that these are related and you can actually define where exactly these rays will go through. Now, this comes in very handy when in the lab many times what you do is that you construct, you want to expand, you want to change the size of the beam.

Why would you that. So, now when you are talking about assembling a microscope or constructing a microscope, we said okay, let us start with a simple microscope that has this principle of that you can use it for detecting in a focal manner. So, the first thing that comes to your mind is that in order for you to actually get the smallest focus, you need to expand this beam. It need to be as wide as the objective lens itself.

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So, just to give you the picture, to start with the laser light is a pretty small. The diameter of the laser light is very small. It is of the order of a few millimeters, in fact we have all seen this, when you have a laser pointer in any presentation, you see that how tiny the laser beam is and to show you the real world objective lens, how it looks like, what I am going to show to you here is few of this objective lenses.

So, each of the objective lenses will have their own characteristics, that is what we are going to see in this lecture as I was telling you before, I am in a real world to get the real picture, I have brought here some of the objective lenses that we use in the lab. So, I have basically got a set of three lenses from two different companies. Two from Olympus and one from ZEISS. Apart from this, you also have microscope that shows Leica and Nikon, making their objective lenses.

So, all of them are doing their specified job exceedingly well. There is nothing that they spend a lot of research and effort to make these lenses and now remember, I talked to you about the size of the lens, right. So they also come at varying different sizes, so one of the lens that we use in the lab that is this. So, this is an objective lens so if you look at the objective lens here, it is kept upside down.

So in real system, you would see that the microscope body is somewhere here on the top and you would be holding this objective lens onto the microscope body, and your samples goes down here. So, in such a case now, the back aperture right, so that you can actually see that it is a pretty wide. In this case, it is about 20 millimeters or so, the clear aperture, and then on the other hand, you also have lenses again from different manufacturer, though for almost ZEISS and it has different properties or sometimes you need to use this against that.

Now, if you look at the back aperture that is pretty different. So, just to give you a comparison where I am going to flip it and then show both of this. It is nothing to do with the manufacturer per se, but the job of these two lenses are very different. We will see that in a minute. You can actually see that, there is another lens from Olympus again, now this back aperture is still different.

So, given this varying varieties of objective lenses and then their corresponding back apertures being different, then when you are constructing a microscope or constructing any localized excitation systems, you need an ability to dynamically change or even to change the diameter of the beam at whim, because depending on the object that you choose, you need to able to use different diameters to get the sharpest focus that you can have.

In such a case, then how do you go about doing this, so it is very simple. So, we do that through the two lens system of the telescopes, so in order to understand that telescope, again,

we would have seen that in the high school, except that the context in which we have seen as telescope is in terms of getting further image of an object that is at a longer distance, make it appear closer. It magnifies large enough and then it gives you the feeling that it is really closer and so on.

To understand that, we would have used an geometric optics, and drawn the ray diagrams that we have just described. Now that kind of a ray diagram is sufficient to even understand some of the really involved optical pathways, and one such being the pathway of simple light microscope itself. Why is it complicated. Now, I am talking about a simple light microscope, when I say is light microscope.

I mean a transmission microscope, bright field transmission microscope, so you have a sample whose transmittance is a function of space, so when you put your sample in your sample plane, and then you shine some light either from the top, down and then collect the light in the bottom or as I have described using these objectives, these are defined to have the illumination coming from bottom and then you collect the light or you see from the top.

Either way, the second configuration that I describe to you is the upright microscope while the other one is a inverter microscope. Either way, what you need to do is to have an illumination path setup such that the sample is uniformly illuminated.

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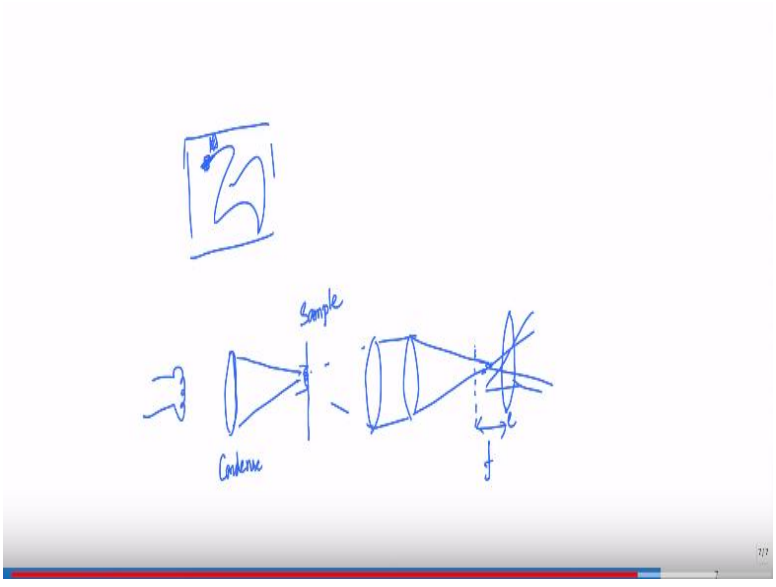

So imagine you have your sample, let us draw it as a square, wherein these lines actually represent, blue line inside represent the change in the contrast, change in the transmissivity of

the sample. This could be the dark regions, which just means that the amount of light that gets transmitted through this sample is in that area, this area is less. So you need to be able to capture that.

So, now if you want to capture that, the first thing that you need to do is able to uniformly illuminate the number of photons that are hitting unit area here, versus an unit area should be exactly the same. If it does not, then you would not be able to tell us the difference between whether it is due to the sample being having this contrast or the illumination itself having the different contrast.

So, to this problem was faced by people earlier, people meaning the original initial microscope had this issue, so, the issue was that in order to create this illumination, we would had some amount of light that can illuminate the sample, so what they would do is that they would take in a filament, and they would take a lens and simply focus that light onto the sample plane.

Okay, that is the idea, very simple idea. So, you have a sample plane, and then you have a light bulb, and this is called as a condenser lens, because it is actually condenses the illumination light on to the sample. The idea here is, I have created the maximum intensity or the flux that I could have actually created, that is what the purpose of illuminating the sample is. So then, once I have created the illumination then all I need to do is to have another lens system where I actually collect this light.

And you form any image through if you are detecting it through an eye piece; what if you are actually doing is that you are making it form an image such that it falls within the f and the lens itself. So, this is the f of the focal length of this eye piece. Now, when you do that what you end up having is that a virtual image, which our eye when you are seeing through focuses on to the retina giving you the image itself.

Now, the problem here is that when you use a very simple lens to actually concentrate the light that is emitted by the bulb on to the sample, what happens is that this serves as the imaging system of the filament itself. So, what you end up having is that the filaments image itself formed here. So, now what you end up seeing is that the image of the filament or part of the filament along with your sample.

So both of them superimposed on each other and that becomes very obstructive and able to decipher out the fine changes in the contrast that might happen. That happens because this per se, the light source per se, the bulb per se is a finite dimensional light sources, it is not like an infinitesimally small light sources where in you have the photons coming in, where there is no real point source per se. So now, such kind of illumination is no longer in use so Mr. Kohler, he was able to come up with a solution to overcome this kind of a problem.

Now, that illumination we call it as a Kohler illumination wherein we do not necessarily image the filament itself or the light filament itself on to the sample and condense the light, but he said okay we can separate these two things. One is that requiring the light, I mean the high intensity of light and then not being able to have the image on the filament of the sample plane. So the idea of segregating the image versus the uniform illumination of the sample plane that gave the name to that.

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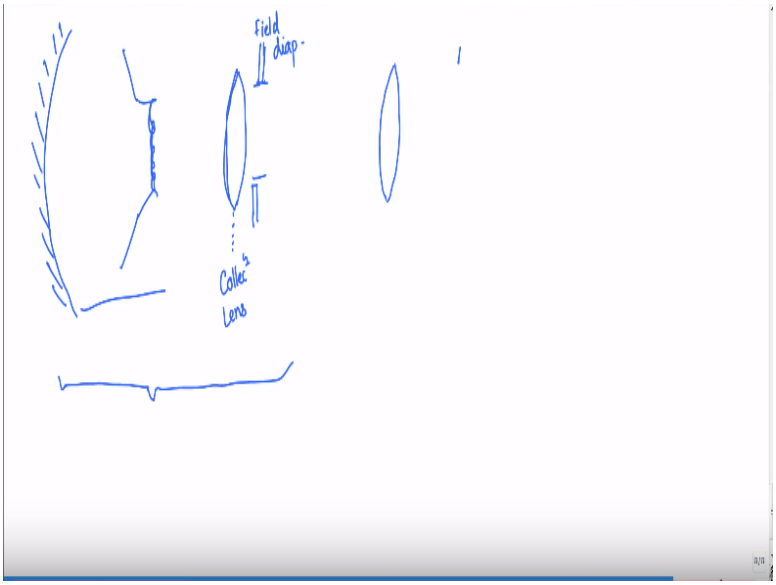

So essentially what it meant is that if you actually take a light bulb with a filament of finite dimension, so he said is that let us put collection lens, not just the condenser lens, the collection system. The modern day you would see a collection system which comprises of a concave mirror. The idea here is that if you have an object here and it is emanating all of this light, so if you collect all of this light and then make it parallel, and in addition you also have at the exit, a collection lens alright.

The job of these two objects, the mirror and the collection lens is to illuminate an aperture, a diaphragm it is called, which is called as a field diaphragm that is kept right after the collection lens. You have an collection lens and an aperture that is kept as field diaphragm, now my claim here is that with such an arrangement and then illuminating that field diaphragm, he was able to create an illumination on the sample plane, that is very uniform.

So, the way he would do that, I am going to list out the parts and then we will see in the next lecture, how we can actually do this diagram and then we will go back to the objective lens, and then see what are all those lenses play in the following lecture. So, he had this collection apparatus and then his argument was now, if I were to use this collection lens and this collection assembly and illuminate a field diaphragm and uniform diaphragm and then use a condenser lens now.

To image not the filament, but this field diaphragm on to my sample plane. Now you see at the field diaphragm, you can approximate the rays are to be parallel because both from this concave mirror that is reflecting the light from the bulb as well as this collection lens, what it does is that this effectively makes them all become parallel because it is a quasi-focus as a result it is quasi-parallel, now all that he had to do was to condenser lens to image it on the sample. We will see that diagrams and then how it translates to the imaging of the sample in the next lecture.