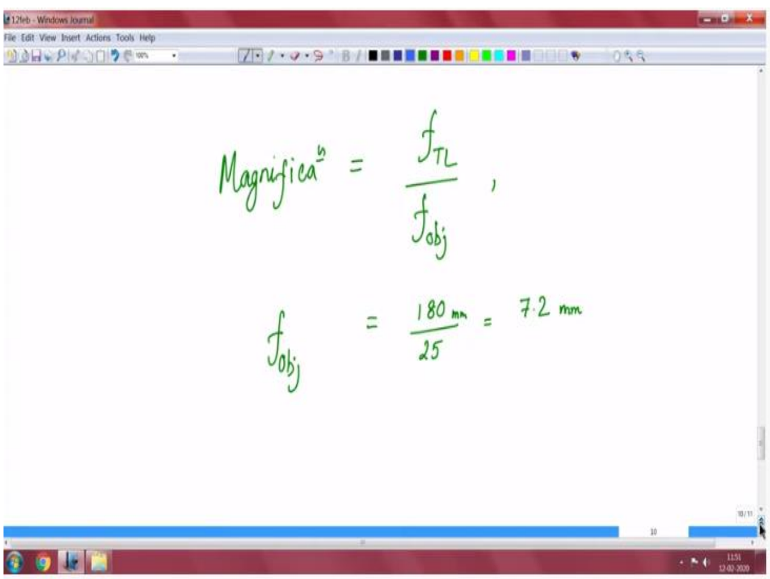
Optical Spectroscopy and Microscopy
Prof. Balaji Jayaprakash
Department: Centre for Neuroscience
Indian Institute of Science – Bangalore

Lecture – 51
Fundamentals of Optical Measurements and Instrumentation

Hello and welcome to this course on optical spectroscopy and microscopy. In the last lecture we were actually looking about the detailed description of the parameters that are present on an objective lens and how do we make use of this when we are actually using an objective lens to construct an equipment that can localize the excitation or the collection of light from a point in space. So that involved understanding what is the focal length, what is the magnification, how the objective lenses are designed and what parameters are kept in mind.

So we will continue on that front to understand more on various facts that are present on the objective lens and then will see how the different operations are corrected in this lens and in a lens and one goes about correcting these aberrations in the lens okay.

(Refer Slide Time: 01:35)



So we were looking at how do we estimate the focal length of the objective and then said what does it mean I mean where does that mean from where do I measure this focal length of the object okay.

(Refer Slide Time: 01:49)

So it turns out that so that focal length that you calculate using that is the focal length of the objective lens from its optical centre and its optical center is defining an optical centre is in such a big object right. The reason why it is big object as we know is because there are multiple lenses present here it is not just one lens that is this cylinder. But there are multiple lenses present at these critical distances and as a combination of this lenses together gives you this property why do you need a multiple lenses we will see in a minute.

But just at least for the time being just take it from me that there are multiple lenses so the geometric centre in that the system becomes very inconvenient to use. So what is convenient to use in this objective lens is the distance of the focal plane from the front surface right. So where my nail is pointing right that is the front surface no that is a very defined point or the plane from where you can actually measure things right.

So from that point how far off is the focal plane that is a very useful parameter right because if I want to keep a sample such that it is the focus, I need to know how far can I need to go alright. So that distance is called as a working distance of the lens. So now this working distance of the lens is not necessarily inscribed on the lens itself. But you go to the manufacturer and then type in this number you often you would be able to get that working distance and then its working distance is of paramount importance.

Because, you can imagine such a kind of an objective lens when it is held its going to restrict and post steric hindrances to the approach of many things towards the sample right. So people want would mention what kind of a working distance that you want in our lab as we would see in the lab sessions, we would be we are focused on imaging structures that are deeper inside the brain. So we would like to have a long working distance objectives and just to since we are working with many different objectives, I mean simultaneously very hard to remember all of them.

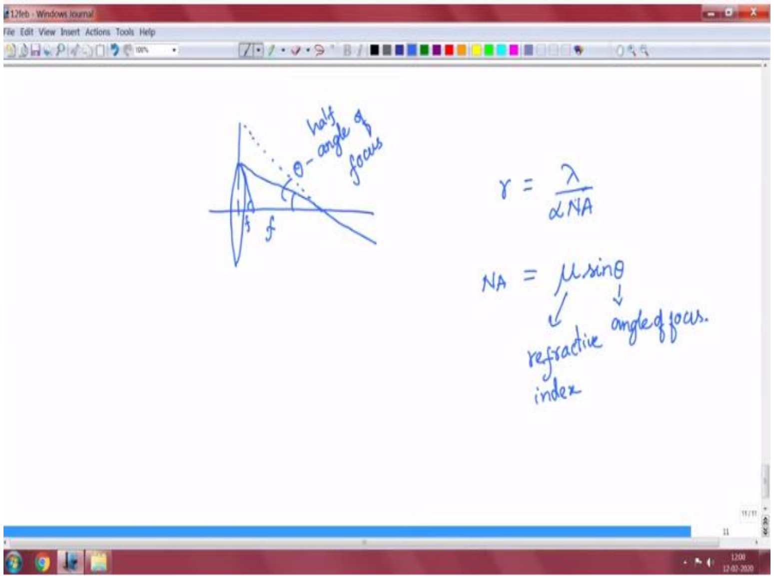
So what we had done is that they have put a small sticker and say which says WD the working distance is 2 millimeter. Now this information we got it from we got it by while ordering the objective itself and also if you happen to have an objective that you did not order but you just inherited or something. Then you just type in this number to them in the manufacturer website or the manufacturer should be able to provide you with this information.

This 2 millimeter which means from its the outermost external surface when starting from the flange all the way to the top from that point to 2 millimeter if you go that is where the focal plane is. So this is 2 millimeter and I am calling it as long working distance simply because of the fact that usually these objective lenses do not have the luxury of having that high a distance. Typically, there are a few hundred microns okay 2 into 300 microns not above that.

And here if you look at it again, we do not see any inscription about the working distance. But of course you can note down this number and then type it in and research then you will see the working distance that you get is 2.1 millimeter okay again for this the sticker has come off in here but then you can take it from me. This is a very large but and it is a low magnification okay 4x and a very large, in the sense of about a centimeter or so okay.

So that is good, but the key here is that why do we need the working distance to be so small while you know clearly its convenient to have more space between the lens and where exactly we keep the sample right. So the reason why we would like to have that to be people who I mean because it is hard to have it longer is because let us look at the what is happening to the objective lens.

(Refer Slide Time: 07:18)



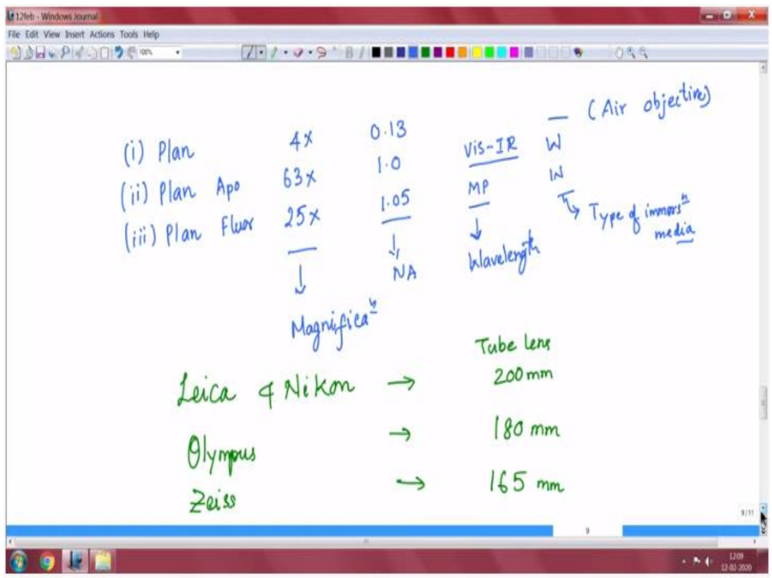
When you keep a sample close to it right or any lens for that matter. So the lens is actually focusing the light beam with an angle theta okay. So we will call this is called as angle of actually half angle of focus but. So now if you have to, I mean we know this that for a given this is a focal length right. So for a given focal length the angle of focus alright the angle of focus if you want to increase you need to increase the size of the objective lens itself or size of the lens.

So that the ray can pan out now like this so that we have a higher rank now that would mean making this making an objective lens even more fatter and fatter okay. So given the size of the exit aperture you want to fix that or the size of the lens then your focal or the angle of focus will be largest the closest that you go to right. So now or in other words the shortest focal length and since we define the focal length with respect to a center or optical center of the objective.

And we use the working distance we need to go as close to the sample as possible to have a higher angle of focus. Now higher angular focus is critical not only to localize the incident photons to a tighter spot because your resolution lambda goes as sorry the resolution not lambda. So the resolution r or d goes as lambda over NA some constant okay. So now you maximize, and the numerical aperture is NA, NA is numerical aperture. We know that we have seen in the class is given by mu sine theta where theta is the angle of focus and mu is the refractive index in which we are trying to focus okay.

We will come to that in a minute that is so clearly if you want to maximize your numerical aperture to get the best possible resolution right you want to go smaller and smaller and particles even to be able to see. So you need to maximize this NA which means you need to maximize your theta the maximum that you can have is about 90 degree where is just completely perpendicular which means you have to come closer and closer. So that is why it is very hard to make an objective lens with a long working distance which means longer focal length but yet have a higher numerical aperture okay.

(Refer Slide Time: 11:32)



Now look at these lenses the we had marked down the numerical aperture previously right and the tallest one here the 25x has a numerical aperture of 1.05 63x again 1.0 and then the 4x is point 1.3 is much 10 times smaller okay and that is why making this objective lens is relatively easier compared to making this or this for and then the cost of these lenses are very high because of the refinement that needs to be made to have a higher numerical aperture and yet have that long working distance possible.

In addition, I mean again its nothing the difference is not per say here about manufacturer it is about I mean every single manufacturer has an objective lens that has equivalent to meet your requirement or either this or this or that you know that is not a problem. So let us concentrate not on that but on the properties themselves okay. So that is why these two lenses are considerably expensive compared to this because of the refinement that even the amount of work that goes in.

So that addresses a few of the points here one is the magnification other is the numerical aperture right numerical aperture we saw was about 1.05 and 1 and that is 0.13. We also talked about the magnification and then infinity correction okay. Apart from this, you also you would also noticed that there are a few other things that we have noted down which is the W that the water I told you is the time type of the immersion medium okay.

It goes directly to the fact that when we when we wrote down the expression of the numerical aperture right here, we said NA is given by mu times sine theta this mu is the refractive index as the refractive index of the media. So when the manufacturer designs an objective lens, they design such that that they assume that you would fill the gap between the objective element objective lenses front element and the sample.

So the gap here alright this gap right so imagine that we have our sample that is present the working distance is about 2 millimeters right. We saw that so the gap between the front element and the 2 millimeters. They expect it to be filled by an immersion media which is having a refractive index close to that of water or you fill it with water that is the idea of water immersion objectives.

Now the same here there is also water and they do not expect anything and it is; in fact, they expect it to be fill meaning they do not, and they expect it to be within air so that is why there is nothing there is a dash there. Now apart from this depending on the nature of the work the immersion objectives themselves can be of 2 types the what particularly the water immersion objectives.

So you see here in this objective lens the front element and things surrounding that are all metal okay while here and here these 2 objectives have a Teflon based material that is coating its front element, I mean so the area around the front element because these are what is called as water dipping objectives as against water general immersion objectives. Water immersion objectives expect them to be you to use water refractive index media and the pure water in some sense. But here because of the nature of the work that we do we often have to dip it in salt solution right.

So to enable that the salt solutions do not leach out on the metal and stuff you have Teflon coat so when people say it is water dipping or immersion you do not have to worry about that there is no optical twist to it, it is just that the protection that is given here for water dipping is to enable the I mean there is a Teflon coat so that you can dip it in a salt solution. Typically, these are these will be having a long working distance.

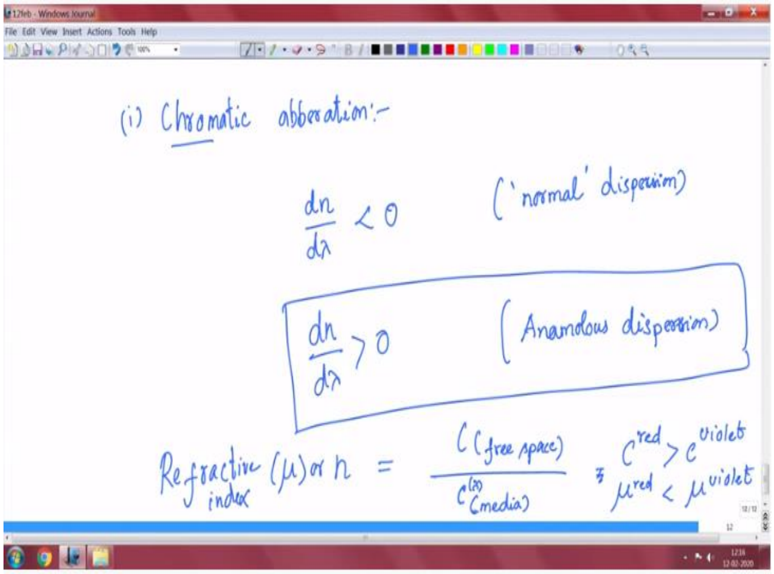
Because, the nature again the nature of the work demands that when you require a salt solution you are typically working with the live sample and we need to be able to image deeper inside a scattering tissues. So except for that correlation there is no optical restriction in terms of water dipping around water or an optical difference between water dipping around water immersion objectives good.

So now those take care of the numerical aperture working distance and the infinity correction that we talked about and the magnification. So that we also explained the W and visible IR and NP that is written on these two tells you the transmittance of the elements that are present here okay the many elements that are present right. So each of them lose some amount of light that you are putting in, but they have ensured they have enabled the transmittance of these objectives through a specialized coating.

These coatings are exactly same as that of the coatings that are presented that are used in dichroic and interference filters, we have talked about these filters and the dichroic in the course before. We will see their principle of function little later in the course but then such kind of materials can be used to enhance the transmittance of light in a particular spectral region and that is what they have done.

They have made sure that they transmit these lights the visible and the infrared light. So just what this objective lens are designed for okay. Now next comes an important kind of characterization of the objective we talked about I mean at least the 3 kinds that we talked about our Plan, Plan Apo and Plan Fluor. So what do these guys what do these names mean.

(Refer Slide Time: 19:30)



These refer to the kind of alterations that the imaging field suffers, or the imaging plane suffers because of lens being an real-world object that is to say that there are variations in terms of the properties of the lens that that comes about because it is a finite sized object. So one of them and of course we are dealing with not one particular wavelength but often multiple wavelengths and so on.

So these are called I mean we are in more specifically we are going to be talking about aberrations that give that comes it comes about because the lenses are real-world object and you need I mean we need to take some special care to make sure that this do not happen conceptually though whenever we write down this expression right the object this one over the object distance minus the image distance gives you the 1 over the focal length we and many other estimates we make some assumptions about the lens properties and this may not be met in real world so that also poses some problem.

So first of the problem are the abrasions are we talk about is called as a chromatic aberration as the name implies these are aberrations caused because of different colors of light that is being sent into the lens and then we are trying to focus it when you send different wavelength of lights two things happen the dispersion there is a dispersion because it is the lens; it is a dispersion media that the extent of dispersion or the dispersion is dependent on the wavelength alright.

So if we were to, I mean the if you had to measure the refractive index as a function of wavelength for different materials, we will see this also in detail later in the course in a special section. But in general the variation of the refractive index where n is the refractive index dn / d lambda is negative all right. So the dn / d lambda is negative for a dominant case of materials like a glass hand and so on and etc.

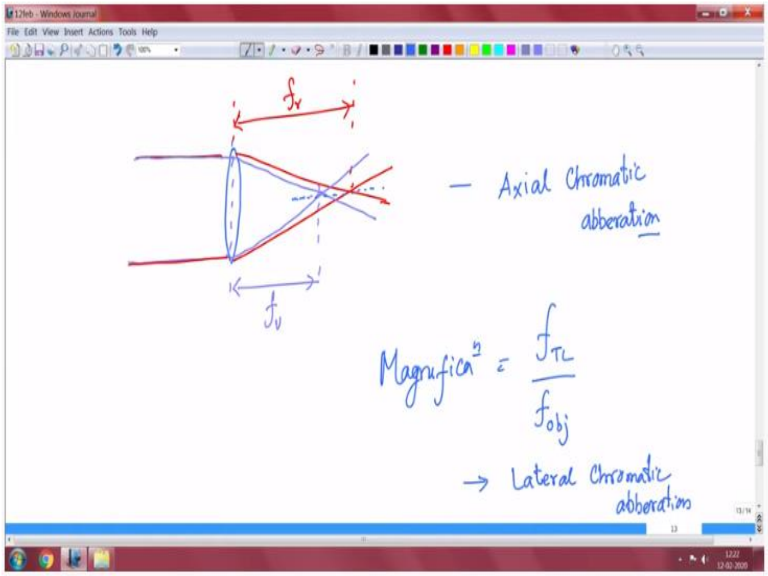
So these are called as a regular or normal dispersing media or dispersion while it is not that there are no materials that have dn / lambda greater than 0 positive. There are some materials you can actually construct them, so such kind of materials are called such kind of dispersion is called as an anomalous dispersion, dispersion in the materials are called anomalous dispersive materials. So now; so this being a special case we are not going to look into that here.

But we are talking about materials with dn / d lambda lesser than 0 which is precisely why you see that the refractive index of the red light is, so this is when you go from red light to the violet light the red light travels faster. So as a result the refractive index, which is defined as the n mean previous lecture, we had written it as mu, but it can be mu or n refractive index of a material is defined as the speed of the ratio of the speed of light in free space divided by the speed of light in that medium given medium.

If the now this is a function of lambda so if the speed of, I mean clearly, we know this the maximum it can be therefore it is the maximum speed that you can have is the speed of light which is so that in a given media so you would expect this ratio to go one and upwards alright. So as the slower I mean denser the media get it gets which means it slows down the material more and more a slow stone the speed the light propagation more and more the refractive index increases.

So in this case when we go towards the longer wavelength because of the dn/ d lambda being negative the red light has higher speed or to say that lower refractive index compared to that of the violet light. So now as a result what you have is that we have C in a given median that like a glass or something C red is larger than C violet okay so because the lambda red is larger than lambda violet and dn / d lambda is negative so since because of this the refractive index of red all right is going to be lesser than mu violet.

(Refer Slide Time: 27:26)



So now imagine what is going to happen when we have a lens and then we pass a red light that is less a purple or a violet light. So for the lack of the purple we have that so even though they both can originate from as a parallel beam of light what you would see is that because the refraction of the violet is more it tends to focus before the red light. So there is a dispersion along the axial direction okay along the axial direction you see different wavelengths coming to focus at different points.

Now such kind that results in an abrasion and such kind of abrasion is called as an axial chromatic aberration. Now because the focal length is different right so for this is the focal length of the violet and this is the focal length of red you would also have something else here the remember in any system right if you have a tube lens being constant now magnification in any system is given by focal length of your; so we have written here the tube lens divided by the focal lens of the objective okay.

So tube length divided by focal length of the objective. Now the focal length changes the magnification also changes. Please note tube lens is another lens whatever the aberrations we talk about in the objective lens you would say that hey look you might also have it there in tube lens too and I mean does it do not they cancel or how do they work.

Now the point here is that the extent of this aberrations are very different between the objective lens and tube lens simply because the object unlike an objective lens tube lens typically are much simpler okay they have they do not mean they can be compound and you will see in a minute that we also have to correct for this some operations sometimes in the tube lens. But I mean there are aberrations because too but there dominant effect happens in the objectiveness nevertheless wherever we have the lens you are going to have the separation and you need to be able to account for it.

And then what is going to happen is that the magnification is going to look different than what you have estimated, and it will be different for different colors purple versus the red. So what you will see is that the effect of this is that not only different colors coming into focus at different planes, but they will also be of a different size or in other words if you look at around the edges you will see a spread of these colors.

That is because of what is called as a lateral chromatic aberrations. So there is one axial chromatic aberration and then lateral chromatic aberration both of them are because of the fact that the there is a wavelength dependent refractive index. The refractive index is wavelength dependent as a result they come into focus at different points this leads to aberrations of two kinds along the axis because two different points in axis.

So I call it as actual chromatic aberration and then because the focal length is different the magnification is also different and then that manifests as the spread in the lateral dimension. So you call it as lateral chromatic aberration. In the next class we will see few more of these abrasions list of lists them all go through what gives rise to these kind of aberrations and then say how one go about correcting it again.

We may not be able to go through in complete detail of working out the mathematical detail of how we can correct for because that will be beyond the purview of the course there will be a detailed optics course in all itself. But what we will see is in principle how one goes about correcting it and what it means for these objective lenses okay and I will see you in the next class. Thank you.