Optical Spectroscopy and Microscopy

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Lec – 50

Fundamentals of Optical measurements and instrumentation

Hello and welcome to this course of Optical Spectroscopy and Microscopy. In the last lecture, we were talking about how to illuminate the sample such that we do not introduce any artificial contrast or in a true sense, we were talking about how to separate the idea of illuminating the sample and still not be able to form the image of the light source itself on to the sample. We were talking about the critical illumination principle whereby the sample when it was illuminated, the image of the filament itself was formed on to the sample plane and that kind of an illumination scheme.

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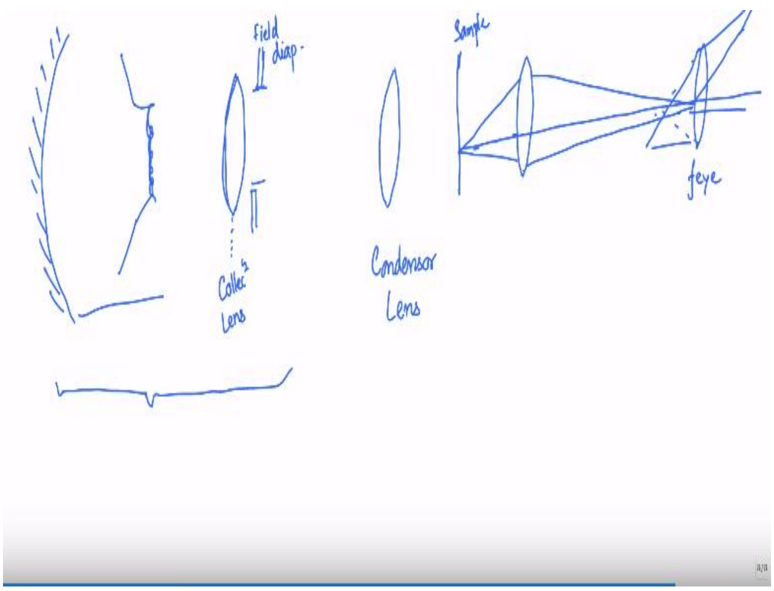

This kind of illumination scheme wherein we use this light source and then use a simple condenser lens to illuminate the sample, intensify the light around the sample is called as the critical illumination. The problem as we saw in the last class is that it introduces artificial contrast. So to avoid that, Kohler came up with this rationale wherein he said that let us not image the filament itself. All we need is the photon, so let us create a uniformly illuminated path. It is an aperture, uniformly illuminated aperture and let us image the uniformly illuminated aperture onto the sample.

That was achieved using collection assembly, which consisted of the light source, reflector and a collection lens placed such that they are all going to illuminate the field diaphragm uniformly. So, once you have this field diaphragm illuminated uniformly, then the job of illuminating the sample becomes much easier wherein he would have a condenser lens image this field diaphragm, the light coming out from the field diaphragm or the field diaphragm itself onto the sample plane.

Now if you think about it since field diaphragm you do not have the image of the filament. The way this is done, the image of the filament is formed at infinity after this lens. So, because they are at focus. We know that we just saw the geometrical optics in the previous class where if you have to place the object or to the focus, then your image will be formed at infinity.

So, the point here is that we do not have to worry about the image per se of the filament, but what we do have is that all the photons that are emanating from here as much as we could collect would be coming through this aperture and it will be bright. That is actually illuminating the sample, so it is uniform illumination. Now, what we could actually have is lens 1, objective lens taking a point, just the way we drew the optical diagram and then imaging that point on to a place that is between within the focal length of the eye piece.

The effect being that you generate an image of this point as a virtual image. Now, that were to be verifying and good until a point where people wanted to correct for various different problems that the lens per se would pose because lens by itself has its own limitations when it comes to various degrees of defects that originate from the focus. So, this is our objective lens by definition because it is close to the sample. In a modern day objective lens, what you would see is that it is far from a one simple lens and that is exactly why when we actually looked at this objective lens, it is such a big object.

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This is not a very simple lens, but it is a pretty extended object. So, now in this pretty extended object, there are quite a few parameters that is written on to this objective lens and what we are going to do is that we are going to understand what each of this is and why is that important.

So to start with, we write down in parallel what this parameters are and how do we make use of them in our system. So, now if you look at the objective lens, the first place is usually they will have the brand name, in this case it is the country of origin, Japan and in this case it is ZEISS. So in this lens, the first one that you see is the country name we saw and then the second is the description of the type of this lens. Now here what we have is XL Plan N. So, let us remember, it is a plan objective. On the other hand, here you see that it is W Plan Apochromate. In here, you see it is plan FL.

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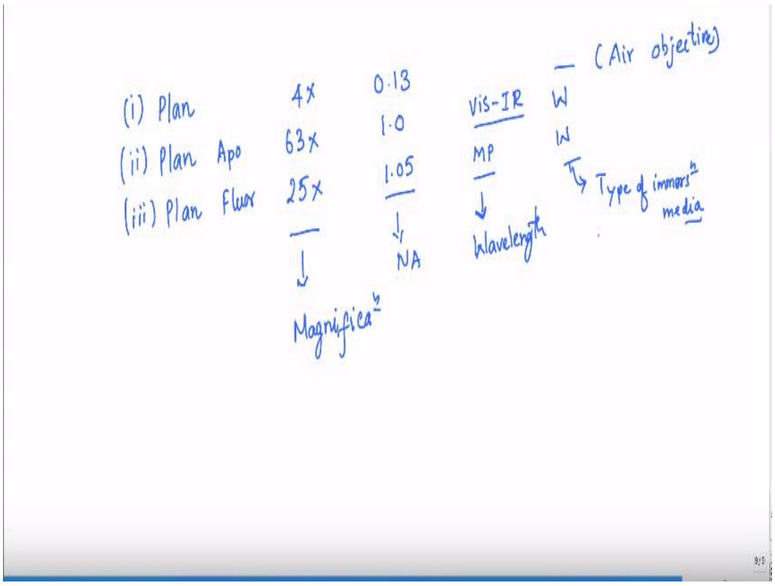

So, we have three kinds of lenses, so let us mark it down. One is Plan, another is Plan Apo and third is Plan Fluor. So now apart from this, we also have few other illustrations on the objective lens, so if you look at the first objective lens, you see that you have a number that written as 25x /1.05 WMP. I am not sure people can see, so it take from 1.05 WMP. On the other hand, so this for want of space, they have written on the other side, where you have 63x 1.0 IR, while the plan FL you have 4x 0.13.

We will concentrate on this numbers first, so our plan had a number written 4x and 0.13, while the plan Apo has 63x 1.0, while on the other hand, this has 25x and 1.05. In addition, you also saw in the Apo, it is a VIS-IR and here it is MP and so now, you can also see apart from this numbers, we also have the letter W mentioned on this XL plan, where WMP coming up on the while W coming up on the objective lens right here, W plan and this one does not have any W here.

Nothing is written here, you can actually search along, nothing will be written here. So, each of them have a very definite meaning, so we will also point out that nothing so. This one has a W and this one has a W. So, now probably you would have guessed by now, this 4x, 63x, and 25x corresponds to the magnification. We will see that in detail. This number by themselves do not have much sense if you have to say few other things and then while the 0.13, 1.0, and 1.05 corresponds to numerical aperture NA, and VIS-IR and MP that tells you that the wavelength regions or the regions that transmission is optimized for.

There is some amount of light that you are going to put through this objective lens, how much of that is going to come out, so that is a very critical parameter because you do not want to eat lot of incident light and so for that reason you need to do that and this W, I am stating this facts here, we will see what that means in principles in a minute. So, this W tells you the kind of immersion medium that you will be using, type of immersion media that we will be using.

So, W here means water, so we will be using a water immersion media and then not mentioning anything, so just like this microscope objective. This one, it does not require any immersion media that is what it means to say that. They are also called as air objective because they expect air to be present and nothing else. So, what are all the different numbers, we said that this different numbers mean represent these different terms, what do they mean. Now, the first thing regarding the magnification.

Now, let us go back to our original diagram, super-simplified microscope system where what we have done is we have taken the one part, part on the left hand side of the sample, the way I have drawn it is there to illuminate the sample and the right hand side part of it is there to image the sample on to your eye or you could actually thinking of having a camera and the system changes.

The eye piece and things like that will change, but the point is, it is for capturing the sample, representation of the sample, which is the image. So, now in this part originally where it was designed in olden days, these objective lenses where designed such that if we were to have a sample just at the vicinity of the focus, they would make the image at about 160 mm, it is about 16 cm, from the back flange of the objective. So what is the back flange.

We will take any of this, but I am going to take biggest one easier to explain. So now, if you look at this objective here or this objective here, now you can see that there are numbers and bodies that are written and then on the top here, the brass what you see is the thread. It is just a fine thread, which can actually take in. So, right now this is a cap lid, so now this were to be the microscope body, you would actually have the lens hanging up like this.

This is the microscope body and then the lens held. So, if that is held through these threads on the objective and the counterpart on the microscope body, now that is called as the flange. Flange that actually holds the objective. So, from that point onwards, it is designed to have the images formed at 160 millimeter. Now, this modern day microscopes are far from that, meaning they are different breed and you know, that they are of different breed because of some inscriptions, we will go back to that inscription a little later after this explanation, and how are they different.

They are different that if you look at the ray diagram, they no longer form at 160 millimeters, but they would form the image only at infinity or in other words, the beam forms the image at infinity. So expect the image to be formed at infinity. In other words, you can say that the beam coming out of those lenses are parallel. So which means, if they form at infinity, but in my lab, I need to be able to see with my eyes many times. So, how do we do that?.

The way we do that is by introducing a new lens called as a tube lens, so and the tube lens together with the objective, acts such that it forms this image, such that it is within the focus of the eye piece, so that we form the same virtual image just like the olden microscope. Now, these objectives that are expected to form the image at the infinity are called as Infinity corrected objectives.

So how do I know, given an objective which is infinity corrected or something else, now again go back and look at these inscriptions. Now, you see here, the number infinity is inscribed on here that tells you that this is an infinity corrected objective, again same here, you see that infinity being inscribed here. This is again an infinity corrected objective, and you see that infinity slash minus because it is an air objective it does not expect anything, but infinity because it is infinity corrected objective.

So that is about infinity correction, but then I taught to you about the tube lens. The tube lens together with the objective lens forming the image such that when you look through the eyepiece, you can form the virtual image in the eye. It can create a virtual image, such that you can form image of the retina of the eye. So, now the magnification right. We wrote down the magnification of the different objective lenses, 4x, 63 x and 25x. Now, that attains the meaning only with respect to the tube lens that we are actually using, so then if I give you this objective, just the simple objective like this and then it says 25x and if you were to construct an imaging system using this objective, would it give you a 25x magnification.

No it is not given. It will give you a 25x magnification if you use this in combination with as specified tube lens. So, now why is that because let us look back at the ray diagram. You know that without the tube lens, there is no image formation by the objective lens at all because it is a parallel beam that is coming out from the back aperture. You have the back aperture and there is no image formation that you can have. The fact that you are seeing some image through the objective lens here is because the objective lens forms an infinite beam, which is then focused by the camera on to the sensor.

There is a lens on the camera. If I take it away, then you will not see any image at all. It is just the lens on the camera allows you to be able to actually form it. That is true when you actually look at any system through the objective. So, now what we have to do is that we need to know, if I have to use this lens with this 25x, then what kind of a tube lens do I need to use it. So to that tube lens that varies from the manufacturer to manufacturer.

Here, we have two of the manufacturers I told you. These are dark in color, and it just says is Japan. It is Olympus objective lenses, and this is ZEISS. I told you apart from this, there are two other manufacturers that manufactures the microscope and the tube lens focal length is very standard. That is fixed. They do not change, so that when somebody changes the objective lens in their own microscope, there is finite definition of what the magnification is.

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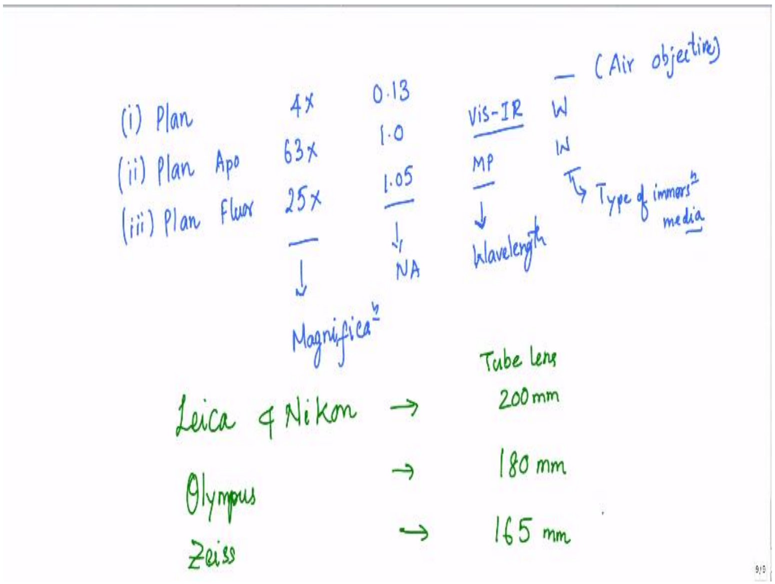

So, just to put out the numbers, so Leica the manufacturer, and Nikon, these objectives I do not have but they define the microscope objective, assuming or having a tube lens of 200 mm in mind. So, all the defined parameters objective of the lens are assuming their tube lens to be of 200 millimeters of focal length and Olympus, the two dark objectives that you have seen, they assume the focal length of their tube lens is 180 millimeter and ZEISS designs their objectives with 165 mm focal length tube lenses. So now, why is this important.

Now remember, I told you that the magnification is irrelevant without something else, because they are form at infinity. There is no image itself, so only with respect to the tube lens, they form the image, so now the objective lens and tube lens together form an imaging system. In a two lens system, and then the magnification there is defined by the ratio of their focal lengths. So, you would get a 25x magnification with this objective lens, only and only if you were to use a 180 mm focal length lens.

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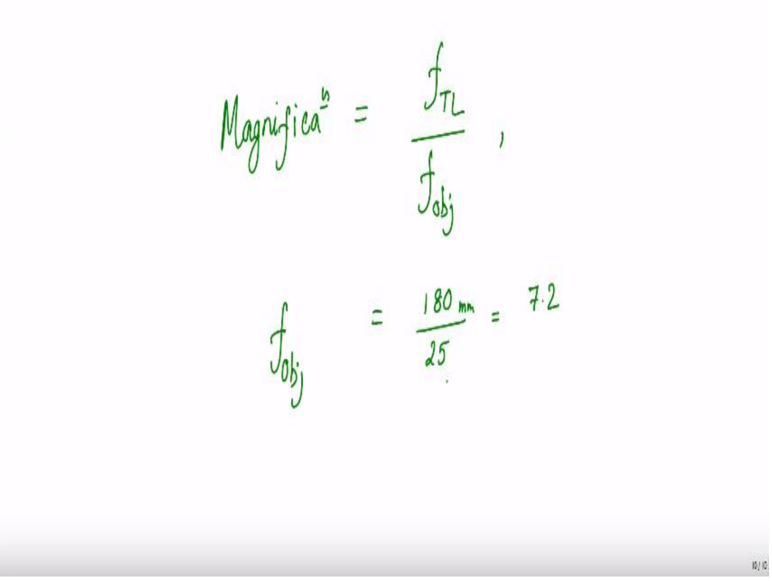

So, if you are to estimate the focal length of the objective lens, so the magnification is defined as the focal length of the tube lens, divided by the focal length of the objective. So for example, if I want to know the focal length of this particular objective, then we will go and plug in this numbers, the focal length of the tube lens is designed around 180 millimeters, so we are writing everything in millimeters, so that is 180 and the focal length of the objective would be 180 divided by 25, which is equal to 7.2 millimeter. Now, that is good.

We know the focal length of the objective. It is very useful in many applications where we have to actually design an imaging system around the objective lens. However, there is one catch to this whole problem, that is I know it is 7.2, but 7.2 from where because if you look at the objective lens, it is a big long fat object. So, it is 7.2 from the front, back or somewhere in the back or somewhere in the middle and how do I know and how do we overcome that. We will see that in the next lecture.