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Lecture-10
Materials Characterization
Fundamentals of Optical microscopy

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Hello everyone! Welcome back to this material characterization course. And 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 and 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 spend a lot of time in solving these problems and you can always come back and ask some questions if you have in the relevant time. So let us go and look at this problems one by one.

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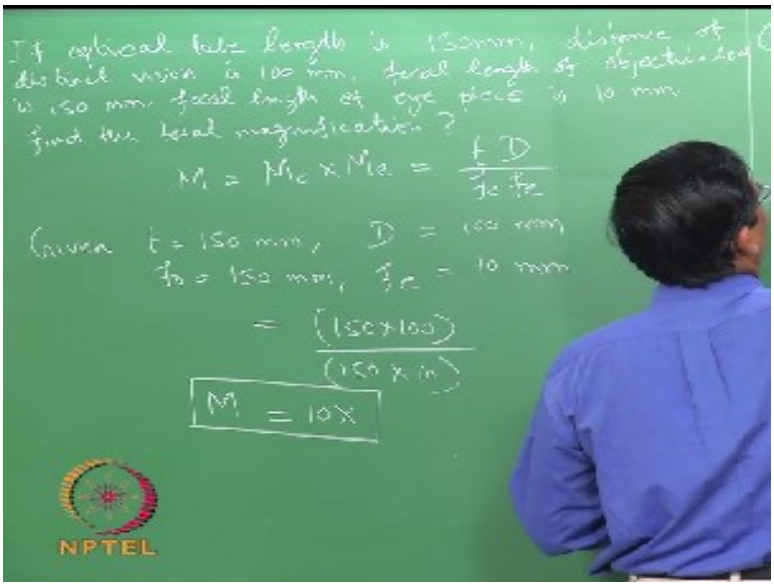

So what I have written is if optical tube length is 150 mm and distance of distinct vision is 100 mm focal length of objective lengths is 150 mm and focal length of eyepiece is 10mm find the total magnification. So you know ,that it is very straightforward problem you have the ready-made formula for this what is that formula? Total magnification m = (magnification of objective)*(magnification of eyepiece); if you recall and it is related like this you can refer back over there lecture notes and slides.

And then this is what it is, so you can simply substitute this. So you probably what you write is given T = 150 mm that is optical tube length T and B is 100 mm that is distance of distinct vision and then you have F_0 focal length of objective is 150 mm and focal length of eyepiece = 10 mm. So you simply substitute this and then you get the total magnification like you get around 10X. So this is one simple problem to find the application of this relation.

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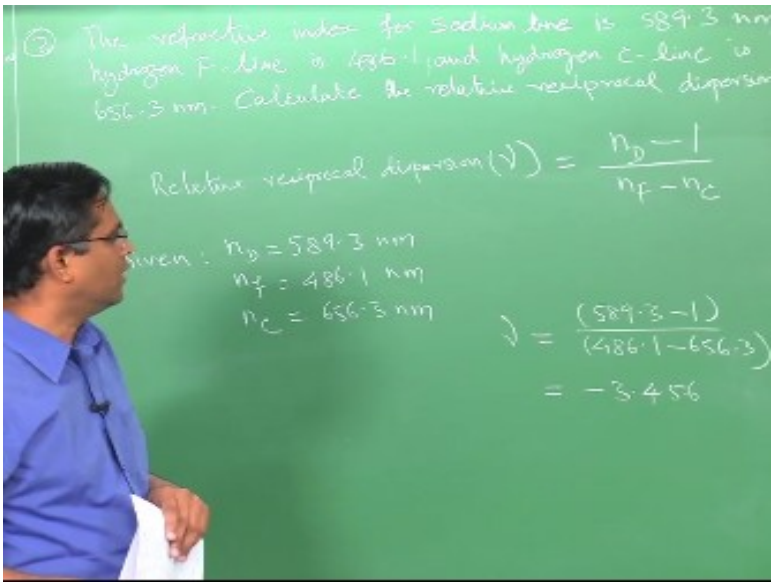

So we move on to the next problem. So the question is the refractive index for sodium line is 589.3 nanometers, hydrogen F line is 486.1, and hydrogen C Line is 656.3 nanometer. calculate the relative reciprocal dispersion. Similarly we have the expression for the relative reciprocal dispersion. What is that expression let us writes ν is equal to; so this is the formula. So simply we can substitute this what is given.

So we can simply substitute this ν equal to. So it is -3.456. We will look at another problem involving reciprocal dispersion or we will use refractive index known refractive index let us look at one more problem.

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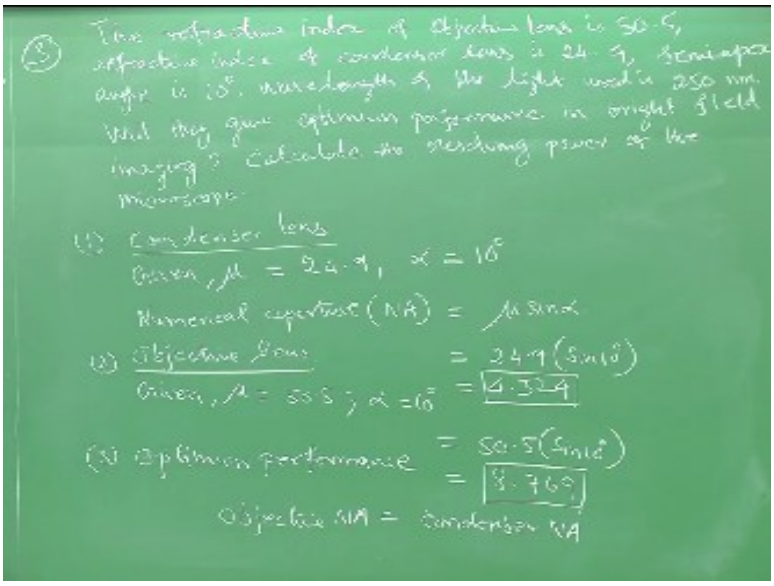

So the problem is: the refractive index of the objective lenses 50.5 and refractive index of condenser lens 24.9 and semi apex angle is 10° wavelength of the light is 250 nanometers. If all these parameters give optimum performance in the bright field imaging; that means all these parameters belonging to a microscope will they give optimum performance in the bright field imaging; also calculate the resolving power of the microscope.

So what do we do now? We see; we take one by one. Condenser lens; given μ=24.9 and you have α = 10° semi aperture angle this for condenser lens. Let us calculate the numerical value for this aperture $\mu \sin \alpha$; you can calculate substitute directly this. So we will see for objective lens given μ =50.5; α=10° and the μ of objective lens in directly substitute here 50.5 sin 10°; you will have 8.769. So you have this for condenser you have this for subject of numerical aperture. So what; is the now look at what is the condition for optimum performance of the microscope 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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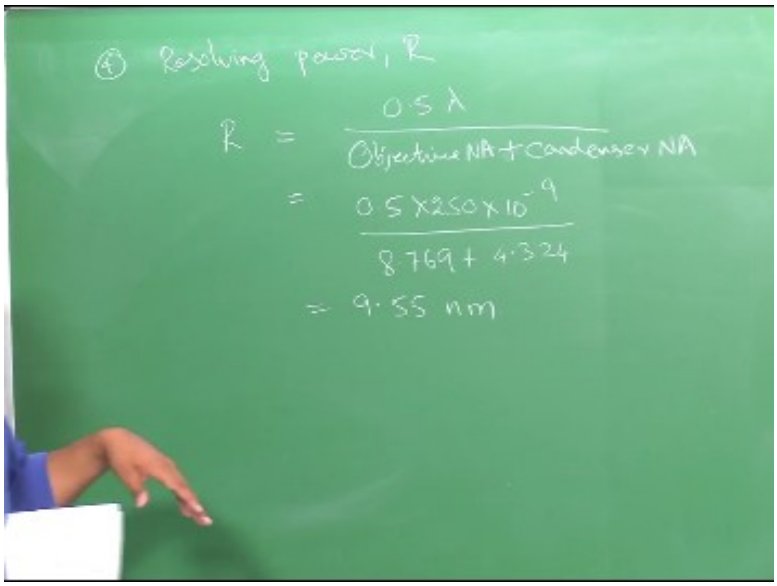

Nevertheless we calculate the resolving power; we have formula : 0.5 times divided by, so this is the answer for this question : resolving power. So this problem gave you some kind of a concept check for getting an optimal performance you need to have a numerical aperture of objective and condenser should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

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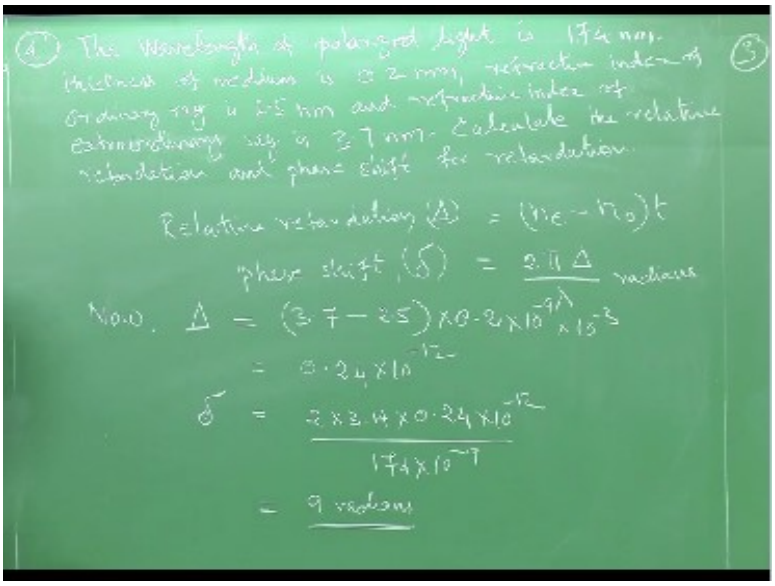

We will now see the next problem that is fourth problem. So the problem here is the wavelength of polarized light is about 174 nanometers, thickness of the medium is 0.2mm, refractive index of ordinary ray is 2.5 nanometers, and refractive index of extraordinary ray is 3.7 nanometer. Calculate the relative 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 variants 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 ray and extraordinary ray. So based on that if you recall the phase shift retardation formula is Δ. So the relative retardation Δ is nothing, (refractive index of extraordinary ray - ordinary ray) *thickness of the medium.

And phase shift $\delta = 2\pi\Delta/\lambda$ radians. So we will simply substitute this straight forward this is 0.2mm so you keep everything in same units so 10^{-9}x so you will work out to be, so I request all of you to check this by unit conversions; and then you will see δ. So this is again a simple substitution here, so you get around 9 radians.

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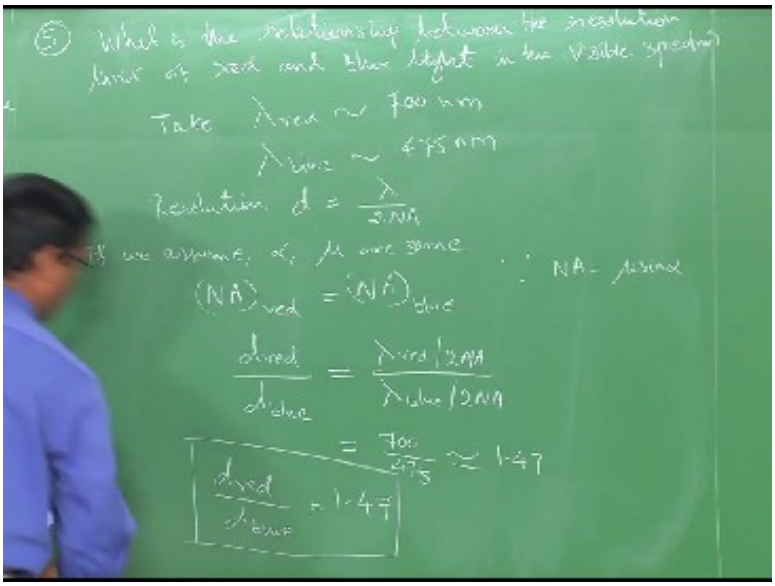

So now we move on to next problem number 5: resolution limit, limit of length; what is the relationship between the resolution limit of red and blue light in the visible spectrum? So very general question you can guess what we can do. So let us take approximately the wavelength λ of red let us say 700 nanometers and λ of blue let us take 475 nanometers we know that resolution limit, you know D=λ/2(NA).

And if you assume this α and μ are same for this of the medium are seen then you can say that red equal to numerical aperture soft blue. So we know that sin α; so let us do that so you take resolution limit of red light divided by resolution limit of blue light; which is nothing but 700/475; 1.47 we can write d_{red}/d_{blue} this is a relation.

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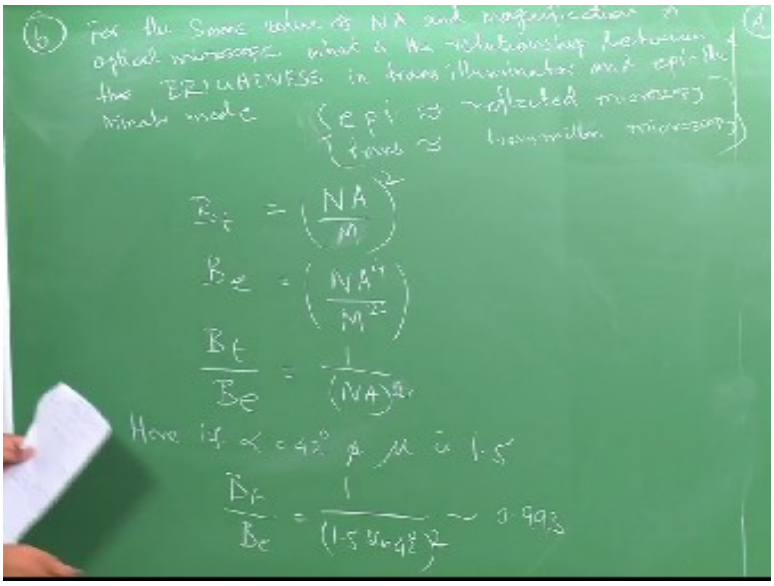

So we will move on to the problem six. So the question is for the same value of numerical aperture and magnification in an optical microscope what is the relationship between the brightest in trans illuminator and epi-illuminator mode. So we have seen these two types of eliminators in optical microscope; we can write for our clarification. So epi-illuminator is generally reflected mode and trans: transmitted transmission microscope.

So we have this, so we have this relation simply. So what is written here is this is for the brightness in the transmission mode in this is far in epi-illumination mode. And then if you do this and then you have these kind of relation I think it should be the two, I think it should be then it is right. So just give that relation between these two illuminators.

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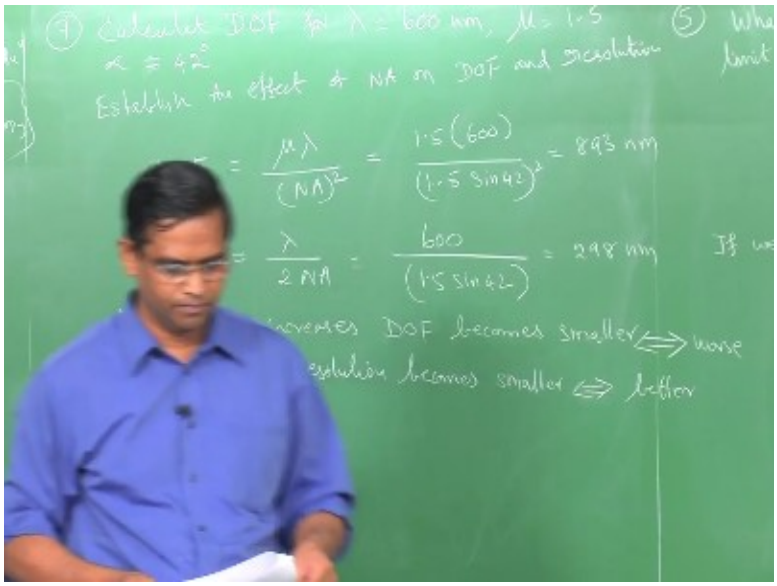

So calculate the depth of focus for the parameters λ=600 nanometers, refractive index is 1.5, semi aperture angle 42° establish the effect of numerical aperture on the depth of focus and resolution. So we have Resolution = λ/2(NA), so what do we see here is and resolution. So it does not, you can see that it is going back to smaller than worse and this is smaller and better. So this is a simple relationship which we have already seen.

So numerically also we can just see that the depth of focus, and its relation with numerical aperture and the resolution is illustrated in this formula. So 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 on to the other microscopy techniques: namely electron microscope. Thank you.

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