Fundamentals of optical and scanning electron microscopy

Dr. S. Sankaran
Department of Metallurgical and Materials Engineering
Indian Institute of Technology, Madras

Module – 01
Unit -2 Optical microscope and its instrumental details
Lecture – 05
Optical microscope demonstration
Bright field imaging
Illumination of opaque specimen

Welcome back. In the last class, we just reviewed the concept of a filters, illumination filters and interference filters and we also looked at the definition of optical path length and so on and before we started the optical microscope topic. And we also extensively looked at the different kinds of lens defects. So, I just started introducing you the equipment, the light optical microscope, I would like to continue to do that.

So, let us look at what I just showed in the last class, the first one I introduced is the metallurgical microscope, which is an inverted type. And I just want you to have a feel of how this equipment look like then we will slowly get into the details of the parts details as well as the operation details as we go along. So, I think we have just seen that. let me speed up this. I also told that this is an attached with in CCD camera, also with some polarizer and analyzers and so on. These attachments we will take it up as and when we deal with the variation of the microscope.

So, what you are now seeing is an illumination stage where the sample is kept. And this is a simple vertical type metallurgical microscope which I introduced in this in the last class. So, this is just to have a look at it again. This is a specimen stage these are all different objectives. And this is your ocular. And another one is which is attached with the image analysis system that also I just introduced. Just to recap, you can see that objective lens again specimen stage. So, these objectives will have 3 or 4 depending upon the microscope ranging from 5 x to 50 x to 100 x. And you can see that all the objectives are marked with their specification of their magnification refractive index details; and depending upon our interest, we can choose any one of these objectives to view the microstructure.

So, this is again a leveling press to keep the resourcement flat on the microscope. And this is what we have seen already, a metallic polished specimen is being pressed with the plasticizer to make it evenly placed so that your reflection can be from the flat surface. Now, this is how the specimen is placed under the objective. And then you choose the lowest one to examine the microstructure details through the eyepiece. You can directly view through the eyepiece as well as you can look at the monitor, computer monitor because it is being interfaced with the CCD camera.

So, either you can look through the ocular directly. Or, you can look at the computer monitor because it is attached with the CCD camera. Now, this microscope is the, this is where I just left in the last class. So, this is optical transmission in microscope which has got two illuminating system, one is halogen lamp this one and this is mercury vapor lamp. So, it is been shown closely for your clarity halogen lamp mercury vapor lamp. These two are just used for a specific application. I will just mention whenever it is applicable. So, have a look at it, how this structure the architecture is very different from the simple vertical microscope.

What you are now seeing is a polarizer, which is being engaged. And then your normal white field illumination you disengage this so that this is kept for the light to pass through. And what you are now seeing is below is a set of a condenser, and filters and apertures belong to different modes of operation.

You can see that it is being numbered 1, 2, and 3, and 4 and 5 and so on. So, depending upon the operation mode, you will turn this condenser and aperture to a particular slot which is given as a white dot here so that you will be able to perform that particular operation. So, in this set of condenser apertures, the one and two is for a phase contrast mode. And three is for, DIC mode that is differential interference contrast mode, we will see in much more detail when we deal with that particular variant of the microscopic technique. And 4 and 5 apertures are meant for bright field illumination. So, you have this condenser upper set of apertures here and then appropriate apertures are been chosen depending upon the mode of operation.

So, this is for your clarity much closer view of this condenser apertures being rotated 1, 2 - for phase contrast mode; so 3 - DIC mode. I will let you know the details of what kind of apertures and condenser details as and when I just talk about the theory of this mode of operation. I just into want to introduce the kind of hardware now you should not think that every microscope variant will have a different, different microscope as the whole it is just that aperture and then condenser set up and filters which makes the variants of the microscopy. So, now again this is a polarizer being engaged and disengaged.

And this is the specimen stage. Remember this is a transmission optical microscope. So, you have a transparent window, where you can keep your very thin transparent sample on this glass lined and then you can start viewing it. So, this is a specimen stage which can be adjusted in x and y movement for with this knob, you can clearly see that x and y movement. So, you should have an idea, this is done just to give you a feel of as if you are actually in the laboratory to operate this microscope. I will also give you the actual experiment so that you will have a complete understanding of operation of this equipment. So, again this is the whole specimen stage is being adjusted, you can see that. This is another axis movement.

Now you see that your all your objectives are kept in the vertical position compare to the a normal a vertical type metallurgical microscope. It is put in a inverted positions, but you can see that you have about six slots. These two slots are kept empty, but other four slots are filled with different kinds of objective aperture. You can also see that the details of the objectives are written in this letters, where the magnification details 34 x and then this is some kind of refractive index details, and then typically this has got 4 x and then 10 x and 20 x and this is 40 x or 50 x.

So, depending upon the kind of microscopes you can have whether it is 4 objectives or 5, up to 6. So, this has got about possibility of keeping 6 objectives, but you have right now, we have it is only 4 objectives. So, if you have up to 50 and 100 x. Eventually, you will have the magnification up to 1000 x because your eyepiece will have 10 x magnifications.

What is that now you are seeing is this is a part called analyzer, the operator is just inserting the analyzer. This analyzer is being used when we operate that microscope in a differential interference contrast mode - DIC mode. And you can do this a constructive and destructive interference preference using this knob, turning this knob you can make the preference of constructive and destructive inference. We will see in appropriate time how it is useful to use these parts.

So, now we will see another set of condenser aperture, just below this shutter. This shutter is meant for operating this bottom illumination which is mercury lamp. Most in this microscope, it is being used only for fluorescence mode when you want to operate perform a fluorescence microscopy. Then this is kept on this is an on position; mercury, it will be on and when you put it back then it is in a halogen lamp mode.

So, here again, you have a another set of condenser apertures similar to what we have seen in the top of or above these objective apertures 1 and 2 for phase contrast, and 3 for DIC, 4 and 5 for bright field illuminations. So, you should keep both the condenser and aperture filters above and below the objectives on a same position to perform a particular mode of operation. For example, if it is if you are going to operate a bright field illumination, either you choose a condenser window, here 4 or 5, similarly we have to rotate this on the top of or above the objective condenser lens also 4 or 5 then only your mode of operation will be correct. It is just for your clarity, this rotation is being showed once again.

So, the next detail we would like see is the focusing knob. This is the bigger one is a coarser focus, and the smaller one is the fine focus. It will basically move the stage towards the objective or either way, you will start focusing the specimen and this is coarser, this is coarser, this is fine, the small one is fine.

And the next detail is the eyepiece illumination, I mean magnification you have 1 X, if you pull this knob out, then it is 1 X; and if it is pull it, it is 1.6 X; if you push it inside, it is 1 X for eyepiece. So, these facilities possible and this is your brightness control. This is more intensity to the lower intensity.

So, this is an ocular piece which is got about which has got some variable I mean 10 X to 15 X. So, now you have some idea about a parts of optical transmission microscope and as well as the simple vertical or inverted microscopes, what are the kind of parts, you have illumination system, you have a specimen stage, you have objective, you have an eyepiece and so on. And if it is integrated with the computer or CCD camera then you will have those also will be part of the optical microscope.

So, now, I would like to show a bright field illumination in this optical microscope in actual experiments. Before I do that I would like to do some board work. In a bright field illumination the primary thing is the reflectivity; reflectivity is the key factor in bright field illumination. So, when you say reflectivity what happens when the light falls on the specimen then the light which is being reflected which are getting entering into the objective then that place will appear bright, so the reflectivity from the object, the more the image quality and so on; so for that, objective also should be completely illuminated. So, for that we will see some of the basics of a condensers and illuminators, especially for opaque specimens like a metallurgical system or material systems.

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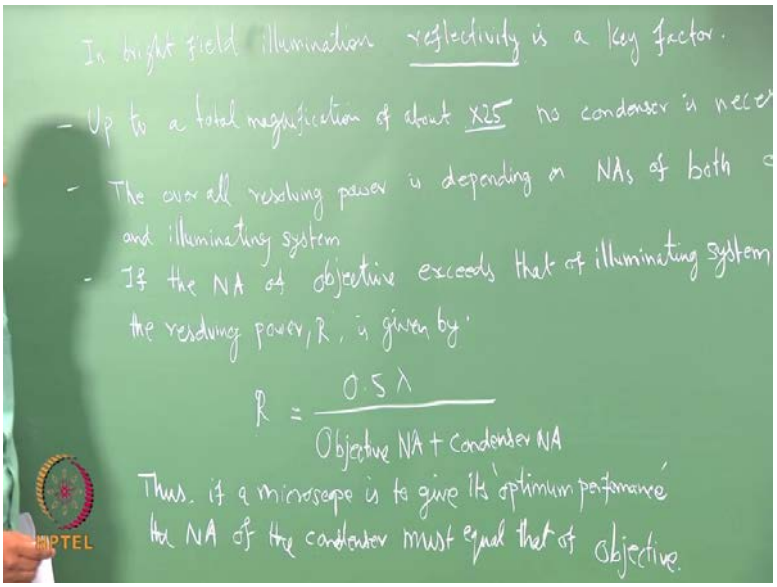

Bright field illumination, reflectivity is a key factor. So, this is your key point. And before we will just exploit this concept, we will look at the condensers and illuminators,

we will talk about illuminators. So, you see up to 25 times of magnifications, you do not require a condenser to illuminate an objective, but beyond that otherwise the objective which is having higher magnification, you need a condenser. So, let us write; no condenser is necessary up to the magnification of 25 X, this is another point. So, the overall resolving power is depending on numerical apertures of both objective and illuminating system.

So, we know that already, just to give connect to the concept of reflection, illumination of objective is also equally important concept. So, for that we are looking at the some of the basics. And so we can write if the numerical aperture of the objective exceeds that of illuminating system then the resolving power we can write is given by R equals 0.5 times lambda divided by objective numerical aperture plus condenser numerical aperture. So, this clearly tells that thus if your microscope is to give its optimum performance, the numerical aperture of the condenser must equal that of objective.

So, let me read it again. We are talking about illuminating the objective at higher magnification that is more than 25 X. The overall resolving power is depending on the numerical apertures of both objective and illuminating system. If the numerical aperture of objective exceeds that of illuminating system then the resolving power R is given by 0.5 times lambda divided by objective of numerical aperture and then condenser numerical aperture. If a microscope is to give its optimum performance, you can put it in the quote the numerical aperture of the condenser must equal to that of objective. So, that takes care of the illumination of the objective then what we talk about this reflectivity also will be optimum.

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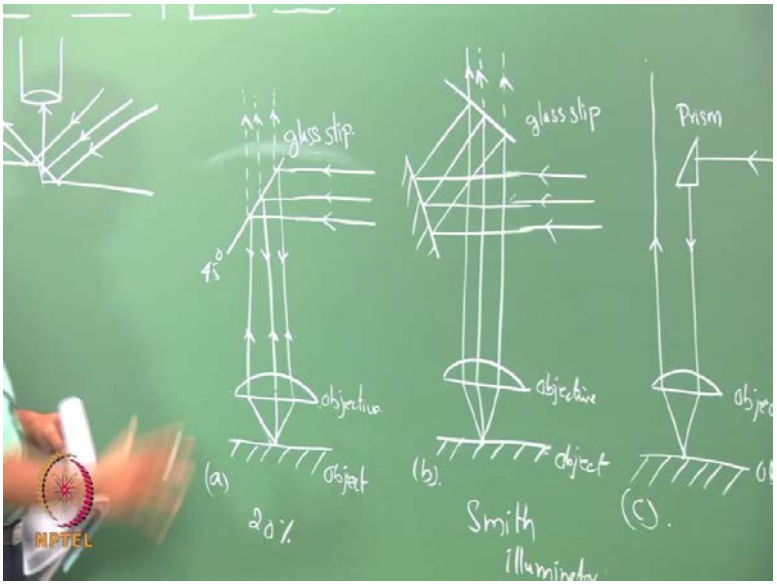

So, in normal microscope, if you talk about the illuminating system, and if there are two things, we will confine our discussion only to the metallurgical microscope, primarily, we will see what the other variations in the other variants are. First, let us take illumination of opaque specimens. See if you look at the working distance of any metallurgical microscope, which we are going to see, I will show you, it is very, very small. So, illuminating the specimen between the objective and the specimen surface is rarely possible. So, oblique illumination can be employed.

So, what happens if you employ an oblique illumination, suppose if you have the surface like this, suppose we are now illuminating like this, this will go, and this will, this will go and suppose you have the objective here, so if it is oblique illumination on a surface like this, if it is not on even surface, the reflected rays not primary reflected rays which will not enter the objective. But only the reflection which is from the step or an uneven surface only will get into the objective only those features will appear bright in the feature. So, see the best is the normal the illumination through the normal direction. We will just see what the illuminations are which are primarily employed in the metallurgical microscope and then we will proceed to the bright field illumination.

Let me draw the schematic. Suppose this is the light ray, which is coming from the source. So, normally it is passes through a glass slip, and then they are all reflected then you have the objective. And then you have this specimen surface. This is object let it ‘a’ is - object. This is objective lens. And then it goes and then it reflected back and it goes to eyepiece. This is an inclined slip illuminator, inclined slip illuminator in a normal conventional metallurgical microscope. The other type of illumination is this. Here the glass slip is kept at 45 degree, and you have this glass slip, and then this turns down, and you have the objective, and you have the object, this is b. And then this goes eyepiece. And this is called smith illuminator. And the third and final one is simple one, the light source comes and then it enters a prism and then directly goes to the objective, and the sample, so this is c.

So, you have these 3 kinds of illumination possible in the conventional metallurgical microscope. This is called inverted I mean the inclined glass slip illumination. And you see that with this only 20 percent of the light is being used for the image formation. And this set up is just because if you use a polarized light, and in this case, if you use a polarized light the plane of polarization get rotated by this glass slip which is overcome by this design that is for smith illuminator. The more intense illumination is possible by introducing a prism in the optical tube, but then it also abstracts the ray path in the tube, but these are the primary illumination paths are being considered for the conventional metallurgical microscope.

So, now, we will look at some of the example of bright field illumination. I will take you to the microscope lab again and then we will see. So what you are now seeing is I am going to just show you the bright field illumination and the identifying the micro structure. There are three samples one is steel specimen, another is cast iron, and another is aluminum specimen. We will see how this bright field illumination gives what kind of information. We will use again the vertical type microscope. And I will take up this a sample preparation techniques in a separate class and this is just I am introducing a specific mode of operation. So, we are talking about a bright field illumination now. So, you should have some idea about how this bright field is looking like.

So, we will keep this, now the sample is being kept the polished sample being kept on inverted microscope. And what you now see this is another I mean this a cast sample. And you can even use the clip to be station in the specimen and then you use the appropriate objective lines to start with it will be at the lowest probably the 5 X objective we use, and you can rotate the turret. And what you now see is selecting the appropriate objective and then I am viewing it on this specimen. And this is for aluminum sample.

So, all these 3 micro structures we are going to look at it that is why it is just shown how the samples are being kept, and then just it is just showing the operational mode how you look at it. Since it is a reflection microscope and as I said it is an opaque sample. So, we will see how the bright field microstructure appears.

So, you see the micro structure is grabbed by the CCD camera. And now you are seeing at in the monitor. This is the heta-Styron and microstructure, where you see, the people who have some materials background will understand what I am saying; otherwise, the people who do not have the material, you do not have to worry, what it is. All that we have to appreciate is you are able to see this microstructure, because of the reflection that the region which is appearing very bright are getting the lights are getting into the objective; the one which is appearing dark, they are escaping the objective that is something you can see. So, here for the people who understand the material the information is this is white cast iron.

And then you have so now you see that you are increasing the magnification you are able to see the third phase details. So, you have basically a two phase; in this case it is pearlite and cementite; the white is cementite, the black is pearlite; pearlite and cementite. And then you increase the magnification to 500 X. So, you start seeing much more detail of this specimen. You see that there is the magnification increases, you can see that the depth of focus is also having some issue, because the specimen flatness is questioned here and you can see that at the end of the corner, you are not able to focus as good as in the center region of this specimen. So, now you go to the 500 X or even more 1000 X, you will see that much more detail. I think it is getting blurred beyond this. We will look at the next, this is better 1000 X.

And this is for the medium carbon steel specimens as a lowest magnification or 100 X you see that ferrite white is ferrite and black is pearlite. As I mentioned, the people do not have the background and materials you do not have to worry, it is just that the phase which is appearing bright that means, the reflectivity of that phase is very high, you can say that. That means the rays which is coming from this white surface are entering into the objective, the one which is appearing dark escape the objective that is all you have to remember and then appreciate. For any specimen, which is having this kind of a surface undulation, you will have this kind of the contrast.

Again this is a 200 X magnification, and you see the detail of the micro structure is getting better and better. So, now you go to 500 X, you see the much more detail inside the white region all close boundaries are being revealed so clearly. And this is 1000 X magnification, you can even see that you are able to resolve this black portion which is perlite and you are able to see much more details of the sample. So, you just see, this is a better 1000 X, you can see much more clearly the details of the black phase.

And so we will now move on to the next sample. This is the aluminum sample is as cast structure you are you are able to see that it is cast a structure the people who do not have this background for this materials I would say them it is a solidified form of aluminum microstructure. You see that we call it these things are called in metallurgical terms are dendrites. And then you can see that the details much more details of this specimen at as you go from a lower magnification to higher magnification much more clear details are visible. You can see that inside this, the other black phase you will be able to see much more details of the micro structural information.

So, with that I would like to move on the next technique. What we have now seen is a bright field illumination in a conventional metallurgical microscope, the kind of information you get what I have just demonstrated. And in the next class, I will start with the first another variant of optical microscope and then I will also similarly take you to this microscopy and then show the actual demonstration of the different contrast which you obtain from the microscope.

Thank you.