Fundamentals of optical and scanning electron microscopy

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Module - 02
Lecture - 10
Tutorial - Problems related to optical microscopy

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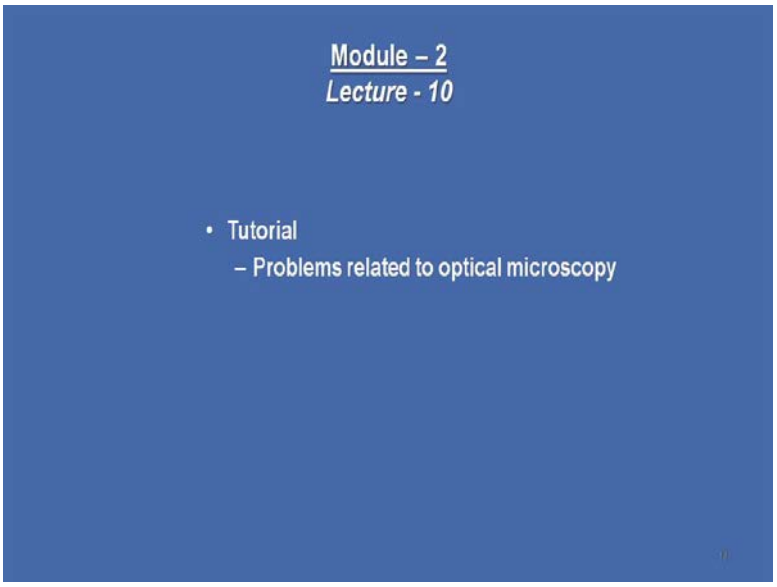

Hello everyone, welcome back to this material characterization course. In the last nine classes, we have just looked at all the optical microscopy and its variants, and various principles and demonstrations, and laboratory exercises, sample preparations everything we have seen. I would like to have one tutorial class for the whole set of optical microscope, and today we will try to solve as many problems as possible, and this exercise will enable you to solve not only the assignments which you are going to go through and also it will be useful in the end semester examinations. So, you spent lot of time in solving these problems, you can always come back and ask some questions, if you have in the relevant time. So, let us go and look at these problems one by one.

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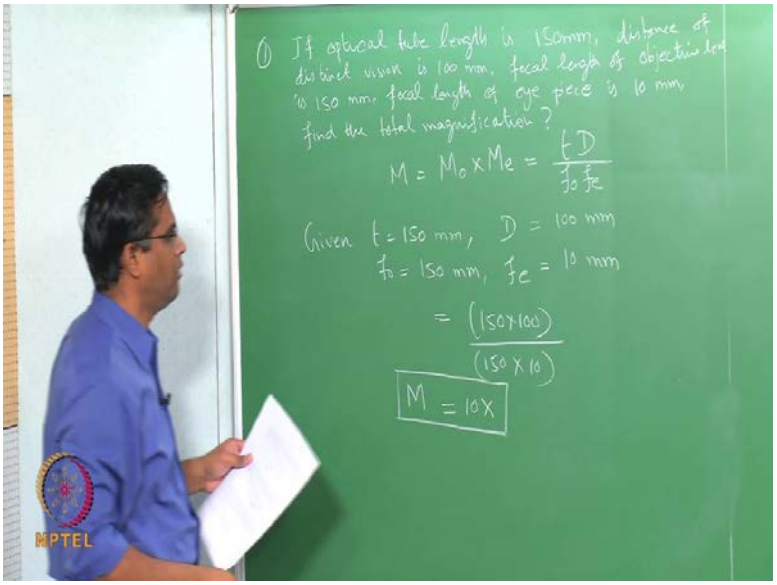

So, what I have written is, if optical tube length is 150 mm, and distance of distinct vision is 100 mm, focal length of objective lens is 150 mm, and focal length of eye piece is 10 mm, find the total magnification. So, you know that it is very straight forward problem. You have the readymade formula for this. What is that formula, total magnification M is equal to magnification of objective times magnification of eye piece, if you recall and it is related like this, you can refer back your lecture notes and slides and then this is what it is. So, you can simply substitute this. So, probably what you write is given t is equal to 150 mm that is optical tube length – t, and D is 100 mm that is distance of distinct vision. Then you have f naught focal length of objective is 150 mm, and focal length of eye piece equal to 10 mm. So, you simply substitute this and then you get the total magnification like you get around 10 x. So, this is one simple problem to find the application of this relation.

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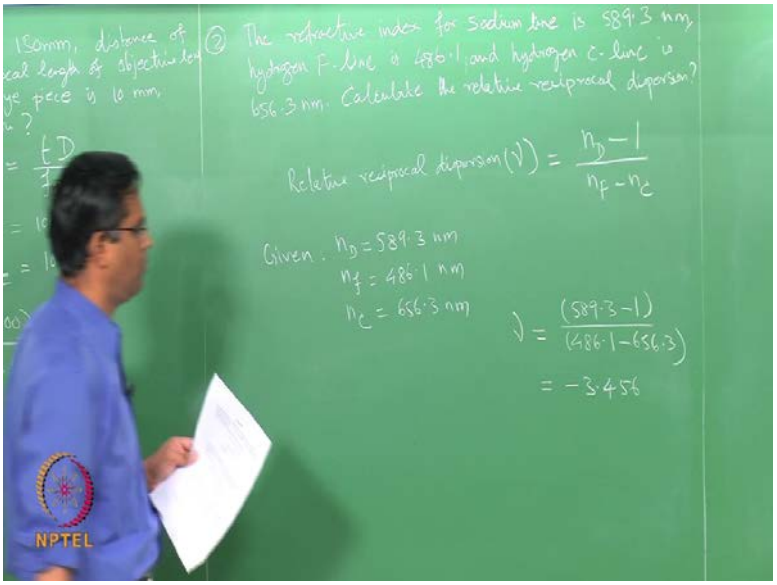

We will move onto a next problem. So, the question is the refractive index for sodium line is 589.3 nanometers, hydrogen F-line is 486.1, and hydrogen C-line is 656.3 nanometers. Calculate the relative reciprocal dispersion. Similarly, we have the expression for the relative reciprocal dispersion. What is that expression? Let us write it is mu is equal to so this is the formula. So, simply we can substitute this, what is given. So, we can simply substitute this mu equal to so it is minus 3.456. We will look at another problem involving reciprocal dispersion or we will use refractive index, knowing refractive index.

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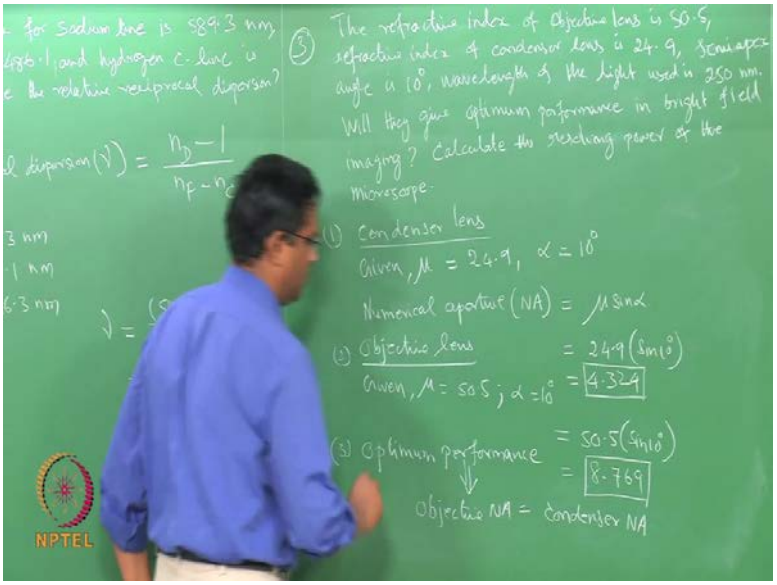

Let us look at one more problem. So, the problem is the refractive index of the objective lens is 50.5, and refractive index of condenser lens is 24.9, and semi apex angle is 10 degree, wavelength of the light used is 250 nanometers. If all these parameters give optimum performance in the bright field imaging that means, all these parameters belonging to a microscope, will they give optimum performance in the bright field imaging; also calculate the resolving power of the microscope.

What we do now, we will see, we will take one by one, condenser lens given mu equal to 24.9 and you have alpha is 10 degree - semi aperture angle, this is for condenser lens. Let us calculate the numerical value for this, which is mu sin alpha; you can calculate substitute directly this. So, we will see for objective lens given mu equal to 50.5, alpha equal to 10 degree and the mu of objective lens we directly substitute here 50.5 sine 10 degree you will have 8.769. So, you have this for condenser, you have this for objective numerical aperture.

So, what is the; now, look at what is the condition for optimum performance of the microscope in light microscope in general. If you recall we had written optimum performance, the condition is so this is a condition that means objective numerical aperture should be equal to condenser numerical aperture. So, in this case, is it equal?

They are not equal. So, these parameters will not contribute to the optimum performance of the microscope; it will not give the optimum performance, because these two are different.

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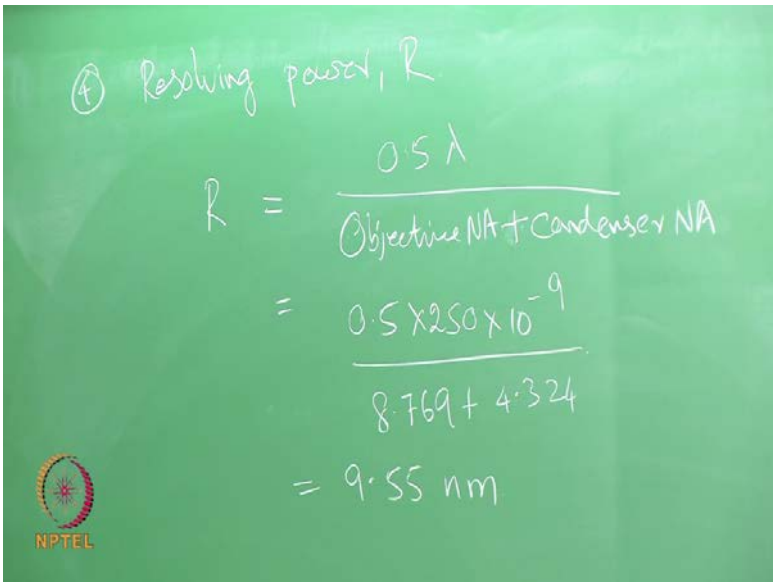

Nevertheless, we will calculate the resolving power. We have formula 0.5 times divided by so this is the answer for this question, resolving power. So, this problem give you some kind of a concept check for getting an optimal performance you need to have a numerical aperture of objective and condenser should be equal, so that concept is a kind of a concept check question. The other thing is simply here a substitution of in the formula.

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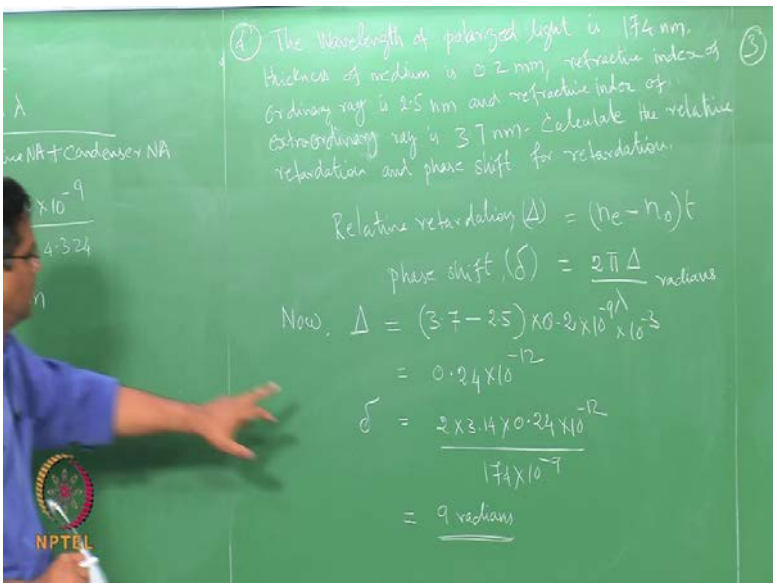

We will now see the next problem that is fourth problem. So, the problem here is the wavelength of polarized light is about 174 nanometers, thickness of the medium is 0.2 mm, refractive index of ordinary ray is 2.5 nanometers and refractive index of extraordinary ray is 3.7 nanometers. Calculate the relative retardation and phase shift for retardation? See, the retardation and the phase shift we have looked at in the fundamentals of light optics as well as when we looked at both the variance of light optical microscope namely phase contrast as well as polarized light microscopy.

So, where we see that the material will split the light into two rays that is ordinary and extraordinary ray. So, based on that if you recall the phase retardation formula is delta. The relative retardation delta is nothing but the difference in the refractive index of extraordinary ray and ordinary ray times the thickness of the medium and phase shift delta is equal to 2 pi delta divided by lambda radians. We will simply substitute this straight forward, this is 0.2 mm. So, you keep everything in same units. So, 10 to the power minus 9 into, you will work out to be, I request all of you to check this by unit conversions and then you will see delta. This is again a simple substitution here. So, you get around 9 radians.

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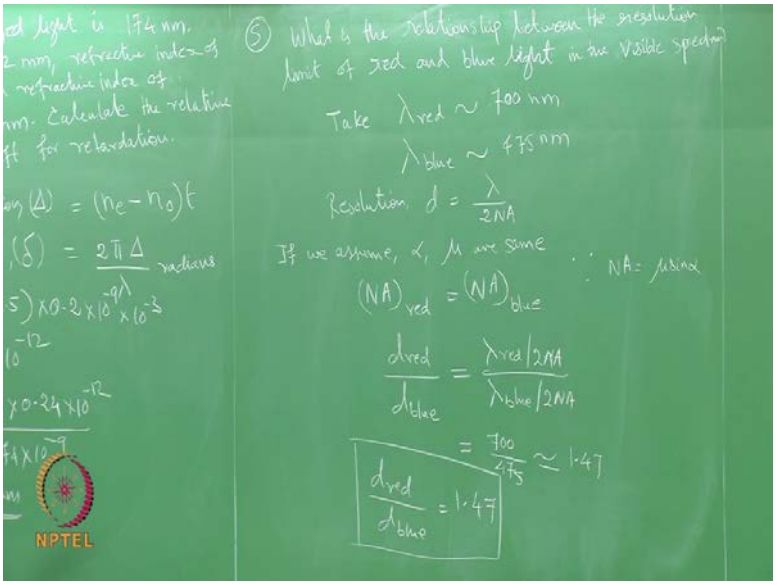

Now, we will move onto next problem, problem number 5 - Resolution limit of red, what is the relationship between the resolution limit of red and blue light in the visible spectrum. So, very general question, you can guess what we can do. Let us take approximately the wavelength lambda of red let us say 700 nanometers, and lambda of blue let us take 475 nanometers. We know that resolution limit d equals lambda by 2 times NA and if we assume that; assume this alpha and mu are same for this, of this medium are same then you can say that red equal to numerical apertures of blue. So, we know that sine alpha. Let us do that, so you take resolution limit of red light divided by resolution limit of blue light which is nothing but 700 divided by 475 - 1.47. We can right d red by d blue, this is a relation.

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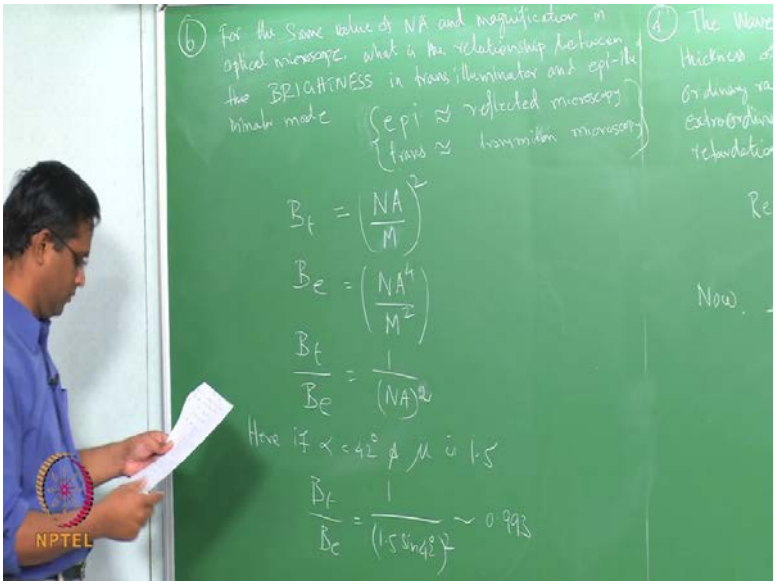

We will move onto problem 6. So, the question is for the same value of numerical aperture and magnification in an optical microscope, what is the relationship between the brightness in trans-illuminator and epi-illuminator mode. So, we have seen this two types of illuminators in optical microscope, we can write for our clarification. So, epi-illumination is generally reflected mode and trans; transmission microscope. So, we have this relation. Simply, what is written here is, this is for the brightness in the transmission mode and this is for an epi-illumination mode and then if you do this and then you have these kinds of relation, I think it should be 2 then it is right. So, just give that relation between these two illuminators.

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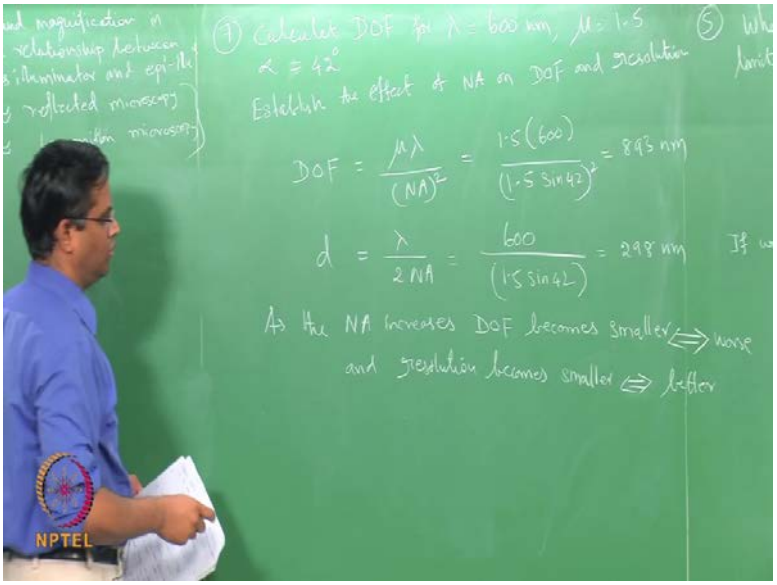

Calculate the depth of focus for the parameters; lambda equal to 600 nanometers, refractive index 1.5, semi aperture angle 42. Establish the effect of numerical aperture on depth of focus and resolution. So, we have resolution is lambda by 2 NA. What do we see here is, and resolution, so it does not, you can see that it is going back to smaller and worse, and this is smaller and better now. This is a simple relationship which we have already seen. So, numerically also you can just see that the depth of focus and its relation with numerical aperture and the resolution is illustrated in this formula. What we will do is, in the next class, we will solve some more problems in the light optical microscopic principles and then we will move onto the other microscopic techniques namely electron microscope.

Thank you.